Multiple myeloma etiology and treatment

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Abstract

Genomic aberrations comprise hallmarks of multiple myeloma (MM), a plasma cell malignancy with an overall poor prognosis. MM is heterogeneous and has different molecularly-defined subtypes according to varying clinical and pathological features. Hyperdiploidy or non-hyperdiploidy has usually been identified as early initiating genetic events that can be followed by secondary aberrations, including copy number changes, secondary translocations, and different epigenetic modifications, which cause immortalization of plasma cell and disease progression. Even though recent advances in drug discovery have offered new perspectives of treatment, MM remains incurable. However, understanding the molecular complexity of MM would allow patients to get precision treatment. Our review focuses on current evidence in myeloma biology with special attention to genomic and molecular variations.

Keywords: Multiple myeloma, cancer genetics, targeted therapy, clinical trial

INTRODUCTION

Multiple myeloma (MM) is an incurable neoplasm of terminally differentiated B lymphocytes called plasma cells, which occurs in bone marrow and secretes immunoglobulin[I]. MM mainly affects elderly people, and the median diagnosed age is 69[I]. It has a poor prognosis, and the 5-year overall survival rate is 48.5%[I].

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Genomic aberrations are central to the development and progression of multiple myeloma\cite{3}. Genomic instability affects all levels of the genome and leads to two types of aberrations: large-scale and small-scale\cite{3}. Large-scale aberrations include insertions, deletions, translocations, and inversions\cite{4}. These aberrations can be revealed in tumor cells during the metaphase of the mitotic cycle using traditional Giemsa banding and spectral karyotyping\cite{5}. Similarly, fluorescence in situ hybridization and other molecular cytogenetic approaches can identify large-scale aberrations in interphase cells\cite{6}. Small-scale aberrations include small insertions and deletions (indels), loss of heterozygosity, copy number changes, and base substitution mutations\cite{3}. Next-generation sequencing (NGS) is a collection of methods that can identify small-scale aberrations and includes whole-genome sequencing and protein-encoding exome sequencing (WES)\cite{7,8}. Recently, transcriptome-wide sequencing has allowed for the identification of subtypes stratified by cells of origin and genomic/epigenetic alterations\cite{9}. To this end, analysis of a 70-gene prognostic signature developed by the University of Arkansas for Medical Sciences has been used and validated to stratify risk for relapse and survival\cite{10}. Furthermore, transcriptome sequencing-based stratification can predict response to MM therapy, as shown with MCL1-M co-expression and bortezomib response\cite{11}.

In the cytogenetic approach, MM initiation and progression involve primary and secondary events. Primary events responsible for plasma cell immortalization are further categorized into two subtypes: hyperdiploid (HRD) and non-hyperdiploid (non-HRD). HRD subtype is correlated with trisomies of the odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, and 21). Non-HRD subtype involves balanced chromosomal translocations, with more than 90% of non-HRD cases affecting the transcriptionally active IgH locus on 14q32. The primary translocations t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20) cause over-expression of oncogenes MMSET/FGFR3, CCND3, CCND1, MAF, and MAFB\cite{12,13}. These primary translocations can be found in about 50% monoclonal gammopathy of undetermined significance (MGUS) patients as an early event, which takes place in the lymphoid germinal center during physiological class-switch recombination and somatic hypermutation\cite{14}. Either directly or indirectly, HRD and non-HRD events can cause dysregulation of the G1/S cell cycle transition point through the over-expression of cyclin D genes, which is a key to an early molecular abnormality in myeloma\cite{15}. The secondary events involved in myeloma progression occur later in the disease and include secondary translocations: t(8;14) linked with MYC overexpression, loss of heterozygosity, copy number variations (CNV), acquired mutations, and epigenetic modifications\cite{1,14}.

One of the pivotal aspects of MM is the recognizable clinical phase linked to each step of MM development. MGUS and smoldering multiple myeloma (SMM) are both early premalignant phases. MGUS is asymptomatic and is characterized by a < 10% plasma cell count in the bone marrow and a progression rate of 1% per year to MM. SMM follows MGUS; it is also an asymptomatic phase with > 10% intramedullary clonal plasma cells and 10% per year progression risk to MM. Thirdly, overt MM presents clinical features of hypercalcemia, renal dysfunction, anemia, and bone disease (the acronym CRAB). Lastly, plasma cell leukemia (PCL) is characterized by extramedullary plasma cell clones and rapid progression to death. Hence, the disease continuity between MGUS, SMM, and MM involves genomic hierarchy, including germline events that increase predisposition to MM, followed by early initiating events, and later gaining of genomic aberrations that ultimately trigger disease progression and treatment resistance\cite{15}.

**GENETIC PREDISPOSITION**

The inherited susceptibility to MM is well established, with an estimated heritability of about 15% and 17% for MGUS and MM respectively\cite{4}. In 2010, a Swedish study comprising 13,896 MM patients revealed first-degree relatives of MM patients having a higher relative risk (RR) to develop MM (RR = 2.1), MGUS (RR = 2.1), acute lymphoblastic leukemia (RR = 2.1) and, to a lesser extent, solid tumors (RR = 1.1)\cite{17}. There is a
4.25-fold risk of MM in first-degree relatives (95% CI: 1.81-8.41) that was observed in the 1961-2003 Swedish national cancer registry data[18]. Similarly, among the first-degree relatives observed in Minnesota and Mayo Clinic cohorts exhibited the increased risk of MGUS (RR = 3.3; 95% CI: 2.1-4.8) and MM (RR = 2.0; 95% CI: 1.4-2.8)[19]. While the familial clustering of MM indicates a genetic predisposition to the disease, only recently (2012) has GWAS identified single-nucleotide polymorphisms associated with MM risk[20]. In addition to identifying multiple risk loci, GWAS has provided innovative insights into genetic-related risk[20]. Inherited variations at loci 2p23.3, 3p22.1, and 7p15.3 are associated with a genetic predisposition to MGUS and involves gene pairs 2p: DNMT3A and DTNB, 3p: ULK4 and TRAK1, and 7p: DNAH11 and CDCA7L[21,22]. Chubb et al.[23] verified that the seven common variant loci 2p23.3, 3p22.1, 3q26.2, 6p21.33, 7p15.3, 17p11.2, and 22q13.1 may account for 13% of the familial risk of MM. Further studies have confirmed more candidate loci summarized in Table 1. Additionally, rare variants such as LSD1/KDM1A, KIF18A, ARD1, CDKN2A, and DIS3 may be contributed to missing heritability[16]. The reliable identification of these susceptible risk variants would be an important advancement in the early detection of MM. Furthermore, it could postulate potential personalized treatments or gene knockdown to limit progression to MM in the future.

The African American (AA) population has a higher prevalence of MGUS and MM than Caucasian Americans (CA) of European ancestry[30,31]. A study by Costa et al.[32] reported a 2.24-fold higher incidence of MM in AA men compared to CA men. Also, MM occurs in the AA population at an early age of 65.8 compared to age 69.8 in the CA population[33]. When considering polygenic risk scores (PRS), people of African ancestry in the top 10% PRS had a 1.82-fold (95% CI: 1.56-2.11) increased risk for MM compared to those with an average risk[34]. Although the confounding factors of healthcare inequalities, lifestyle, and environmental factors are significant, racial genetics is crucial in the etiology of MM in the AA population[20].

A study involving GWAS analysis revealed a stronger association between the 7p15.3 (rs4487645) locus and MM in AA[35]. The expression quantitative trait locus analysis on the biological function of the 7p15.3 (rs4487645) risk locus showed that the C risk allele is linked to elevated CDCA7L (cell division cycle-associated 7 like)[36]. The elevated CDCA7L attributes to the emergence of an IRF4 binding site on the 7p15.3 enhancer[37], hence, connecting the germline risk of MM to a genetic pathway IRF4-MYC.

Similarly, an NGS study about acquired somatic mutations in MM/myeloma has underlined new insights into racial differences between AA and CA patients. It demonstrated higher mutation frequency in genes ABI3BP, ANKRD26, AUTS2, BCL7A, BRWD3, DDX17, GRM7, IRF4, MYH13, PARP4, PLD1, PTCHD3, RPL10, RYR1, SPP2, STXBP4, and TP53 among AA than in CA myeloma patients[3]. Besides, myeloma MM-associated translocations t(11;14), t(14;16), and t(14;20) also play a critical role in racial AA vs. CA disparity[38].

**TRANSLOCATIONS IN MYELOMA**

**Translocation (4;14) (p16.3;q32.3)**

The translocation t(4;14) is seen in 15% of MM cases and has a poor prognosis[39,40]. This translocation results in the over-expression of two genes: FGFR3 (70% of cases) and MMSET (all cases)[41,42]. FGFR3 up-regulation results in the ectopic expression of the FGFR3 tyrosine kinase receptor types[43]. MMSET is a methyltransferase protein. Its up-regulation leads to enhanced methylation of histone H3K36, which modulates the expression of several genes. MMSET also regulates the methylation of histone H4K20, subsequently affecting the recruitment of tumor protein p53-binding protein 1 (TP53BP1) at the site of DNA damage[44]. Both MMSET and FGFR3 over-expression up-regulate CCND2 and in some instances CCND1.
## Table 1. Susceptible loci for multiple myeloma

<table>
<thead>
<tr>
<th>Study</th>
<th>Locus</th>
<th>Candidate gene</th>
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<tbody>
<tr>
<td>Broderick et al. [21], 2012</td>
<td>2p23.3</td>
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<td>22q13.1</td>
<td>CBX7</td>
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<td>Duran-Lozano et al. [29], 2021</td>
<td>13q13.3</td>
<td>SOHLH2</td>
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via unknown mechanisms [12]. Notwithstanding t(4;14) being associated with a poor prognosis of MM, treatment of MM with bortezomib, a proteasome inhibitor (PI), results in an increased survival rate in these patients [45].

**Translocation (6;14) (p21;q32)**

The translocation t(6;14) is rare and is present in only 2% of MM patients [1]. It has a neutral prognosis. This translocation causes the juxtaposition of CCND3 to the IGH enhancers, thus directly up-regulating CCND3 expression [46].

**Translocation (11;14) (q13;32)**

The translocation t(11;14) is the most frequent translocation present in MM (15%-20% patients) [1]. It has a neutral prognosis, although t(11;14) patients show significant heterogeneity and may present as PCL [14]. This translocation up-regulates a cyclin D gene in the CCND1 form. Gene studies demonstrated that the over-expression of CCND1 and CCND3 results in the deregulation of common downstream transcriptional events [47]. The central role of cyclin D gene deregulation in MM provided insight into research on cyclin D inhibitors in vitro. Human trials on cyclin D inhibition therapy for MM are also under consideration [48].
Translocation t(14;16) (q32.3;q23)
The translocation t(14;16) shows up in 5%-10% MM patients. This translocation is associated with a poor prognosis; however, a large retrospective analysis of 1003 patients with t(14;16) revoked its prognostic significance. The t(14;16) gives rise to over-expression of the MAF gene splice variant c-MAF, which is a transcription factor that up-regulates a couple of genes, including CCND2, by directly binding to its promoter. MAF up-regulates the expression of APOBEC3A and APOBEC3B, two DNA-editing enzymes, in MM tumors carrying t(14;16). This leads to a mutational pattern termed as APOBEC signature with a high mutation rate.

Translocation t(14;20) (q32;q12)
The translocation t(14;20) is the rarest of 5 major translocations detected in only 1% MM patients, and has a poor prognosis. However, paradoxically, long-term stable disease is found in the MGUS and SMM stages. It results in over-expression of the MAF gene paralog - MAFB. Mutant MAFB is seen in 25% of patients with MM harboring t(14;20). Microarray studies have shown that MAFB over-expression results in CCND2 deregulation like c-MAF. Tumors with t(14;20) have the APOBEC mutational signature, which is induced by the up-regulated APOBEC4.

Secondary translocation affecting MYC
Secondary translocations are independent of class-switch recombination and occur later in the disease. The c-MYC proto-oncogenes at 8q24 is the key target of secondary translocations. c-MYC over-expression is associated with poor prognosis and has a robust correlation to high levels of serum β2 microglobulin. The most common secondary translocation in MM is t(8;14), involving the IGH at 14q32.3. The other partner loci in the remaining 40% MYC translocations include IGL at 22q11.2, IGK at 2p11.2, FAM46C at 1p12, FOXO3 at 6q21, and BMP6 at 6p24.3. Importantly, all these translocations are unbalanced and associated with kataegis, which is a pattern of localized hypermutation linked with the deregulation of APOBEC activity near the translocation breakpoints. As APOBEC works on single-stranded DNA exposed around the translocation locate, kataegis occurs next to the point of chromosomal rearrangements.

COPY NUMBER VARIATIONS
CNVs involve either gain or loss of DNA. It comprises focal deletions/amplification, chromosomal arm loss/gain, and hyperdiploidy. CNVs contribute to genomic instability either via over-expression of proto-oncogenes or loss of tumor suppression genes. Therefore, CNVs act as important driver events in MM development and progression.

Hyperdiploidy
HRD is defined by a chromosome count greater than the diploid number of chromosomes (> 46). In MM, HRD involves trisomies of the odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, and 21) and is noticed in approximately 50% of MM cases. The underlying mechanism for HRD is unknown, but one hypothesis suggests that single disastrous mitosis causes the gain of all chromosomes rather than their serial gathering over time. However, the contribution of HRD to myelomagenesis is unknown. In addition to the dysfunction of cyclin D genes, GEP studies have validated the involvement of many protein synthesis genes in hyperdiploid tumors. These include MYC, NF-kB, and MAPK signaling pathways. From a prognostic perspective, HRD is associated with more favorable survival outcomes than hypodiploidy. Furthermore, patients harboring trisomy 3 and trisomy 5 have better overall survival in comparison to trisomy 21. In contrast, HRD MM with co-existent cytogenetic lesions like del(17p) t(4;14) and gain of 1q has a poor prognosis compared to HRD MM alone.
Gain of 1q
The gain of 1q arm is present in 30 to 40% of MM cases and is associated with a poor prognosis\[14\]. The amplification process involves 1q12 pericentromeric region instability due to hypomethylation and jumping translocation of the whole 1q arm\[65\]. Gene studies have shown a minimally amplified region between 1q21.1 and 1q23.3 carrying candidate oncogenes including CKS1B, ANP32E, BCL9, PDZK1, ADAR1, PSMD4, ILF2, IL6R, and MCL1\[14\]. The protein phosphatase 2A inhibitor ANP32E involved in chromatin remodeling and transcriptional regulation is independently associated with short survival\[67\]. The identified specific inhibitors of the candidate genes and pathways may help in the treatment of patients with 1q gain\[14\].

Loss of 1p
Loss of 1p is present in 30% of MM cases and may involve whole arm deletion or interstitial deletion. 1p loss correlates to poor prognosis\[46\]. 1p12 and 1p32.3 are two important regions involved in myelomagenesis\[1\]. Both these regions experience hemizygous or homozygous deletions\[14\]. Tumor suppressor gene FAM46C is located on 1p12, and its expression has been verified as positively correlated with ribosomal proteins, eukaryotic initiation, and elongation factors involved in protein translation\[9\]. Similarly, FAF1 and CDKN2C are located on 1p32.3\[10\]. The protein encoded by FAF1 is involved in apoptosis initiation via the Fas pathway, while CDKN2C is a cyclin-dependent kinase 4 (CDK4) inhibitor which negatively regulates the cell cycle\[1\]. 1p32.3 deletion correlates to a poor prognosis in MM patients undertaking an autologous stem cell transplantation (ASCT) and a neutral prognosis in those receiving non-intensive treatment\[68\].

Loss of chromosome 13/13q
Loss of chromosome 13 is present in 45%-50% of MM cases, and primarily in non-HRD tumors. 85% of cases involve whole 13q arm deletion whereas 15% encompass interstitial deletions\[14\]. The minimal deleted region located between 13q14.11 and 13q14.3 also contains some genes related to MM progression, including RB1, RCBTB2, RNASEH2B, EBPL, mir15a, and mir161. The under-expression of RB1, a tumor suppressor gene, results in negative cell cycle regulation\[69\]. In 90% of cases, del(13/13q) occurs concurrently with t(4;14) as determined by conventional cytogenetic studies and is linked with poor prognosis\[69\]. In the absence of concurrent lesion, del(13/13q) lacks prognostic significance. Hence, the correlation between del(13/13q) and poor prognosis can only be seen in some patients with other high-risk genetic lesions\[70\].

17p deletion
The chromosome 17 deletion is a late disease event. It is hemizygous and involves the whole p arm\[14\]. The most common gene deregulated in 17p deletion is the tumor suppressor gene TP53\[71\]. GEP has shown that monoallelic 17p deletions in MM samples exhibit remarkably lower TP53 compared to non-deleted samples\[57\]. TP53 influences DNA repair, cell cycle arrest, and apoptosis in response to DNA damage as a transcriptional regulator\[14\]. In MM, 17p deletion is related to more extramedullary involvement, an aggressive disease phenotype, and a shortened life span. It is hypothesized that PCL is the main consequence of TP53 dysfunction\[72,73\].

Miscellaneous chromosomal gains & losses
Focal CNVs have extracted the list of potential driver genes affected by these changes. Gain of 8q24.21 can be discovered in 14% MM patients and disturbs MYC genes\[8\]. A gain of 11q13.2 is found in 15% of patients and involves the oncogene CCND1. CCND1 is also affected by chromosomal translocations and somatic mutations\[1\]. 11q deletion is detected in 7% MM cases and downregulates tumor suppressor genes BIRC2 and BIRC3\[97\]. Deletion of 14q occurs in 38% of cases and involves TRAF3 (tumor suppressor gene)\[1\]. 16q deletion is another common event (in 35% myeloma cases) and reduces the expression of the tumor suppressor genes CYLD and WWOX (implicated in apoptosis)\[97\]. Del(8p) and del(12p) are independent
adverse prognostic markers\textsuperscript{[14]}. Del 8p downregulates the \textit{TRAIL} gene. \textit{TRAIL} gene is linked with TNF-induced apoptosis. Its downregulation facilitates the immune escape of malignant clones from cytotoxic T lymphocytes and natural killer cells\textsuperscript{[74]}.

**DEREGULATED CELLULAR PATHWAYS**

Several signaling pathways are dys-regulated in MM and contribute towards pathogenesis by influencing proliferation, apoptosis, survival, migration, and drug resistance\textsuperscript{[75]}. 

**NF-\(\kappa\)B pathway**

NF-\(\kappa\)B is a group of transcription factors that play important roles in cell proliferation, differentiation, and survival, as well as in inflammation and immunity\textsuperscript{[76]}. The NF-\(\kappa\)B pathway is active in 50\% of MM cases and involves both plasma cells and bone marrow stromal cells (BMSCs)\textsuperscript{[77]}. Activation of NF-\(\kappa\)B within MM cells involves either activation of oncogenes or inactivation of tumor suppressor genes in the pathway\textsuperscript{[78]}. Genes encoding components of the NF\(\kappa\)B pathway include TRAF3, CYLD, LTB, IKBKB, CARD11, BIRC2, BIRC3, and TRAF3IP1\textsuperscript{[54]}. The NF-\(\kappa\)B pathway does not influence the survival in MM\textsuperscript{[14]}. The pathway involves the proteasome protein complex, thereby suggesting the role of proteasome inhibitors in MM treatment\textsuperscript{[78]}.

**Cell proliferation pathways**

The cell proliferation pathways in MM include the MAPK pathway, the JAK-STAT pathway, and the phosphatidylinositol-3 kinase (PI3K) pathway.

*The MAPK pathway*

The MAPK pathway is a chain of proteins that communicate signals from cell surface receptors to the DNA in the cell nucleus\textsuperscript{[79]}. The pathway is activated from inflammatory cytokines TNF-\(\alpha\), IL-6, and IGF-1 and, in return, triggers the downstream kinase cascades RAS, RAF, MEK, and MAPK, thus regulating gene expression. Two dominant oncogenes involved in this pathway include \textit{NRAS} and \textit{KRAS}\textsuperscript{[80]}. Their mutations are frequently subclonal and are involved in disease progression. \textit{RAS} mutations indicate a poor prognosis, aggressive phenotype, and shortened survival\textsuperscript{[51]}. The involvement of \textit{RAS} mutations across various cancers has given insight into the research on therapeutic inhibitors within this area\textsuperscript{[14]}. Likewise, activation of mutation in the BRAF-MAPK signaling pathway, which encodes serine/threonine-protein kinase suggests the potential use of BRAF inhibitors in MM patients with BRAF mutations\textsuperscript{[81]}.

*The JAK-STAT pathway*

The JAK-STAT pathway is activated in both MM cells and BMSCs in approximately 50\% of cases\textsuperscript{[82]}. Cytokine IL-6 signaling induces JAK-STAT activation and myelomagenesis\textsuperscript{[14]}. The over-activation of STAT3, a STAT family transcription factor, causes over-expression of Bcl-x an anti-apoptotic protein, and therefore triggers chemoresistance\textsuperscript{[69]}. The \textit{in vitro} inhibition of STAT3 with atiprimod, curcumin, and the JAK2 kinase inhibitor AG490 have already shown fair results for inhibition of IL-6-induced MM survival\textsuperscript{[84]}. In addition, STAT3 inhibition has shown sensitization of the U266 cell line to apoptosis from conventional chemotherapy agents\textsuperscript{[85]}. Hence, these results highlight the prospective conjoined role of STAT3 inhibitors and conventional chemotherapy in myeloma treatment\textsuperscript{[14]}.

*The PI3K pathway*

PI3K-Akt is a signal transduction pathway that supports cell growth and survival in response to extracellular signals\textsuperscript{[86]}. The PI3K (phosphatidylinositol 3-kinase) gets activated with IL-6 and IGF-1 action on tyrosine kinase receptors, leading to phosphorylation of the serine-threonine-specific kinase AKT (serine/threonine kinase). AKT, in return, activates its downstream genes, including mTOR, GSK-3B, and FKHR, therefore
regulating cell proliferation and apoptosis resistance\cite{14}. The phosphorylated AKT is a marker indicative of pathway activity, which is observed in approximately 50% of MM cases\cite{87}. Therapeutic targeting of PI3K is an area of interest in MM research\cite{14}.

**Cell cycle deregulation**

The deregulation of the G1/S cell cycle transition point via cyclin D gene overexpression is central to an early molecular abnormality in MM\cite{14}. Additionally, the defect of negative cell cycle regulatory genes is another major event that destabilizes cell cycle regulation. CDKN2C (Cyclin-Dependent Kinase Inhibitor 2C) downregulation either by 1p deletion or DNA methylation deregulates the G1/S transition\cite{68}. Similarly, CDP inhibitors p15, p16, and p18 are important in the regulation of progression through the cell cycle. Studies have shown that hypermethylation and homozygous deletions of p15, p16, and p18 genes lead to uncontrolled growth and MM progression\cite{88}. Treatment with the demethylating agent 5-deoxycytidine restores p16 protein expression and induces G1 growth arrest in MM cell lines\cite{75}. p21, another potent cyclin-dependent kinase inhibitor, binds to and inhibits the activity of CDK2, CDK1, and CDK4/6 complexes. It protects the MM cells from apoptosis by the induction of cell cycle arrest and subsequent DNA repair, hence inducing resistance to apoptosis by chemotherapy and radiotherapy\cite{89}. Furthermore, RB1 (tumor suppressor gene) inactivation also affects the G1/S transition and may occur because of monosomy 13, homozygous deletion, or mutational inactivation\cite{57}.

**Defective DNA repair**

The DNA repair score is a predictive factor for progression-free and overall survival of MM patients. The score’s strength is based upon the influence of aberrant DNA repair in MM\cite{90}. The understanding of DNA repair mechanisms in MM is important for developing therapeutic approaches based on the concept of synthetic lethality. It states that a combination of deficiencies in two genes (e.g., gene X and a DNA repair gene) causes cell death, whereas a deficiency in only one of the genes (gene X) does not\cite{3}. For example, poly ADP-ribose polymerase (PARP) inhibitors are used to treat solid tumors deficient in BRCA1 and BRCA2 function, which are important for maintaining the error-free homologous recombination (HR) pathway of DNA repair. PARP is a family of proteins involved in several cellular processes (e.g., DNA repair, genomic stability, and programmed cell death). PARP1 expression is linked with shortened survival and high-risk disease in MM patients\cite{65}. PARP inhibitors have given promising results in cancers with defective HR-mediated DNA repair mechanisms, as MM backbone drugs proteasome inhibitors (e.g., bortezomib) affect the apoptotic sensitivity of MM cells\cite{66}. Therefore, bortezomib-induced impairment of homologous recombination in MM cells can pharmacologically sensitize them to PARP inhibition, resulting in synthetic lethality\cite{66}. We recently found that a noncoding RNA MALAT1 is critical for PARP1 binding to LIG3 to mitigate an alternative end-joining DNA repair pathway and may serve as a novel therapeutic target for MM\cite{92,93}.

**Abnormal RNA editing**

Post-transcriptional RNA processing is important for the maintenance of genomic stability in MM\cite{94}. MM patients may harbor mutations in genes controlling RNA processing and protein translation. DIS3 gene on 13q22.1 encodes an exonuclease involved in regulating the abundance of RNA species. In MM patients, loss of DIS3 function is linked to monoallelic mutation or deletion. Exosomes play a vital role in regulating the mRNA pool. Therefore, loss of DIS3 activity may contribute to oncogenesis of MM due to protein translation deregulation. Similarly, the role of FAM46C in translational control and recurrent mutation in myelomagenesis is of biological relevance\cite{95}. RNA processing includes splicing pattern modification of transcripts involved in DNA repair\cite{96}. This alternative splicing of DNA repair depends upon the proper activity of RNA-binding proteins (RBPs)\cite{97}. Genetically aggressive myeloma patients who have 1q21 amplification usually have 1q21-induced over-expression of the RBP-ILF2 (interleukin enhancer-binding
factor 2). As ILF2 is a key regulator in HR repair in MM, high ILF2 expression promotes resistance to genotoxic reagents by modulating the translocation of YB1 (Y-box binding protein 1). Therefore, blocking the ILF2 signaling pathway may improve the effect of DNA-damaging agents in MM therapy.[98]

Deregulated plasma cell differentiation
IRF4 (interferon regulatory factor 4), also known as MUM1, is involved in the regulation of interferon transcription and B cell proliferation and differentiation[14]. An in vitro RNA-interference study discovered that IRF4 is necessary for the survival of MM cell lines[99]. IRF4 is also important therapeutically, as the backbone MM drug lenalidomide indirectly downregulates IRF4 by downregulating cereblon, the primary target of the CRBN-IKZF1/3-IRF4-MYC pathway[100,101]. IRF4 acts as a transcription factor for BLIMP1, another transcription factor pivotal in plasma cell differentiation. A study by Chapman et al.[7] identified 2 out of 38 patients with MM harboring an identical mutation (K123R) in the DNA-binding domain of IRF4. The same study group also harbored loss of function mutations in BLIMP1 usually identified in diffuse large B-cell lymphoma[7,102]. However, the role of differentiation pathway dysfunction in myelomagenesis needs further investigation as MM is a malignancy of terminally differentiated plasma cells.

Bone disease in myeloma
Bone disease in MM is associated with shorter overall survival and presents as focal/diffuse pain, pathological fractures, cord compression, and hypercalcemia. It is common in patients with hyperdiploidy, t(4;14), and MAF translocations[103]. A recent GEP study has identified approx. 50 genes linked with bone disease, with DKK1 and FRZB being the most prominent. DKK1 and FRZB are Wnt pathway inhibitors and induce osteoblast differentiation inhibition and increase bone resorption via RANKL/OPG ratio imbalance[104,105]. The antibody against DKK1 is an important therapeutic area to approach bone disease in MM patients. Anti-DKK1 antibody has resulted in improved bone disease outcomes and myeloma cell growth inhibition in pre-clinical models[106].

EPIGENETIC MODIFICATIONS
First, genomic instability is the hallmark of MM, and dysfunctional DNA damage response is one of the many driving contributing factors[3]. SIRT6 (NAD-dependent deacetylase) is highly expressed in MM cells and is linked with poor prognosis. Its expression is an adaptive response to maintain genomic stability. SIRT6 interacts with the promoter area of transcription factor ELK1 and ERK signaling-related genes. SIRT6 also downregulates the MAPK pathway gene expression and signaling. Moreover, the inactivation of ERK2 signaling increases DNA repair via checkpoint kinase 1 and confers resistance to DNA damage[107]. RecQ helicase, a DNA unwinding enzyme, is involved in maintaining chromosome stability. MM cells have a higher expression of RECVQ1, which is associated with poor prognosis. RECVQ1 over-expression helps MM cells escape from cytotoxicity of melphalan and bortezomib. On the contrary, knockdown of RECVQ1 suppresses cell growth and stimulates apoptosis in MM cells; RECVQ1 depletion promotes double-strand breaks on DNA in MM cells and sensitizes them to PARP inhibitors. RECVQ1 downregulation can also be induced by DNMT inhibitor treatment through dysregulation of miR-203 in MM. Hence, PARP inhibitors combined with DNMT inhibitors constitute an important therapeutic approach for MM patients[3,108].

The HOXA9 gene encodes a DNA-binding transcription factor involved in cell differentiation, morphogenesis, and gene expression regulation. It is regulated by histone methyltransferases, and knockdown of it in MM cell lines incurs a competitive disadvantage as compared to those with intact HOXA9 gene expression. This indicates the role of HOXA9 expression in myelomagenesis and the utilization of epigenetic changes for devising new therapeutic targets in MM[7].
Secondly, studies have revealed that miRNAs may act as both tumor suppressors and oncogenes in various cancers. Substantial work has been done to investigate the role of miRNAs in MM. Studies indicate that miRNAs can negatively regulate genes and pathways relevant to myelomagenesis via transcriptional control through promoter methylation\[14\]. For example, miR-137 maintains genomic instability in an aurora kinase A (AURKA)-dependent manner, while miR-22 regulates DNA ligase III in MM\[109,110\]. In short, miRNA deregulation is a key contributor to malignancy, and further research will unravel potential treatment targets.

Third, DNA methylation regulates gene expression and contributes to MM progression from MGUS to PCL. DNA methylation is found at higher frequencies in promoter regions, repeat sequences, and transposable elements of genes. MM has a recognized pattern of global DNA hypomethylation and gene-specific hypermethylation affecting cell adhesion, proliferation, the stromal-clone relationship, cell cycle progression, and transcription, predominantly in t(4;14) tumors, resulting in MMSET gene overexpression\[111\].

**CLONAL HETEROGENEITY**

Intraclonal heterogeneity is a common feature of MM and occurs in the milieu of selection events in the tumor microenvironment\[1\]. The clonal evolution in MM follows the Darwinian model, which involves the random acquisition of genetic changes that offer a survival advantage\[14\]. WES sequencing analysis shows that clonal heterogeneity begins from a premalignant stage and follows either linear or branching evolution patterns. Linear evolution involves the emergence of a new subclone or predominance of a pre-existing subclone, resulting in the stepwise acquisition of driver mutations. Branching evolution involves the emergence of one or more subclones via divergent mutational pathways, while other subclones decline in frequency or disappear\[2\]. Another factor is clonal stability, where similar clonal and subclonal heterogeneity is found before and after treatment, which would equally repopulate the tumor. The study of intraclonal heterogeneity is important to improve the understanding of disease pathogenesis, as the genetic aberrations in the predominant clonal population at the time of sampling may not apply to all subclonal populations. Thus, such heterogeneity may explain relapse and drug resistance to anticancer treatments\[14\].

**BONE MARROW MICROENVIRONMENT**

A complex interaction exists between malignant plasma cells and non-malignant stromal cells in the bone marrow microenvironment. This interaction involves adhesion molecules and autocrine/paracrine cytokine signaling. The cytokines secreted by the stromal cells include IL-6, VEGF, IL-1b, IL-10, TNF-a, TGF-b, MMP-1, osteoprotegerin (OPG)/RANKL MIP-1a, FGFs, and IGFR\[112\]. IL-6 is the most significant with a role in B cell differentiation; however, in MM, it induces proliferation and apoptosis inhibition. The IL-6 receptor has two subunits: IL-6Ra and gp130 (a transmembrane signal transducer). IL-6 combines with IL-Ra, which then mediates signals via gp130. IL-Ra subunit has an agonist action. In contrast, gp130 may competitively inhibit the growth-promoting effects of IL-6/IL-6R complex at higher concentrations\[113\]. IL-6-IL-6R interaction activates 3 downstream pathways: STAT1/STAT3 pathway, STAT3/STAT3 pathway, and Ras/MAPK pathway\[114\].

Similarly, VEGF, FGFs, and HGFs play a role in angiogenesis and IL-1b, RANKL, and HGFs in osteoclast activation. TNF-a, IGFs, IL-1b, and VEGF have a direct effect on MM cells\[75\]. Some factors secreted by bone marrow are known to influence the efficacy of chemo and radiation therapy and have a role in disease progression. For example, MM cell interaction with fibronectin in the extracellular matrix up-regulates p27, which induces drug resistance\[119\]. Likewise, the binding of MM cells to hyaluronic acid synergizes IL-6 signaling and reduction in adhesion molecules CD56; very late antigen 4 facilitates the transition to the
extramedullary phase\textsuperscript{[75]}.  

**MULTIPLE MYELOMA TREATMENT**

ASCT is standard care for MM. However, the foremost decision in MM patient management is ASCT eligibility. Patients less than 65 years of age with no severe comorbidities are usually eligible for ASCT. Furthermore, no definitive clinical data is available to support that ASCT is better in the early stage of disease than in later/relapsed cases\textsuperscript{[114]}. All transplant-eligible MM patients must receive primary induction therapy. The induction therapy combination regimens are given in Table 2.

Lenalidomide is a derivative of thalidomide, which is also an immunomodulatory drug (IMiD) but has more powerful anti-tumor and anti-inflammatory effects. It induces MM cell growth arrest, binding inhibition to BM-ECM and stromal cells, and downregulation of IL-6 and NF-\(\kappa\)B\textsuperscript{[117]}. While lenalidomide has a partial response rate of 24%-29% in treatment-refractory MM patients, combinatory lenalidomide and dexamethasone has peaked partial remission to an additional 29% in the lenalidomide-responsive patient group\textsuperscript{[118]}.  

Over time, more powerful triplet combinations of lenalidomide/dexamethasone with a monoclonal antibody (elotuzumab - anti-CD319, daratumumab - anti-CD38) or a PI (bortezomib, carfilzomib, ixazomib) have evolved with significant improvement in the progression-free survival (PFS) and overall survival (OS)\textsuperscript{[119,120]}. Combination treatment strategies apply the concept of using therapies with distinct mechanisms of action\textsuperscript{[121]}. The triple combination therapy trials of proteasome inhibitor and monoclonal antibodies are summarized in Tables 3 and 4.

Once remission is achieved, stem cells are harvested via apheresis. Maintenance therapy after transplantation includes (1) oral lenalidomide - 10 mg/day for the first 3 months; (2) oral Ixazomib - 3 mg on day 1, 8, and 15 in 28-day cycles in cycles 1 through 4 and increased to 4 mg from cycle 5 if tolerated; and (3) intravenous bortezomib - 1.3 mg/m\textsuperscript{2} on days 1, 4, 8 and 11 every 3 months.

Despite these treatment advancements, a considerable number of MM patients have shown resistance to PI, IMiDs, and monoclonal antibodies. A retrospective study has revealed that refractoriness results in only 5.6 months median OS in MM patients\textsuperscript{[120]}. Hence, there is an urgent need to devise more effective therapeutic interventions for this patient population\textsuperscript{[121,122]}.  

**Conventional chemotherapy**

Conventional chemotherapy can serve as salvage therapy in relapsed/refractory MM (RRMM) patients non-responsive to the triple-drug combination therapies. Due to intense toxicity, these cytoreduction agents are used for short periods of time and serve best as a bridge to more effective therapies\textsuperscript{[121]}. A study of dexamethasone without thalidomide administration with an infusion of cisplatin, doxorubicin, cyclophosphamide, and etoposide [D(T)PACE] resulted in an overall response rate (ORR) of 49%, median PFS of 5.5 months, and OS of 14 months\textsuperscript{[123]}. Among patients that proceeded to ASCT, median PFS was 13.4 months, and OS was 20.5 months. Another study compared the outcome of three chemotherapy regimens (1) dexamethasone, cyclophosphamide, etoposide, and cisplatin; (2) bortezomib, thalidomide, dexamethasone, cisplatin, doxorubicin, cyclophosphamide, and etoposide (VTD-PACE); and (3) cyclophosphamide, vincristine, doxorubicin, and dexamethasone (CVAD) in RRMM. The three salvage regimens demonstrated similar overall RR (55%), PFS (4.5 months), and OS (8.5 months)\textsuperscript{[124]}.  


Table 2. Induction therapy for transplant-eligible patients

1. Lenalidomide/Dexamethasone
2. Bortezomib/Thalidomide/Dexamethasone
3. Bortezomib/Lenalidomide (Revlimid)/Dexamethasone (VRd, RVd)
4. Bortezomib/Doxorubicin/Dexamethasone
5. Bortezomib/Cyclophosphamide/Dexamethasone (CyBorD, VCD)
6. Daratumumab/Bortezomib/Thalidomide/Dexamethasone (dara-VTD)

Table 3. Triple combination monoclonal antibody with lenalidomide and dexamethasone trials

<table>
<thead>
<tr>
<th>Trial title</th>
<th>Trial ID</th>
<th>Phase</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMN006</td>
<td>NCT03695744</td>
<td>II</td>
<td>Daratumumab + Bortezomib + Dexamethasone</td>
</tr>
<tr>
<td>CANDOR</td>
<td>NCT03158688</td>
<td>III</td>
<td>Daratumumab + Carfilzomib + Dexamethasone</td>
</tr>
<tr>
<td>Phase III trial comparing Poma, Dixa with/without Dara in RRMM with 1 prior therapy but not Lenalidomide &amp; PI</td>
<td>NCT03180736</td>
<td>III</td>
<td>Daratumumab + Pomalidomide + Dexamethasone</td>
</tr>
<tr>
<td>VELCADE</td>
<td>NCT02541383</td>
<td>III</td>
<td>Daratumumab + Bortezomib + Thalidomide + Dexamethasone</td>
</tr>
<tr>
<td>CASTOR</td>
<td>NCT02136134</td>
<td>III</td>
<td>Daratumumab + Bortezomib + Dexamethasone</td>
</tr>
<tr>
<td>POLLUX</td>
<td>NCT02076009</td>
<td>III</td>
<td>Daratumumab + Dexamethasone + Lenalidomide</td>
</tr>
<tr>
<td>ALCYONE</td>
<td>NCT02195479</td>
<td>III</td>
<td>Daratumumab + Melphalan + Bortezomib + Prednisolone/Dexamethasone</td>
</tr>
<tr>
<td>Phase II single-arm study of Elotuzumab with Lenalidomide + Dexamethasone in newly diagnosed or RRMM</td>
<td>NCT02159365</td>
<td>II</td>
<td>Elotuzumab + Lenalidomide + Dexamethasone</td>
</tr>
<tr>
<td>Phase II study of elotuzumab in combination with Poma, Bort, &amp; Dixa in RRMM</td>
<td>NCT02718833</td>
<td>II</td>
<td>Elotuzumab + Pomalidomide + Bortezomib + Dexamethasone</td>
</tr>
<tr>
<td>Single arm open-label anti-SLAMF7 mAB therapy after ASCT</td>
<td>NCT03168100</td>
<td>II</td>
<td>Elotuzumab + Bortezomib + Lenalidomide + Dexamethasone</td>
</tr>
<tr>
<td>ELOQUENT 3</td>
<td>NCT02654132</td>
<td>II</td>
<td>Elotuzumab + Pomalidomide + Dexamethasone</td>
</tr>
<tr>
<td>HRMM</td>
<td>NCT01668719</td>
<td>I/II</td>
<td>Bortezomib + Lenalidomide + Dexamethasone +/- Elotuzumab</td>
</tr>
<tr>
<td>ELOQUENT 2</td>
<td>NCT01239797</td>
<td>III</td>
<td>Lenalidomide + Dexamethasone +/- Elotuzumab</td>
</tr>
</tbody>
</table>

RRMM: Relapsed refractory multiple myeloma; PI: proteasome inhibitor, Poma: pomalidomide; Dixa: dexamethasone; Bort: bortezomib; mAB: monoclonal antibody.

Table 4. Proteasome inhibitor combination therapy trials

<table>
<thead>
<tr>
<th>Trial title</th>
<th>Phase</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFM2005-01</td>
<td>III</td>
<td>Bortezomib + Dexamethasone vs. Vincristine + Doxorubicin + Dexamethasone</td>
</tr>
<tr>
<td>DSSM-XI</td>
<td>II</td>
<td>Bortezomib + Cyclophosphamide + Dexamethasone</td>
</tr>
<tr>
<td>GIMEMMA</td>
<td>III</td>
<td>Bortezomib + Thalidomide + Dexamethasone vs. Thalidomide + Dexamethasone</td>
</tr>
<tr>
<td>GEM05-MEN0565</td>
<td>III</td>
<td>Bortezomib + Thalidomide + Dexamethasone vs. Thalidomide + Dexamethasone vs. Chemotherapy + Bortezomib</td>
</tr>
<tr>
<td>IFM2013-04</td>
<td>III</td>
<td>Bortezomib + Thalidomide + Dexamethasone vs. Bortezomib + Cyclophosphamide + Dexamethasone</td>
</tr>
<tr>
<td>HOVON-65/GMMG-HD4</td>
<td>III</td>
<td>Doxorubicin + Bortezomib + Dexamethasone vs. Vincristine + Doxorubicin + Dexamethasone</td>
</tr>
<tr>
<td>IFM2009</td>
<td>III</td>
<td>Bortezomib + Lenalidomide + Dexamethasone +/- ASCT</td>
</tr>
<tr>
<td>CASSIOPEIA</td>
<td>III</td>
<td>Daratumumab + Bortezomib + Thalidomide + Dexamethasone vs. Bortezomib + Thalidomide + Dexamethasone</td>
</tr>
<tr>
<td>ENDEAVOR</td>
<td>III</td>
<td>Carfilzomib + Dexamethasone vs. Bortezomib + Dexamethasone</td>
</tr>
<tr>
<td>A.R.R.O.W</td>
<td>III</td>
<td>Weekly vs. Biweekly Carfilzomib + Dexamethasone</td>
</tr>
<tr>
<td>ASPIRE</td>
<td>III</td>
<td>Carfilzomib + Lenalidomide + Dexamethasone vs. Lenalidomide + Dexamethasone</td>
</tr>
<tr>
<td>DKd</td>
<td>1b</td>
<td>Daratumumab + Carfilzomib + Dexamethasone</td>
</tr>
<tr>
<td>TOURMALINE-MM1</td>
<td>III</td>
<td>Ixazomib + Lenalidomide + Dexamethasone vs. Bortezomib + Dexamethasone</td>
</tr>
</tbody>
</table>
Bendamustine is a bifunctional alkylating agent. A retrospective study of bendamustine monotherapy and corticosteroid combination has resulted in 3% “very good” partial response, 33% partial response, 26% stable disease, and 20% progressive disease, along with a median PFS of 7 months and OS of 17 months in RRMM\textsuperscript{125}. The combination regimens of bendamustine with thalidomide, lenalidomide plus dexamethasone, and bortezomib plus dexamethasone have also demonstrated good tolerability and improved efficacy in early trials of RRMM\textsuperscript{126,127}.

Histone deacetylase inhibitors (HDACi) target the effects of epigenetic modification and have demonstrated positive outcomes in RRMM patients, especially when used in combination with PIs. In the phase III PANORAMA-1 trial, RRMM patients received panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone. The results demonstrated a clinically significant improvement with a median PFS of 11.99 months vs 8.08 months\textsuperscript{128}. Similarly, the PANORAMA-2 trial tested panobinostat combination therapy in bortezomib-refractory patients with a subsequent 34.5% ORR and 6 months median response duration\textsuperscript{129}. Another HDACi vorinostat was tested in the VANTAGE 095 trial involving heavily pretreated RRMM refractory to bortezomib and immunomodulators. A combination of vorinostat and bortezomib resulted in an ORR of 17%, median response duration of 6.3 months, PFS of 3.1 months, and OS of 11.2 months\textsuperscript{130}. Furthermore, the phase III VANTAGE 088 trial compared the outcome of vorinostat plus bortezomib with the bortezomib group alone. The study’s results included a PFS of 7.63 months vs 6.83 months and an ORR of 56.2% vs 40.6%\textsuperscript{131}.

**Salvage ASCT**

Salvage ASCT is an important therapeutic choice for RRMM. Several retrospective studies have demonstrated post-reinduction salvage ASCT success in MM patients who relapsed after first ASCT or RVD-alone treatment\textsuperscript{132}. Although most patients with RRMM were not candidates for salvage ASCT due to age and comorbidities, those who underwent salvage ASCT exhibited a PFS of 7 to 22 months. The foremost factor predicting improved PFS and OS after salvage ASCT is the duration of remission after initial ASCT\textsuperscript{121}.

**Selinexor**

Selinexor is an oral, slowly reversible, first-in-class, potent selective inhibitor of nuclear export compound that specifically blocks exportin 1 (XPO1). The Food and Drug Administration has approved selinexor for RRMM patients who have had 4 previous therapies and disease refractoriness to 2 PIs, 2 IMiD agents, and anti-CD38 mAb\textsuperscript{133}. The Selinexor trials are summarized in Table 5.

**Immunotherapies for multiple myeloma**

Advances in cellular immunotherapy - CAR (chimeric antigen receptor) T-cell therapy, B cell maturation antigen (BCMA)-targeted therapies, and bispecific T cell engager (BiTE) and tri-specific T cell engager (TiTE) - have good prospects in MM therapy\textsuperscript{120}. In CAR T-cell therapy, T cells are modified to express CARs genetically through introducing fusion proteins that have an antigen recognition region and a co-stimulation domain. CAR T-cells targeting BCMA, CD138, CS1 glycoprotein antigen (SLAMF7), and light chains are in active development for RRMM treatment\textsuperscript{134}. BCMA is a type of surface receptor, which belongs to the tumor necrosis factor superfamily. It is expressed in advanced B cell differentiation stages, and predominantly in plasma cells. Several BCMA-targeted therapeutics, including antibody-drug conjugates (e.g., belantamab mafodotin GSK2857916), CAR-T cells, BiTEs, and TiTE have also resulted in incredible clinical response in RRMM\textsuperscript{135}. Tables 6 and 7 summarize immunotherapy clinical trials for MM.
Table 5. Selinexor combination trials

<table>
<thead>
<tr>
<th>Trial title</th>
<th>Phase</th>
<th>Drug combination</th>
<th>Dose</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>STORM Trial</td>
<td>II</td>
<td>Selinexor + Dexamethasone</td>
<td>Selinexor - 80 mg oral day 1 &amp; 3 of each week; Dexamethasone - 20 mg prior to each dose</td>
<td>Partial response 26% Clinical benefit rate (CBR) 39% Median response duration 4.4 months Median PFS 3.7 months Median OS 8.6 months Pt with molecular response had median OS of 15.6 months</td>
</tr>
<tr>
<td>NCT02336815</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOSTON Trial</td>
<td>III</td>
<td>Selinexor + Dexamethasone + Bortezomib (S/Vd)</td>
<td>Selinexor - 100 mg once weekly; Dexamethasone - 40 mg weekly; Bortezomib - 1.3 mg/m²</td>
<td>PFS 13.93 months in S/Vd arm vs. 9.46 months in Vd arm</td>
</tr>
<tr>
<td>NCT03110562</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Further, 10 combination therapies of Selinexor in 11 treatment arms are under investigation in the STOMP Trial (NCT02343042).

Table 6. BCMA and non-BCMA CAR - T cell clinical trials in MM

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>Phase</th>
<th>No. of Pt.</th>
<th>Dose</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-BCMA CAR-T cell (NCT02215967)</td>
<td>I</td>
<td>12</td>
<td>0.3, 1.0, 3.0, 9.0 × 10⁶ CAR cells/kg</td>
<td>PR 3, SD 8, sCR 1</td>
</tr>
<tr>
<td>bb2121 Anti-BCMA CAR-T cell</td>
<td>I</td>
<td>33</td>
<td>50, 150, 450, or 800 × 10⁵ CART cells</td>
<td>ORR 85%, sCR 12, CR 3, VGPR 9, PR 4, SD 4, PD 1</td>
</tr>
<tr>
<td>bb21217 Anti-BCMA CAR-T cell (NCT03274219)</td>
<td>I</td>
<td>8</td>
<td>150 × 10⁴ CAR T cells</td>
<td>sCR 1, VGPR 3, PR 2, -ve MRD 3</td>
</tr>
<tr>
<td>LCAR-B38M Anti-BCMA CAR-T cell (ChiCTR-ONH-17012285)</td>
<td>I</td>
<td>17</td>
<td>0.21-1.52 × 10⁶ CAR T cells/kg</td>
<td>ORR 88.2%, sCR 13, VGPR 2, NR 1</td>
</tr>
<tr>
<td>LCAR-B38M Anti-BCMA CAR-T cell (NCT03090659)</td>
<td>I/II</td>
<td>57</td>
<td>0.07-2.1 × 10⁵ CAR T cells/kg</td>
<td>ORR: 88%, CR 39; VGPR 3, PR 8, -ve MRD 35</td>
</tr>
<tr>
<td>LEGEND-2 Trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCARH125 Anti-BCMA CAR-T cell (NCT03430011)</td>
<td>I/II</td>
<td>19</td>
<td>50-150 × 10⁴ CAR T cells/kg</td>
<td>sCR 2, CR 1, VGPR 2, PR 2, MR1</td>
</tr>
<tr>
<td>EOLVE Trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT053 Anti-BCMA CAR-T cell (NCT03915184)</td>
<td>-</td>
<td>16</td>
<td>0.5-1.8 × 10⁶ CAR T cells</td>
<td>ORR 100%, CR 2, PR 4, VGPR 6</td>
</tr>
<tr>
<td>MCARH171 Anti-BCMA CAR-T cell</td>
<td>I</td>
<td>11</td>
<td>72, 137, 475, 818 × 10⁵ CAR T cells</td>
<td>ORR 84%, VGPR 2</td>
</tr>
<tr>
<td>CT013A Anti-BCMA CAR-T (ChiCTR000018137)</td>
<td>I</td>
<td>9</td>
<td>1, 3, 6 × 10⁵ CAR T cells/kg</td>
<td>ORR 100%, CR 4; VGPR 1, PR 4</td>
</tr>
<tr>
<td>CD3g &amp; 4-1BB Anti-BCMA CAR-T cell (NCT02546167)</td>
<td>I</td>
<td>25</td>
<td>1.5-5.0 × 10⁷ CAR T cells</td>
<td>sCR 1, CR 1, VGPR 5, PR 5</td>
</tr>
<tr>
<td>P-BCMA-101 CAR-T cell (NCT03288493)</td>
<td>I</td>
<td>12</td>
<td>48-430 × 10⁵ CAR T cells</td>
<td>sCR 1, nCR 1, VGPR 1, PR 2</td>
</tr>
<tr>
<td>CD4⁺: CD8⁺ BCMA CAR-T cell (NCT03338972)</td>
<td>I</td>
<td>7</td>
<td>5-15 × 10⁵ CAR T cells</td>
<td>ORR 100%</td>
</tr>
<tr>
<td>Anti-CD19 non-BCMA CAR-T cell (NCT02195406)</td>
<td>I</td>
<td>10</td>
<td>1.1-6.0 × 10⁶ CAR T cells</td>
<td>VGPR 6, PR 2; PD 2</td>
</tr>
<tr>
<td>Anti-CD138 non-BCMA CAR-T cell (NCT01886976)</td>
<td>I/II</td>
<td>5</td>
<td>0.44-1.51 × 10⁷ CAR T cells/kg</td>
<td>SD 4, PD 1</td>
</tr>
<tr>
<td>κ light chain non-BCMA CAR-T cell (NCT05088920)</td>
<td>I</td>
<td>16</td>
<td>0.2-2.0 × 10⁶ CAR T cells/m²</td>
<td>4 SD of 7 MM</td>
</tr>
</tbody>
</table>

ORR: Overall response rate; VGPR: very good partial response; CR: complete response; PR: partial response; sCR: stringent complete response; MRD: minimal residual disease.

Targeted therapy for multiple myeloma
Clonal heterogeneity and clonal competition in the MM cancer cells have signified the role of targeted therapy (precision medicine) in patient management. Therefore, the identification of driver mutations is central to designing personalized targeted therapy. In addition, novel vaccines and immune-checkpoint...
Table 7. BCMA targeted ADC and bispecific T-cell therapy clinical trials

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>Phase</th>
<th>No. of Patients</th>
<th>Dose</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belantamab mafodotin (GSK2857916)</td>
<td>I</td>
<td>35</td>
<td>3.4 mg/kg every 3 weeks</td>
<td>ORR 60%, sCR 2 (6%), CR 3 (9%), VGPR 14 (40%), mPFS 12 months, mDOR 14.3 months</td>
</tr>
<tr>
<td>DREAMM-1 (NCT02064387)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DREAMM-2 (NCT03525678)</td>
<td>II</td>
<td>196</td>
<td>2.5 or 3.4 mg/kg every 3 weeks</td>
<td>2.5 mg/kg cohort: ORR 30 (31%), sCR/CR 3 (3%), VGPR 15 (15%), PD 56 (58%), mPFS 2.9 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.4 mg/kg cohort: ORR 34 (34%), sCR/CR 3 (3%), VGPR 17 (17%), PD 55 (56%), mPFS 4.9 months</td>
</tr>
<tr>
<td>BCMA/CD3 (AMG 420) (NCT02514239)</td>
<td>I</td>
<td>42</td>
<td>0.2-800 μg/day, 4 weeks infusion + 2 weeks off, for up to 5 cycles. Average 2.5 ± 2.6 cycles</td>
<td>ORR 31%, sCR 14%, CR 7%, VGPR 4.8%, PR 4.8%</td>
</tr>
<tr>
<td>BCMA(bivalent)/CD3 (monovalent) (CC-93269) (NCT03486067)</td>
<td>I</td>
<td>19</td>
<td>0.15-10 mg/day for a 28-day cycle (D1, 8, 15, and 22 for Cycles 1-3; D1 and 15 for Cycles 4-6; and on D1 for Cycle 7). Median 4 cycles Median DOT 14.6 weeks</td>
<td>12 patients w/dose of ≥ 6 mg: ORR 10 (83.3%); sCR/CR 4 (33.3%), VGPR 7 (58.3%)</td>
</tr>
<tr>
<td>BCMA/CD3, IgG2a backbone (PF-06863136)</td>
<td>I</td>
<td>17</td>
<td>Once weekly non-continuous infusion in 6 dose-escalation groups</td>
<td>Minimal response 1 (6%), SD 6 (35%), PD 9 (53%)</td>
</tr>
<tr>
<td>BCMA/CD3 (REGN5458) (NCT03761108)</td>
<td>I</td>
<td>7</td>
<td>6 mg/kg, 16 weekly doses + maintenance 12 doses per 2 weeks</td>
<td>ORR 4 (53.3%)</td>
</tr>
</tbody>
</table>

PD: Progressive disease; SD: stable disease; mDOR: median duration of response; mPFS: median progression-free survival; ORR: overall response rate; VGPR: very good partial response; CR: complete response; PR: partial response; sCR: stringent complete response; MRD: minimal residual disease.

Table 8. Targeted therapy in multiple myeloma

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Targeted therapy</th>
<th>Mutations</th>
<th>Targeted therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. KRAS mutation</td>
<td>Selumetinib [136]</td>
<td>5. BRAF mutation</td>
<td>Vemurafenib [130]</td>
</tr>
<tr>
<td>2. NRAS mutation</td>
<td>Cobimetinib [137]</td>
<td>6. BCL-2 mutation</td>
<td>BCL-2 Inhibitors - Venetoclax - Navitoclax</td>
</tr>
<tr>
<td>4. MEK mutation</td>
<td>MEK inhibitor [142] - Trametinib - Cobimetinib</td>
<td>8. del 1p (CDKN2C), t 11;14 (CCND1), t 6;14 (CCND3)</td>
<td>Palbociclib [143]</td>
</tr>
</tbody>
</table>

9. Immune Checkpoint Inhibitors- Nivolumab, Atezolizumab [144]

Inhibitors address another area of therapy development based on mutational landscapes. This would enable powerful therapeutic combinations for high-risk MM patients previously treated with a non-personalized approach. Table 8 includes examples of therapies targeting specific mutations in MM.

CONCLUSION

Genetic studies in MM patients have revealed mutational landscapes and a clearer understanding of disease pathophysiology and molecular heterogeneity. Hence, instead of a single treatment approach, a series of genetically-targeted treatment combinations based on the genetic subtypes would be effective. However, further studies using single-cell RNA sequencing technology are required on MM patient samples to extend our knowledge of clonal evolution and to precisely identify resistance mechanisms for novel therapeutic target identification. With current drug development, including antibody-drug, MM patients will eventually develop drug resistance. Obviously, there are patients either intrinsic-resistant or acquired-resistant to multiple drug treatments. There are very active drug development and clinical trials ongoing to develop bispecific antibody-drug conjugation to overcome multiple drug resistance, including single antibody-drug
treatment.

**DECLARATIONS**

**Authors’ contributions**

Review paper writing and editing: Sadaf H, Hong H, Maqbool M, Emhoff K, Lin J, Yan S, Anwer F, Zhao J

**Availability of data and materials**

Not applicable.

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**Conflicts of interest**

Jianjun Zhao has a consulting role for Curio Science. Faiz Anwer has a consulting or advisory role for Seattle Genetics, Incyte Corporation Speakers’ Bureau, Company: Incyte Corporation; receives travel and accommodations expenses from Seattle Genetics, Incyte; receives honoraria from Incyte, Company: Seattle Genetics; and received research funding from Seattle Genetics, Company: Celgene, Acetylon Pharmaceuticals, Millennium, Astellas Pharma and AbbVie. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

**Ethical approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

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