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# Current perspectives on interethnic variability in multiple myeloma: Single cell technology, population pharmacogenetics and molecular signal transduction

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#### ABSTRACT

Multiple myeloma (MM) is an aggressive cancer characterised by malignancy of the plasma cells and a rising global incidence. The gold standard for optimum response is aggressive chemotherapy followed by autologous stem cell transplantation (ASCT). However, majority of the patients are above 60 years and this presents the clinician with complications such as ineligibility for ASCT, frailty, drug-induced toxicity and differential/partial response to treatment. The latter is partly driven by heterogenous genotypes of the disease in different subpopulations. In this review, we discuss emerging single cell technologies and applications in MM, population pharmacogenetics of MM, resistance to chemotherapy, genetic determinants of drug-induced toxicity, molecular signal transduction, as well as the role(s) played by epigenetics and noncoding RNAs including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) that influence the risk and severity of the disease. Taken together, our discussions further our understanding of genetic variability in 'myelomagenesis' and drug-induced toxicity, augment our understanding of the myeloma microenvironment at the molecular and cellular level and provide a basis for developing precision medicine strategies to combat this malignancy.

#### Introduction

B and T type of cells (lymphocytes) present in bone marrow, bloodstream, intestine etc. play a key role in human biology by forming a part of the defense system. These lymphocytes respond to an infection and mature to plasma cells which are responsible for making antibodies that help the body fight against cancerous cells and germs in a healthy human. Multiple myeloma (MM) is characterised by a condition in which the normal plasma cells become cancerous and grow indefinitely with considerable fatality and morbidity. With a better understanding of the disease and the evolution of new therapies, the scope of MM has shifted from "untreatable" to "treatable", although there is still no cure

for MM. Despite this, alarming data from the Global Cancer Observatory indicates 176,404 new cases and 117,077 deaths globally in the year 2020 [1]. Furthermore, the global burden of new incident cases is maximum in Asia and followed by Europe, North America, Latin America and the Caribbean, Africa and Oceania [1].

Emerging technological advancements in immunotherapy have expanded the scope of treatment modalities. Several checkpoint blockade therapies have been tested in patients including by targeting TIGIT [2], LAG3/GAL-3 [3], Tim-3 [4] and PD-L1 [5]. Interestingly, differential outcomes of MM patients treated with bortezomib-based therapies were associated with CTLA-4 polymorphisms [6]. Significantly lower overall survival (46.3% vs 83.3%) and disease-free survival

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(0% vs 57.4%) was seen in patients with rs733618 GG SNP when compared to those with GA+AA following bortezomib-based therapy. Furthermore, rs4553808 GG+GA group showed higher incidence of grade 3/4 non-hematologic adverse events compared to the AA group. In contrast, Kasamatsu et al. [7] did not find statistical significance between clinical variables and CTLA-4 polymorphisms.

CAR T-cells have emerged as a novel frontier in recent years for treating hematological malignancies. Recently, in a Phase 1 trial (NCT02546167) with 25 refractory/relapse MM patients, Cohen et al. [8] found that BCMA-directed CAR T-cell decreased the residual MM cell expression of BCMA in responders. However, progression of MM in non-responders was associated with elevated BCMA expression. Overall, this trial suggested that BCMA-CAR T-cell infusions were clinically beneficial in heavily pre-treated MM patients. A major limitation to BCMA-CAR T-cells is relapse and primary resistance to single-target therapy. This limitation can be overcome by using bispecific CAR T-cells as was shown by Mei et al. [9] in a Phase 1 trial (ChiCTR1800018143) with 23 patients using BCMA-CD38 bispecific CAR T-cells. Studies have also shown that abundance of immunosuppressive cancer associated fibroblasts (CAFs) in the tumor microenvironment can limit the effectiveness of BCMA-CAR T-cells. Indeed, by targeting CAFs, Sakemura et al. [10] showed that dual-targeting of MM cells and an immunosuppressive player (CAFs in this instance) can overcome the resistance of single-target CAR T-cells. The status of CAR T-cells in hematologic malignancies has been lucidly discussed elsewhere [11–13].

Factors for disparities in patient outcomes include resistance to chemotherapy, age, gender, race/ethnicity, socioeconomic status, healthcare access, geographic location, comorbidities, disease state, prognostic risk category and associated amyloidosis. Indeed, these peculiarities of MM have been discussed lucidly elsewhere [14]. In addition to this, ongoing research has highlighted a key role played by genetic disparity among populations to influence pathogenesis of MM including prognosis, survival, responsiveness to chemotherapy, survival and overall outcomes of treatment.

The human population can be sub-classified into distinct groups (based on race/ethnicity) with distinct genetic profiles (e.g. Asians, Hispanic, Caucasian, etc). Till date, early diagnosis of cancer remains a major impediment to curing cancer. It is, therefore, imperative to identify predisposing genetic factors that can lead to various cancers. One way to achieve this is by identifying commonly mutated genes which renders a population more susceptible to aid early diagnosis and prognosis of MM. For example, BAP1 is a common germline mutation with high incidences of malignant mesothelioma as we have noted earlier [15]. Similarly, single nucleotide polymorphisms in specific genes can also adversely affect the risk and severity of cancer in different subpopulations with varying ethnicities as we have observed previously for testicular cancer [16]. Therefore, identifying genetic determinants of MM risk in different populations in response to chemotherapy will prove critical for improving patient outcomes. Indeed, significant work has been done to elucidate the molecular underpinnings of MM with a goal to identify prognostic and predictive biomarkers as well as specific targets, that could improve the treatment efficiency [17]. However, due to interethnic variability, it has become challenging to treat MM with the same treatment for everyone as it influences the responsiveness of patients to standardized treatments. In this review, we outline the molecular aspects involved in MM along with population pharmacogenetics to appreciates the interethnic variability amongst different sub-populations and harness the power of population pharmacogenetics with the goal of achieving precision medicine in MM therapy.

# Single cell technologies and multiple myeloma

Cell-to-cell variability in key biomolecules and bioenergetics (metabolic reprogramming) is known to be observed in the tumor microenvironment in various cancers [18]. Dissecting this cellular heterogeneity within the cancer milieu, especially in hematological malignancies such as MM, is a prerequisite for understanding the genesis of a cancer cell as a biological system, its homeostatic regulation and response to external perturbations [19]. Clonal evolution and functional heterogeneity are known to exist in MM subpopulations and are of prognostic significance [20]. Indeed, single cell sequencing technologies can facilitate the analysis of genetic polymorphisms at a single cell level [20]. In an ideal clinical setting, single cell suspensions must be prepared immediately after sample collection from a patient. Often, due to logistical limitations of experimental designs, the clinicians and researchers cannot assess the freshly collected samples. Such delays could potentially alter the overall results of a scRNA seq analysis. To overcome this, Chen et al. [21] showed that cryopreserving MM samples could preserve both, gene expression profiles and phenotype composition of the sample. In the context of tumor evolution in MM, Dutta et al. [22] recently observed that single-cell studies can shed light on the phenotypic and mutational features of single cells in the peripheral blood, immune microenvironment and bone marrow tumor, thus, lending opportunities for precision medicine in MM. Recently, He et al. [23] used single-cell RNA sequencing (scRNA-seq) on bone marrow samples obtained from 18 MM patients and demonstrated the heterogeneity of MM as well as intra-tumor heterogeneity with myeloma cells being dominated by a major clone. Interestingly, a functional assay has been developed to investigate the ex vivo sensitivity of single MM cells to various standard-of-care drugs based on measurement of the mass accumulation rate of the cells, thus, serving as a predictor of drug response in MM [24].

Chen et al. [25] reported PopAlign, a platform based on mathematical modelling, to automatically identify cellular subpopulations in complex heterogeneous mixtures of single cells and assess gene expression using MM patient-derived disease signatures. Importantly, Smets et al. [26] reported deep profiling of immune cells of MM patients at single cell resolution using mass cytometry by time-of-flight (CyTOF) which enabled the simultaneous quantification of over forty markers in MM. Overall, single cell technologies have shown great promise in unravelling cellular heterogeneity, in dissecting disease pathogenesis and stratification/progression, as well as predictors of therapeutic response [27]. When applied to MM, single cell technologies are emerging as an important tool that will facilitate better understanding of MM clonal diversity and cellular heterogeneity to improve therapeutic approaches to combat the disease.

# Applications of single cell technologies in MM

Recently, Cohen et al. [28] conducted a prospective, single-arm, multicenter clinical trial (NCT04065789) that employed scRNA sequencing technology to identify PPIA (peptidylpropyl isomerase A) as a novel therapeutic target which conferred resistance to proteasomal inhibitors such as carfilzomib. Importantly, cyclosporin-based PPIA inhibition and CRISPR-Cas9 deletion of PPIA sensitized MM cells to proteasomal inhibitors. Furthermore, scRNAseq was also used to determine the temporal abundance of immune cells in the dynamic tumor microenvironment which evolves with disease progression [29]. In another study, using a combination of bulk and scRNA sequencing technologies, it was found that MM cells with a favorable cytogenetic translocation such as t(11;14), were associated with early stage MM whereas cells with t(4;14) translocation were associated with late stage MM. Additionally, a 20-gene signature was also developed which predicted survival independently [30]. Another contributing factor for MM progression is the abundance of myeloid cells in the microenvironment. Indeed, Meng et al. [31] showed using scRNAseq that myeloid-derived S100A9, which is pro-inflammatory, promoted TNFSF13B /TNFRSF13B-dependent survival and proliferation of MM cells.

In another clinical study (NCT02541383), paired single-cell transcriptomic data were generated for MM cells, bone marrow (BM) immune cells, and stromal microenvironment in MM. It was found that MM-specific inflammatory mesenchymal stromal cells were spatially colocalized with immune and tumor cells which transcribed genes that promoted immune modulation and tumor survival driven by proinflammatory cytokines. Populations of CD8<sup>+</sup> stem cell memory T-cell and interferon-responsive effector T-cells were identified as putative drivers of stromal cell-activating cytokines. Collectively, it was reported that antitumor treatments were inefficient in reversing BM inflammation which could lead to persistence of MM [32]. Interestingly, Frede et al. [33] showed using scRNA transcriptome and chromatin accessibility that differential enhancer recruitment and transcriptional reprogramming were promoted while developmental potential was stunted in malignant cells. This created a distinct complement for immunotherapeutic targeting, such as CXCR4, that could serve to overcome treatment resistance in MM.

Recent vears have seen a shift towards developing precision medicine strategies for combating cancer. However, a recent scRNAseq study showed that profiling in BRAF-mutated myeloma patients receiving BRAF/MEK inhibitor-based therapies was associated with rapid changes in cellular states. For instance, drug-resistant clone detection was preceded by transcriptional regulation which induced dependency on oxidative phosphorylation. At the time of relapse, oxidative phosphorvlation was activated and was inversely correlated to MAPK activation. Analysis of metabolic flux revealed that oxidative phosphorylation was a preferred source of drug-persistent MM cells. Taken together, this study highlighted that cancer cells may actively adapt to targeted therapies via epigenetic adaptations, alterations in cell transcriptional states and metabolic rewiring [34]. In contrast, Xu et al. analysed temporal consecutive samples from a 63-year-old female patient over a period of 37 months from diagnosis to death and found that RUNX3 was a potential driver of refractory/relapsed MM. In contrast, Mahdipour-Shirayeh et al. [35] recently used sciCNV (single-cell inferred chromosomal copy number variation) to identify MM subclones with +8q22-24 which, in turn, could upregulate protein synthesis and mRNA processing of MYC and MYC-target genes. Overall, the study provided a pipeline for scRNAseq that enabled paired profiling of chromosome copy number variation (CNV) and transcriptomes of single cells which could facilitate accurate and rapid deconstruction of CNV effects on cellular programming in cancer. Similarly, Johnson et al. [36] used Diagnostic Evidence GAuge of Single Cells (DEGAS, a novel framework for deep transfer learning that transfers disease information from patients to cells) analysis on MM single cell transcriptomes and reported that PHF19<sup>high</sup> MM cells were associated with disease progression.

## Population pharmacogenetics of MM

Despite the availability of high dose chemotherapy and evolution of the existing remedies for MM treatment, the disease still remains incurable. Multiple myeloma is a genetically heterogeneous disease and the complexity escalates as the disease progresses to a more aggressive stage. Most MM cells are responsive to immunomodulatory drugs and proteasomal inhibitors; however, not all elicit an equal response to these inhibitors [17]. MM thus represents a challenging disease in understanding its biological intricacy. The role of different catalysts and how they affect the growth, differentiation, activation and inhibition mechanisms of plasma cells are all associated with specific genetic distinctiveness [37]. Therefore, understanding MM pharmacogenetics will not only help to study the effect of a patient's response to the drug(s) but also enable us to determine the role of interethnic variability by studying population pharmacogenetics. We describe below the population pharmacogenetics of MM in diverse populations/ethnicities across the globe for the benefit of the reader and summarise the various studies in Table 1.

## Studies in American population

Schriber et al. [38] investigated differences in obtaining ACHT

## Table 1

List of gene alleles res	ponsible for ethnic	: variability in	different po	pulations.

Sr. No.	Population	No. of Patients	Gene	Gene Allele	Refs.
1.	American	137	KRT81 XPO5	rs3660 C/C rs11077 A/C or C/	[41] <sup>)</sup>
		626	exon 9 of the IL- 6r gene	rs2228145	[42]
		134	IL-6	-373 9A/9A genotype -572	<sup>(</sup> [43]
		108	BAX	position rs1042265 (A	[44] <sup>)</sup>
			RIPK1	variant) rs9391981 (C	
			CASP9	variant rs751643 (G variant)	
		108	CD4	rs11064392 (AG/ GG)	[45] <sup>)</sup>
		108	ANRIL	rs2151280	[187]
		135	SLC7A5	rs4240803	[199]
2.	Brazilian	106	p53	variant codon 72	[46]
		123	MTR	A2756G	[47]
3.	Italian	137	MICA	-129 Val/Val	<sup>(</sup> [56]
				-129 Val/Met	
				-129 Met/Met	
4.	Polish	144	IRF4	rs872071 (G	[57] <sup>)</sup>
			CRBN	variant) rs711613	
				(A variant)	
		54	CXCR4 CXCL12	rs2228014	[58]
				rs1801157	
		135	BSG MCT1/	rs4919859 C	<sup>(</sup> [59]
			SLC16A1	rs8637 G	
				rs7556664 A	
				rs7169 T	
				rs1049434 A	
		132	bFGF	rs308395 (G>C)/-	[60] <sup>)</sup>
				921G	
		222	RANK	rs1805034 (C	[61])
				variant	
			RANKL	rs/325635 (A and	
			TH OF	G variants	
			TACI	rs34562254 (A	
		100	DOMAG	variant)	<b>[</b> (01)
		100	PSMA6	CG+GG genotypes	[62]
-	D 11	0.40	NOD2/CARD15	3020insC	r.cor)
5.	Danish	348	ILIB	C-3/3/1 (wild type	[63]
		348	ILIB NEVD1	C-3/3/1 (1 variant	[64]
		290	NFKDI	-94IIIS/deIATIG	[02].
		240	CD2EAD	(who type	[66]
		340	DJEAP PAL (introp 1.1	rs4572514	[00]
		348	IL-16	T-31C	[67])
		348	EBCC2 EBCC3	K7510 (C variant	[68])
			CD3EAP	T241M (T variant)	1901
			0202111	G-21A (A variant)	
		348	HSPE	rs4693608 (A	[69] <sup>)</sup>
				allele, wild type	
				rs6535455 (T	
				variant)	
6.	British	181	TNF-α	-308 A (variant	[72])
		222	GSTP1	-105 (Val -105 (Ile)	[73] <sup>)</sup>
		270	LIG4	A3V T9I	[74]
		544	CINP CETP	rs7011 rs289747	[201]
			ALDH1A1	rs610529	
			CDKN1A GAN	rs3829963	
			VEGF ALDH1A	rs2608555	
				rs699947 rs168351	
		665	CDCA7L	rs4487645	[98]
7.	German	665	CDCA7L	rs4487645	[98]
		163	HNF1B	rs7501939	[75]
		aa.c=	SLC30A8	rs13266634	
		2267	TERT TERC	rs2242652	[76]
				rs12696304	
		983	FBXO33 INTU	rs8014839	[200]
			BCL6 IL17RB	rs4618330	

(continued on next page)

#### Table 1 (continued)

Sr. No.	Population	No. of Patients	Gene	Gene Allele	Refs.
				rs1903216	
				rs4687753	
8.	Hungarian	373	FOPNL	rs72773978	([77]
		211	PSMB1	rs12717 (G/G	[78] <sup>)</sup>
				genotype)	
9.	French	602	FOXM1	-	[79]
10.	Australian	90	PON1 BB GST	Slow acetylation	[80]
			T1 null NAT2	genotype	
11.	Russian	69	IL-6	CC genotype	<sup>(</sup> [81]
12.	Chinese	67	CCND1	G870A (GG	[82] <sup>)</sup>
				variant)	
		129	MDR1	C1236T (CC	[84] <sup>)</sup>
				genotype C3435T	
				(CC genotype)	
		827	NCOA1 HLA-I	rs79480871	([86]
				rs6457327	
		40	VDR	TaqI (C allele) BsmI	[89] <sup>)</sup>
				(A allele)	
13.	Korean	196	MTHFR	677CC 1298CC	[90]
		117	NQO1	NQO1*2/*2	[92]
				genotype	
14.	Indian	75	VDR	FokI	[94]
		75	VDR	ApaI a allele FokI f allele	<sup>(</sup> [95]
				BsmI b allele	

(autologous hematopoietic cell transplantation, n = 28,450) and disease-related outcomes (n = 24,102) in American MM patients. They found that utilization rate of AHCT was highest in non-Hispanic whites (22.6-37.8%) followed by non-Hispanic blacks (12.2-20.5%) and Hispanics (8.6-16.9%). However, post-transplantation, ethnicity/race was not identified to be a determinant of treatment outcomes. Landgren et al. [39] conducted a study in 12482 serum samples of myeloma patients representing the US population which included 2331 blacks, 7051 whites, 625 unclassified patients, and 2475 Hispanics. It was reported that 365 patients were suffering from MGUS (monoclonal gammopathy of undetermined significance). Importantly, the frequency of MGUS in Hispanics (1.8%) and whites (2.3%) was less as compared to blacks (3.7%) as were the characteristics with a higher risk of progression to MM. Furthermore, higher MGUS prevalence was seen in North/Midwest (3.1%) when compared to South/West regions (2.1%) regions of USA, suggesting etiologic implications.

Although newer therapeutic agents have consistently reported improvement in patient survival as shown in a recent study by Costa et al. [40] in American populations, the prevalence of MM in non-Hispanic men (black and white) was significantly higher than Hispanics and non-Hispanic black women. Importantly, new agents showed improvement in 5-year survival of patients regardless of their race/ethnicity or age. Similarly, 10-year survival improved in patients under 65 years, but not in those above 75 years. In patients between 65 and 75 years, 10-year survival improved in Hispanics and non-Hispanic whites, but not in non-Hispanic blacks. Interestingly, KRT81 (target of MM miRNA clusters) single nucleotide polymorphism (SNP), rs3660 C/C variant had lesser protein translations, which prolonged overall survival in MM and similar results were seen in XPO5 (component of miRNA biogenesis pathway) SNP, rs11077 with A/C or C/C variants [41].

Trans-signalling by soluble IL-6 receptor (sIL-6r) enhances IL-6 signalling, which, in turn, affects cells it would not under homeostasis. Indeed, from a cohort of 626 MM patients, it was found that a combination of amplification of 1q21 chromosome and SNP rs2228145 minor allele C upregulated sIL-6r levels. This was associated with shorter overall survival and it also helped in identifying MM patients with intermediate risk [42]. Similarly, variant allele with SNP at position 572 in IL-6 promoter region showed 2-fold higher risk of plasma cell neoplasm when compared to population (odds ratio-2.4) or family controls (odds ratio-1.8) while -373 9A/9A genotype showed decreased risk when compared to most common genotype [43]. Variation in genes involved apoptosis and cell cycle play a key role in development of a MM. Indeed, in a cohort with 182 non-Hispanic Caucasian women from Connecticut, Hosgood et al. [44] found that BAX (rs1042265), CASP9 (rs9391981) and RIPK1 (rs751643) genes were associated with risk of MM. While the C variant at rs9391981 and A variant at rs1042265 had lesser risk of MM, the G variant at rs7516435 had a higher risk of MM. CD4 signaling moieties are found on the surface of the immune cells and are made up of glycoproteins. In a cohort of 108 Caucasian (women) MM patients, Lee et al. [45] established susceptibility to MM in part is associated with genetic variation in CD4. Importantly, two SNPs in CD4 and LAG3 genes were identified with a higher MM risk, with rs11064392 variant having the strongest association.

#### Studies in Brazilian population

To confirm the effect GSTM1, GSTT1, and p53 genes on the development of MM, Ortega et al. [46] recruited Brazilian MM patients (n =106, ethnicity not known). It was reported that the expression levels of all three genes were similar in both patients and controls. However, elevated levels of GSTM1 null, P53 PP+AP and GSTM1 null plus P53 PP+AP genotypes were observed at stage III when compared to stages I and II. Thus, susceptibility to MM is not influenced by P53, GSTM1 and GSTT1 genotypes. It is interesting to note that absence of GSTM1 detoxification pathway and codon 73 P53 polymorphism tend to promote myeloma progression in Brazilian population. Lima et al. [47] studied 123 MM patients from south-eastern Brazil (25 African-American and 98 Caucasians) to investigate the relationship between polymorphisms of MTHFR (Methylenetetrahydrofolate reductase) gene (A1298C and C677T), TYMS (thymidylate synthase) gene (2R->3R), MTR (methionine synthase) gene (MTR A2756G) and MTRR (methionine synthase reductase) gene (MTRR A66G) and altered risk of MM. It was found that individual carriers of MTR 2756 variant allege G had 2.31-fold higher risk of myeloma. In contrast, no such phenomenon was seen in polymorphisms of TYMS, MTRR and MTHFR genotypes. Similar results for MTHFR polymorphisms were seen in Italian Caucasians [48].

# Studies in African-American population

Epidemiological studies indicate that African-Americans (AA) are twice as likely as European-Americans (EA) to be diagnosed with MM. Indeed, Rand et al. [49] reported that the variants in 7p15.3, 22q13.1 and 17p11.2 were associated with myeloma risk in African-Americans and people of European ancestry, while 3p22.1 variant was associated only with European Ancestry. Similarly, a combined meta-analysis in people with African-American and European ancestry revealed variations in five regions (2p23.3, 17p11.2, 3p22.1, 7p15.3, 22q13.1) which are associated with increased risk of MM. In conclusion, researchers reported that the risk variants across sub-populations with differential underlying genetic basis can supplement identification of key alleles. In contrast, Baker et al. [50] reported no significant difference in incidence of somatic copy number variations and the incidence of high-risk disease on the basis of gene expression profiling in 115 African-Americans and 353 European-Americans. Instead their data revealed differences in distribution and frequency of molecular alterations in the study populations wherein the frequency of translocation of IgH was significantly lower in AA as compared to EA populations.

Landgren et al. [51] studied 28,636 MM patients (4,855 AA, 23,781 Caucasians) to compare the survival rates in both the arms. AA myeloma patients were reported to have better prognosis when compared to Caucasians. More enhanced survival benefit was seen in Caucasians after they were administered newer therapies such as IMiDs, autologous transplant and bortezomib which can be representative of the inequalities in access to modern medicine. Despite this, the survival in AA was still similar or better than Caucasians. Importantly, higher mortality

in AA, to a large extent, reflects higher incidence (2-3 fold) of MM in AA. Similarly, higher BMI consistently contributed to mortality in a cohort of 239,597 AA in USA, indicating that obesity is risk factor for myeloma in AA [52]. MGUS is a harmless condition for most and can lead to MM over time. From a cohort of 749,020 AA and 3,248,795 white veterans, Landgren et al. [53] identified 3-fold greater prevalence of MGUS in AA than whites (2046 total MGUS cases). Since a similar cumulative risk of myeloma was seen in AA (17%) and whites (15%), therefore, etiologic factors of MGUS must be investigated to better understand the racial disparity of myeloma. Interestingly, HIV infection, which is more prevalent in AA, is a well-documented risk factor in the etiology of MGUS [54]. Another factor for poor prognosis and higher tumor burden in MM patients is comorbid renal insufficiency. It is interesting to note that in a cohort of 59 AA and 59 non-AA, it was found that AA patients with newly diagnosed MM and treated with novel modern medicinal agents showed more significant improvement of renal function when compared to their non-AA counterparts, regardless of their response to myeloma [55].

# Studies in Italian population

Zingoni et al. [56] investigated the role of MICA (MHC class-I related Molecule-A, NKG2D ligand) genetic polymorphisms and sera concentration of MICA in the MM progression in 137 MM patients from Rome (ethnicity not known). They reported that MICA-129Val/Val patients had a higher frequency of relapse along with a high concentration of soluble MICA in the serum. This indicates that MICA polymorphism directly impacts the relapse of disease post-chemotherapy. It was also observed that patients with MICA-129Met/Met genotype had lowest NKG2D levels, suggesting that MICA-129Met/Met has a greater affinity towards NKG2D as compared to MICA-129Val/Val and plays a key role in NKG2D downregulation which leads to an escalation in the concentration of the malignant plasma cells. Thus, MICA dimorphism (Met to Val) affects its ability to optimally recognise NKG2D and that soluble MICA can be used as a prognostic marker in patients with MICA genotype. No consensus exists on the role played of MTHFR in myelomagenesis. Therefore, Chiusolo et al. [48] studied two most common polymorphisms of MTHFR A1298C and C677T to investigate its influence on MM by enrolling 100 Caucasian patients from Central Italy. Their data suggested that none of the variant alleles played a key role either in higher risk or protection from MM. On the contrary, they found that hypermethylation of p16 is a frequent aberration and may contribute to myeloma pathogenesis.

# Studies in Polish population

A study conducted by Butrym et al. [57] in 144 Polish MM patients (ethnicity not known) found that the frequency of rs872071 G allele polymorphism of IRF4 (Interferon regulatory factor 4) gene was more in patients, especially in women while the carriers of rs711613 A allele polymorphism of cereblon showed better response to treatment, especially those with thalidomide; thus suggesting the prognostic relevance of polymorphisms in cereblon and IRF4. Constitution activation of JAK/STAT3 cascade by CXCR4/CXCL12 interaction plays a key role in tumorigenesis. Indeed Mazur et al. [58] studied the correlation between disease progression and susceptibility with polymorphisms in the genes encoding for CXCL12 (rs1801157) and CXCR4 (rs2228014) in a cohort of 172 Polish volunteers (54 patients and 118 controls). They found that the frequency of the CXCR4 T variant was less in patients and was associated with disease progression while patients the frequency of CXCL12-3'A variant in less advanced MM was low and had overall better survival and favorable progression of the disease. Similarly, basigin (BSG or CD147) promotes cancer cell growth by regulating transport of lactate anions via MCT1 (monocarboxylic acid transporter 1, SLC16A1) and the role of SNPs of BSG and SLC16A1 in myeloma was studied by Lacina et al. [59] in a cohort of 135 Polish volunteers. It was found that alleles rs1049434 A, rs7556664 A and rs7169 T were associated with better overall survival while rs4919859 C, haplotype CG and rs8637 G were associated with worst progression free survival. The frequency of alleles rs8259 A, rs8637 G, CG haplotype and rs4919859 C was more common in Stage II and III of ISS (International Staging System). Finally, the rs8259 A allele was also correlated with elevated levels of β-2-microglobulin and creatinine. Taken together, variants of SLC16A1 and BSG affect survival and may have an important role in MM pathogenesis. Another genetic determinant of disease progression after 1st line chemotherapy is the carriership of bFGF (basic fibroblast growth factor) gene polymorphism, rs308395 (G>C, -921 position in promoter region), which was seen more frequently stage I and II when compared to those in stage III according to the Durie-Salmon criteria in a cohort of 132 MM patients (ethnicity not known) [60]. Recently, it was found that SNPs in RANK, RANKL (RANK ligand) and TACI were associated with progression and development of myeloma in 222 Polish MM patients (111 M+111 F). The C variant of rs1805034 (RANK SNP) had better survival (prominent in female populations) and had a lower median age (64 vs 65.5 years) at diagnosis. While the A variant of rs7325635 (RANKL SNP) showed lack of early myeloma progression and lower PFS, the G variant in women resulted in higher Ca<sup>+2</sup> levels in blood. Interestingly, the A variant of rs34562254 (TACI SNP) was frequently seen in advanced cases at diagnosis [61]. While PSMA6 CG+GG genotypes had shorter OS, increased risk of progressive disease and higher risk of death, NOD2/CARD15 3020insC increased sensitivity to bortezomib along with a reduced risk of renal dysfunction in 100 newly diagnosed cases from Poland (ethnicity not known) [62].

#### Studies in Danish population

A number of studies were conducted by Vangsted et al. in Danish MM patients treated with autologous stem cell transplantation (ASCT) and high dose melphalan (between August 1994 and August 2004). A total of 348 patients (ethnicity not known) were recruited and we present their findings herein. IL1B (Interleukin 1 beta) gene polymorphisms in a subset of Danish MM patients showed that the noncarriers of promoter haplotype TGT (-31(T), -1464(G), -3737(T)) and C-allele of IL1B C-3737T (wild type) are responsive to treatment and benefit with better outcomes (longer overall survival (OS) and time to treatment failure (TTF) duration) of INF- $\!\alpha$  and high dose treatment, which is partly related to the NF-kB signalling. However, no correlation between outcomes and genotype was established in patients with MM relapse treated with bortezomib or thalidomide [63]. In contrast, carriers of TGT haplotype and variant T-allele IL1B C-3737T had reduced risk of MM (relative risk of 0.59 and 0.58 respectively) and there was no association between NF-KB polymorphism (NFKB1- 94 ins/del) and risk of MM [64]. Furthermore, INF-a treatment was beneficial in Danish MM patients who carried the NFKB1-94ins/delATTG (wild type) polymorphism homozygously when compared to the carriers of variant allele [65]. This strongly suggests NFKB1 polymorphism should be explored as a prognostic marker in MM patients on INF- $\alpha$  maintenance therapy after a high dose therapy. In another study, it was found that variant alleles in chromosome 19q13.3 spanning the region in CD3EAP (exon1 to exon3-6) and RAI (intron1-1 to RAI intron1-3) were associated with prolonged OS and TTF. None of the haplotypes correlated with OS and TTF better than strongly linked SNPs CD3EAP G-21A (rs967591) and RAI-intron1-1 (rs4572514). Although, association of CD3EAP G-21A and RAI-intron1-1 was independent of NFKB1-94 ins/del, it was found that homozygous ins-allele carriers who were also carriers of CD3EAP G-21A and RAI-intron1-1 had the longest OS. A combination analysis revealed that the functionality of CD3EAP or RAI is related to the availability of NF- $\kappa$ B [66]. Interestingly, the frequency of variant allele IL-16 T-31C in Danish MM patients was increased. Although, no significant role was played by IL-1 $\beta$  T-31C polymorphism in Danish MM patients, it was found that variant C-allele carriers had a longer OS when compared to carriers of homozygous wild-type allele TT. No such

association was seen between polymorphisms of other proinflammatory cytokines (IL-10 C592A, IL-6 G-174C, COX-2 T8473C COX-2 A-1195G, PPARgamma2 Pro(12)Ala, NFKB1 ins/del) and OS [67]. They also reported 1.9-, 1.8-, 1.3-fold longer TTF in carriers of variant A-allele of CD3EAP G-21A, variant T-allele of XRCC3 T241M and variant C-allele of ERCC2 K751Q respectively when compared to homozygous wild type carriers. Importantly, CD3EAP G-21A polymorphism also affected OS, ERCC2 K751Q polymorphism showed prolonged TTF only in women while the carriers of alleles XRCC3 T241M and ERCC2 K751Q in combination showed 2.8-fold prolonged TTF [68]. Finally, homozygous carriers of heparanase SNP rs6535455 variant T-allele showed prolonged survival as compared to hetero- and homozygous carriers of wild-type C-allele (0.3 hazards ratio) while homozygous carriers of heparanase SNP rs4693608 wild-type A-allele had a greater tendency to vertebral fractures when compared to variant G-allele carriers [69]. This means that heparanase gene can affect MM outcomes by influencing bone morbidity. Finally, Mølle et al. [70] studied 113 treatment courses of ASCT and 136 treatment courses of induction chemotherapy in MM patients. They found that risk of infection was not associated with G-463A myeloperoxidase (MPO) promoter polymorphism while G-129MPO promoter type leads to sepsis with an incidence of 0.30 during ASCT. In contrast, FCGR3A, FCGR2A and FCGR3B polymorphisms were not associated with any infections.

# Studies in British population

Davies et al. [71] reported that in a cohort of 198 white patients from Leeds, polymorphisms that were associated with elevated production of TNF- $\alpha$ /LT- $\alpha$  were at a higher risk of developing MM (odds ratio 2.05) and MGUS. There was no significant impact of TNF- $\alpha$  /LT- $\alpha$  polymorphisms on the overall survival (53.8 months) of patients. In contrast, a study conducted in 181 patients (ethnicity not known) showed that TNF locus with high producing haplotypes were not associated with a higher risk to MM. Notably, the data showed that variant allele A of TNF- $\alpha$  SNP at position -308 had a decreased risk of MM (odds ratio-0.57) [72]. Polymorphisms in GSTP1 (Glutathione S-transferase P1), a phase II metabolising enzyme, at codon 105 influences outcomes of chemotherapy. While 105Val variant allele showed longer PFS (progression free survival) in standard dosage regimens, better PFS in high-dosage remines was seen only in patients with homozygous 105Ile allele variant in cohort of 222 MM patients [73]. NHEJ (Non-homologous end joining) is an important step in antigen receptor gene arrangement involved in the etiology of lymphoproliferative diseases. Roddam et al. [74] identified LIG4 (NHEJ DNA ligase IV) as a potential polymorph that had an impact on several lymphoproliferative diseases, includin MM. The two LIG4 polymorphs (A3V and T9I) were investigated for both C and T transitions. The CT genotype of A3V showed 2-fold decreased risk of developing MM and similarly the TT and CT genotypes of T9I were associated with 4- and 1.5- fold decreased risk of developing MM respectively.

## Studies in German population

Rios-Tamayo et al. [75] evaluated the impact of 58 GWAS (genome-wide association studies) variants in 936 patients collected from IMMENSE (International MM Research) and independently in 700 patients from University Clinic of Heidelberg, Germany. It was found that the variant rs7501939 located in HNF1B gene had an unfavorable OS. Further analysis revealed that, in men, SLC3048 SNP rs13266634 had a gender-specific association with OS (each copy resulted in poor OS). The role of TERT (telomerase reverse transcriptase) and TERC (telomerase RNA component) genes in cancer development is put forth by several epidemiological and biological evidence. Interestingly, in a cohort of 2267 German patients (ethnicity not known), Campa et al. [76] found that carriers of rs2242652 (TERT variant) were less susceptible to MM. Furthermore, the length of telomere was found to be

longer in patients when compared to controls. Thus, variants that decrease the efficacy of the telomerase complex reduces the length of telomere ends, which in turn can act as biomarker of decreased risk of MM.

### Studies in Hungarian population

Kiss et al. [77] aimed to derive the relation of FOPNL (fibroblast growth factor receptor 1 oncogene partner N-terminal like gene) SNP rs72773978 with the clinical outcomes in 373 Hungarian MM patients. The genotype distribution of SNP rs72773978 was TT: 2 (0.5%), AT: 43 (11.5%), AA: 328 (87.9%) and the allele frequency in MM patients was  $6.3 \pm 1.8\%$ . Analyses of cytogenetic abnormalities revealed trisomes (hyperdiploid) in 24.9% (89 patients) while IgH translocation was seen in 28.6% patients. In patients who received non-proteasomal inhibitor-based therapy, adverse overall survival was attributed to carriership of the AT and TT genotypes (minor allele). However, use of proteasomal inhibitors reversed the adverse survival in patients. This suggests that the adverse effect of FOPNL SNP rs72773978 could serve as a prognostic marker which is associated adverse survival in patients receiving non-proteasomal inhibitor- based chemotherapy. In another study with a cohort of 211 Hungarian MM patients, Varga et al. [78] found that suboptimal response to bortezomib treatment was predictable in carriers of PSMB1 rs12717 minor allele. This is because of decreased activity of proteasomes in response to bortezomib treatment which in turn promotes escape mechanisms in myeloma cells to cope with misfolded proteins which are abundant.

#### Studies in French population

Miannay et al. [79] studied the alteration of transcription factors in MM by isolating plasma cells from 602 MM patients and 9 controls from IFM (Intergroupe Francophone du Myélome), France. AP-1 activity was found to be lower in all MM cells compared to normal plasma cells. Further, MM was associated with transcription factor FOXM1 (forkhead box protein M1). Finally, poor OS in patients was attributed to elevated FOXM1 activity which is related to upregulation of FOXM1 gene. Thus, we can infer that FOXM1 can be used as one of the markers of survival in MM.

## Studies in Australian population

Lincz et al. [80] studied 90 Australian patients (Caucasians) with MM to investigate the role of polymorphism in xenobiotic enzymes [N-Acetyltransferase (NAT1, NAT2), Glutathione S-transferase (GST T1 null, GST M1), cytochrome P450 (CYP1A1) and Paraoxonase 1 (PON1 BB]. It was found that the incidences of PON1 BB, NAT2 slow acetylation and GST T1 null genotypes were significantly higher when compared to control (n = 205) while no such differences were seen in CYP1A1, NAT1 and GST M1.

## Studies in Russian population

Iakupova E.V et al. [81] studied 69 Russian patients with varying severity of MM. In the study, 308G alpha–>A polymorphism and 174G alpha–>C polymorphism of TNFα and Il-6 gene promoter region respectively were tested to understand their association with MM. They found that TNFα gene had no association with progression either with the clinical variant of the disease or with predisposition to MM. Furthermore, the CC genotype of IL-6 gene was absent in aggressive MM patients and had a frequency of 0.35 in low-progression MM patients. Thus, the CC genotype of the IL-6 gene in myeloma patients from Bashkortostan is associated with mild clinical signs.

## Studies in Chinese population

Cell cycle regulators such as Cyclin D1 are involved in the pathogenesis of several cancers. Wang et al. [82] designed a case-control study in 67 Chinese patients which suggested that Cyclin D1 G870A SNP was associated with a higher risk of developing MM (odds ratio-4.679) especially in individuals over 60 years of age. Yin et al. [83] studied 115 Jiangsu Han patients with MM to determine the correlation of SNPs of MDR1 gene and haplotype variants with susceptibility to MM. They found that there was no significance in distribution of genotypes and alleles in MDR1 loci (C3435T, C1236T and G2677T/A) and similarly no susceptibility to MM was found in diplotype analysis. However, haplotype analysis revealed that T-G-T haplotype frequency increased significantly when compared to controls, indicating that this haplotype might be associated with higher susceptibility to MM in Jiangsu Han population. In contrast, Xiao et al. [84] investigated the association of MDR1 loci (C3435T, C1236T and G2677T/A) in 129 MM patients from Jiangsu Han and reported that at locus C3435T, patients without T allele (CC) had shorter progression free survival (PFS, 29 vs 60 months respectively) than patients with T allele (TT and CT). Furthermore, CC genotype at C1236T had shorter PFS that the TT genotype (28 vs 48 months respectively). They found no association between overall survival and MDR1 polymorphisms. Mei et al. [85] detected hyperdiploidy in 102 patients (50.7%) and its co-incidence with high-risk cytogenetics [del(17p13), +1q21 and adverse t(14q32)] was seen in 68 patients (33.8%) in cohort of 201 Chinese (ethnicity not known) myeloma patients. Progression-free survival and 2-year overall survival were better for hyperdiploidy when compared to those without hyperdiploidy (43 vs 20 months). Furthermore, high-risk cytogenetics without co-existing hyperdiploidy was associated with worst prognosis of MM.

SNPs in NCOA1 region (rs79480871) is strongly associated with MM while that of HLA-I region (rs6457327) is readily associated with MM in Chinese Han population when compared to controls (827 MM and 709 healthy participants) [86]. Lu et al. [87] enrolled newly diagnosed MM (n = 940) patients and reported that patients with IgG isotype had more survival benefits with bortezomib as opposed to patients with IgD isotype. But, no significant difference in benefits was observed in either isotype when patients were treated with older therapies such as vincristine combined with dexamethasone or adriamycin or melphalan combined with prednisone. In another study, Yu et al. [88] investigated the application of chromosome aberration 1q21 in 86 Chinese patients who were newly diagnosed with MM. Amplification of 1q21 was seen in 40 patients (46.5%), among which 11 with atleast 4 copies and 29 with 3 copies of 1q21. Further analysis revealed that amplification of 1q21 with 4 or more copies served as a prognostic factor for adverse events in MM and 1q21 gains even predicted a poor overall survival in patients receiving Bortezomib-based treatment regimens. Finally, Vitamin D receptor gene polymorphisms, TaqI (C allele) and BsmI (A allele) had higher frequency and increased risk of MM in Chinese MM patients (*n*=40, ethnicity not known) [89].

# Studies in Korean population

Many malignancies are associated with MTHFR polymorphism. Moon et al. [90] evaluated MTHFR polymorphisms C677T and A1298C in different cancers. The study was done in 484 Koreans (ethnicity not known) including MM patients (n = 196). They reported that the levels of 1298CC and 677CC genotype of MTHFR were elevated in patients as compared to the control groups. Moreover, the study also highlighted that MTHFR 1298CC and 677CC genotype could have a combined effect on the risk of developing CML (chronic myelogenous leukemia), multiple myleoma and ALL (acute lymphoblastic leukemia). The role of NQO1 (NAD(P)H:quinone oxidoreductase 1) in cancer prevention is known and summarized by Oh et al. [91]. In a study conducted in 117 myeloma patients, it was found that NQO1\*2/\*2 genotype reduced the risk of MM in Koreans (ethnicity not known) [92]. Similarly, Kang et al. [93] CYP1A1\*1/\*2B and CYP1A1\*1/\*2A genotypes were associated with lower risk of MM in a study conducted in 116 myeloma patients from Korea. However, they found that polymorphisms (null types) of GSTT1 and GSTM1 were not associated with a risk to MM as reported in Caucasians [80].

#### Studies in Indian population

Binding of Vitamin D with its receptor VDR (Vitamin D receptor), carries out several biological processes including proliferation and differentiation. VDR gene polymorphisms (FokI, BsmI and ApaI) are drivers of many cancers, including MM. Shafia et al. [94] studied 75 MM patients belonging to the ethnic Kashmiri population. Interestingly, only Fokl polymorphism was associated with increased risk of development and progression in the Kashmiri population. Furthermore, patient carriers of the ff genotype of FokI had an increased risk of developing MM. Similarly, in another study with 75 Indian MM cases, ApaI a, FokI f and BsmI b alleles and genotypes Aa+aa, Ff+ff and Bb+bb were associated with a higher MM incidence and risk respectively. Furthermore, 25-hydroxy vitamin D levels were inversely correlated with severity of disease [95].

## Studies in populations from multiple countries

Interestingly, Dumontet et al. [96] found that SNPs of genes regulating DNA repair and drug metabolism can be used to distinguish subgroups of 169 MM patients from Lyon, France and Edmonton, Canada with different efficacy/toxicity profiles. SNPs in CYP3A4, ABCB1 and TP53BP2 were attributed with response to VAD (vincristine, adriamycin/doxorubicin and dexamethasone) induction therapy, while SNPs in GSTT2, ALDH2 and BRCA1 were attributed with response to high dose melphalan. Similarly, polymorphisms in RAD51, CYP1A1 and PARP were attributed with disease progression and polymorphisms in CYP1A1 and ALDH2 were correlated with overall survival. Finally, polymorphisms in CDKN1A, BRCA1 and XRCC1 were attributed with the incidence of severe mucositis after high dose melphalan. Recently, in a study with 1960 controls and 3056 MM patients from 8 countries, it was found that IL10 SNP, rs3024496 had worse OS and higher risk of MM development [97].

SNP at chromosome 7p15.3 is associated with risk of MM. Indeed, in a study with 848 patients (183 from UK and 665 from Germany, ethnicity not known), rs4487645 had highest risk of MM at 7p15.3, and is associated with allele specific cis-regulation of CDCA7L (MYC-interacting gene). Furthermore, rs4487645 is mapped within binding for IRF4 in a strong enhancer element that is upstream of CDCA7L. Taken together, 7p15.3 exerts its effects *via* an extended pathway which involves MYC and IRF [98]. Similar results were obtained by Li et al. [99] wherein rs4487645 G>T upregulated CDCA7L expression by activating the promoter region. They also reported that higher CDCA7L levels led to adverse survival of patients and that proliferation of MM can be slowed by downregulating CDCA7L which induced apoptosis. Thus, the role of IRF4-mediated CDCA7L expression in myeloma is implied and that germline variation can contribute to susceptibility to MM in different populations.

# Molecular aspects of MM

Pathogenesis of MM is complex and involves abnormalities in signalling pathways, translocation of chromosomes and alteration in bone marrow microenvironment. This can lead to alteration in the pathology, making it even more challenging to treat. While various cellular functions are carried out by signaling pathways, genetic defects within the tumour can lead to up/downregulation of protein/gene expressions which are key components of signalling machinery and can therefore lead to aggressive forms of malignancy. Further, deregulation in epigenetic mechanisms and genetic lesions have revealed variations and mutations in the expression levels of various genes associated with the MM pathogenesis, especially mechanisms associated with modification in histone, methylation of DNA, and non-coding RNA which are discussed herein.

### Signal transduction pathways in MM

A signalling cascade can be described as a series of sequential steps (either activation or inactivation), that ultimately lead to a biological response. A number of cellular signalling machinery are involved in regulating the fate of cells in multiple myeloma microenvironment. The cell's ultimate response to a stimulus is representative of cumulative effect of the primary signalling machinery and the gamut of their cross-talk. We depict these peculiarities in Fig. 1.

# PI3K/Akt/mTOR Pathway in MM

The PI3K/Akt/mTOR cascade plays an important role in mediating survival, proliferation and drug resistance in multiple myeloma. PI3K/ Akt/mTOR activation occurs through phosphorylation of Akt by PCDGF (PC cell-derived growth factor) [100]. PI3K/Akt pathway inhibition has shown cytotoxic and apoptotic effects in MM cells, indicating that it can act as one of the target pathways to treat MM[101]. Interestingly, Peterson et al. [102] reported DEPTOR (mTOR-interacting protein) overexpression in a subset of MM with c-MAF/MAFB or cyclin D1/D3

translocations and that higher DEPTOR expression is necessary to maintain activation of PI3K. Furthermore, inhibition of DEPTOR (which binds to mTOR) induces cytotoxicity [103]. Hsu et al. [104] indicated two mechanisms for activation of PI3K/AKT: first through RAS signaling which is independent of p85 and the second via p85 and STAT3-containing complex. However, studies that controlled PI3K activation highlighted the role of PI3K inhibitor like LY294002, which regulated cell cycle arrest [105]. In addition, IL-6 is also known to play a key role in the activation of PI3K-mediated proliferative response [106] which is followed by activation of GSK-3β, Bad and FKHR (downstream targets), suggesting AKT activation which is important for proliferative response is mediated by IL-6 [107,108]. mTOR (mammalian target of rapamycin) inhibitors activate AKT kinase by upregulating IGF-1 (insulin-like growth factor-I)/IRS-1 (insulin receptor substrate-1)/ PI3K (phosphatidylinositol 3-kinase) cascade [109] and during mTOR inhibiton, heightened AKT expression inhibits VEGF (vascular endothelial growth factor)-IRES (Internal ribosome entry site) activity [110]. p1108 inhibition in the PI3K/ p1108 signaling induces autophagy and triggeres cytotoxicity in INA-6 and LB MM cells [111]. Gan et al. [112] reported that NVP-BEZ235, a dual class I PI3K and mTOR inhibitor can be used to treat osteolytic bone disease in patients. Interestingly, potent and selective inhibiton of AKT by TAS-117 triggered anti-myeloma effects and the cytotoxicity of proteasomal inhibtors (Carfilzomib, Bortezomib) was enhanced in combination with TAS-117[113].



**Fig. 1.** Signal transduction pathways in MM: Interactions of VEGFR and IGF1R with their corresponding ligands VEGF and IGF1 leads to activation/phosphorylation of PI3K. This sequentially activates PDK-AKT-TSC1/2-mTOR cascade. Similarly, docking of GRB-SOS complex in response to activation by upstream molecules activates the RAS-RAF-MEK1/2-ERK1/2 signalling. The Wnt ligand binds to frizzled activating the Wnt-β-catenin pathway. β-catenin accumulates and translocates into the nucleus and regulates genes involved in adhesion, differentiation, proliferation and migration. Axin scaffolds β-catenin to induce proteasomal degradation. NF-kB is activated by upstream CD40, TAC1 and LTβR.

# NFKB Pathway in MM

NF-kB (Nuclear factor kappa-beta) regulates expression of many genes which modulate the survival, growth and metastasis of cells along with cell cycle regulators and angiogenesis and is overexpressed in MM [114]. It promotes bone degradation *via* TRAF6, p62, IkB $\alpha$  and CYLD signalling cascades [115]. Moreover, proteins like IGF1 produced in bone marrow are known to indirectly activate NF-kB in MM [116]. Pharmacological inhibition of NF-kB signalling by IKK2 inhibitors [117] and proteasomal inhibitors [118] overcomes cytoprotective effects of NFKB-mediated drug resistance, survival and growth.

# JAK/STAT pathway in MM

Activation of JAK/STAT3 results in enhanced metastatic and tumorigenic potential. IL-6/STAT3 promotes expression of Hsp90 (heat shock protein) alpha and beta and is indicative of a positive loop that promotes survival of MM cells. This is reinforced when coupled with simultaneous MAPK inhibition [119]. Thus, combined inhibition of IL-6/STAT3 and MEK/ERK can induce apoptosis in MM cells [120]. Similarly, TG101209 (JAK2 inhibitor) is preferentially cytotoxic towards CD45+ myeloma cells [121] and tetracyclic pyridone 6 (P6, a pan-JAK inhibitor) prevents growth of MM cells and MM-derived cells [122]. Additionally, increasing levels of cAMP (cyclic adenosine monophosphate) promotes cellular death by downregulating Mcl-1 (myeloid cell leukemia-1) via the JAK/STAT3 signalling [123] and elevated levels of TJP1 (tight junction protein 1) leads to higher sensitivity of proteasomal inhibitor against MM. This occurs by suppressing the EGFR/JAK1/STAT3 cascade which decreases the expression of LMP2 and LMP7 (immunoproteasome subunits) [124].

# RANK/RANKL/OPG signalling pathway in MM

Patients are often diagnosed with bone lysis in MM, which is a function of the activation of RANK/RANKL/osteoprotegerin (OPG) signal transduction which causes an increase in function and survival of osteoclasts [125]. Interactions between RANK/OPG and RANKL plays an important role in the osteoclast activation and MM cells survival. Moreover, a negative correlation between RANKL and OPG levels has been reported in literature which increases the chances of bone destruction and induction of osteolysis in MM [126–128]. Thus, bone destruction in MM can be overcome by overexpression of OPG. AMGN-0007 (a recombinant OPG molecule) was clinically tested in 28 multiple myeloma patients and was found to inhibited bone resorption [129].

# MAPK signalling pathway in MM

MAPK is a pro-survival signalling cascade (RAS-RAF-MEK1/2-ERK1/ 2) and aberrant high expression is often seen in cancerous cells. RAS mutation is the single most common mutation in MM and is associated with high tumour burden [130]. Interestingly, shorter progression free survival and overall survival is only associated with KRAS mutations and not NRAS mutations [130]. Similarly, FGFR3 not only plays an oncogenic role when overexpressed, but also acts as a target for mutations that allow FGFR3 to play a RAS-like role in tumor progression [131]. Yang et al. [132] found that higher CRP (C-reactive protein) secretion leads to bone destruction. In addition, the CRP bound to surface CD32 of myeloma cells activated p38 MAPK and Twist (transcription factor) which increased secretion of cytokines responsible for osteolysis. Similarly, cellular growth is also triggered by IL-6 via a Ras-dependent MAPK cascade [133]. Interestingly, in a subset of MAF expressing myelomas, MEK inhibitors can induce apoptosis [134]. In a Phase 2 trial, single agent AZD6244 (75 mg, twice/day for 28 days, 3 cycles) was found to be tolerable and had minimal activity in 36 heavily pre-treated patients [135].

## *Wnt*/ $\beta$ -catenin signalling

 $\beta$ -catenin activates the Wnt signalling cascade which is involved in proliferation, migration and differentiation of myeloma cells [136].

Production of Wnt inhibitor DKK1 (Dickkopf-1) by myeloma cells inhibits osteoblast differentiation and is therefore associated with lytic bone lesion in MM patients [137]. In contrast, Qiang et al. [138] reported that inducing Wnt/ $\beta$ -catenin in bone microenvironment can serve as an efficient strategy to treat MM and MM-triggered bone abnormalities. Finally, *in vivo* studies by Edwards et al. [139] suggests that although Wnt cascade activation promotes tumor growth in extraosseous sites, but it prevents myeloma growth in bone and development of myeloma bone disease.

## Role of epigenetics in MM

Abnormalities in histone modifications have gained attention in the recent years and have helped to uncover the importance of activities and their correlation with histone post-translational modifications. H3K36me3 (histone H3 lysine 36 trimethylation) regulates DNA mismatch repair in humans by interacting with mismatch recognition protein  $hMutS\alpha$  through a direct interaction with hMSH6 PWWP domain [140]. Pawlyn et al. [141] detected SET2D gene mutations in both relapsed and newly diagnosed patients indicating that understanding of the effects and mutations in Histone H3 lysine 36 methylation can help to mark MM pathogenesis. Interestingly, Keats et al. [142] reported that there is uniform deregulating in transcripts originating from WHSC1/MMSET/NSD2 gene in all t(4;14)<sup>POS</sup> patients. Furthermore, alternative splicing was a key mechanism for expression of RE-IIBP (response element II binding protein), MMSET I (multiple myeloma SET domain containing protein), MMSET II and Exon 4a/MMSET III transcripts. Similarly, clinical impact of t(4;14) translocation in myeloma is significant and is independent of FGFR3 expression [143]. Kuo et al. [144] reported that catalysis of H3K36me2 by NSD2 in t (4;14)-positive MM cells created a chromatin landscape that selected a transcription profile promoting oncogenicity. Furthermore, KAP1 (a corepressor) and histone deacetylase (HDAC, characterised by decreased H3 acetylation and increased H3K9 trimethylation) promote MMSET-induced repression of miR-126, which leads to increased levels of c-MYC and enhanced proliferation of t(4;14)-positive MM cells [145]. Interestingly, the efficacy of chemotherapy to increase survival and inhibit growth is enhanced by decreasing MMSET expression as studied by Shan et al. [146]. Elevated mRNA expressions of EZH2 at diagnosis in myeloma patients is associated with high-risk clinical features and poor outcomes. Inhibiting EZH2 by small molecule inhibitors such as UNC1999 and EPZ005687 leads to arrest of cellular growth and is followed by apoptosis [147]. Huang et al. [148] found that Glp and G9a (homologous methyl transferases) are overexpressed in cancer and methylate p53 at Lysine at 373, resulting in demethylation. Interestingly, during DNA damage, levels of tumor-suppressing p53 modified at Lys(373)me2 remains unchanged, despite overall increase in total p53 levels. This is indicates that Lys(373)me2 correlates with inactive p53. Overall, their data present a novel methylation site in p53 which is mediated my methylases and present G9a as a potential inhibitory target. Similarly, KDM3A-KLF2-IRF4 axis promotes myeloma cell growth and can sever as potential target for myeloma treatment [149]. It is reported that EP300 and CREBBP, genes encoding for histone acetyltransferase are frequently mutated in myeloma patients and can alter cellular processes such as DNA repair, cell cycle progression, p53 activity and apoptosis [141]. Mithraprabhu et al. [150] found that overexpression of class I HDAC (in particular HDAC1) was much higher in MM patients and indicated poor prognosis in MM. A study conducted by Rizq et al. [151] showed UNC1999, dual inhibitor of EZH1/EZH2 showed better anti-myeloma activity when combined with proteasomal inhibitors (bortezomib) which repress the transcription of EZH2 by abrogating RB-E2F pathway. Moreover, UNC1999 increased the expression of tumor suppressor gene NR4A1 which resulted in suppression of MYC and this suppression was more pronounced in combination with bortezomib. Houde et al. [152] suggested the role of DNA hypomethylation of JAG2 promoter in the pathogenesis of MM and that JAG2 enhanced VEGF, IL-6 and IGF-1 secretion. Finally, Turner et al. [153] reported that ABCG2 is regulated by promoter methylation, is functional and expressed in MM cells. Importantly, it is overexpressed following chemotherapy, this suggests that it has the potential to contribute to intrinsic drug resistance.

### Role of non-coding RNAs (ncRNA) in MM

ncRNAs are those regions of the genome which are not transcribed into proteins but are involved, nonetheless in regulating transcriptional status of genes in the genome by transcriptional repression [154]. The role of ncRNAs, such as lncRNAs, microRNA (miRNAs), ceRNAs, circRNA, in MM biology has been recognized in the literature [155]. Fig. 2 depicts the interplay between noncoding RNAs and signalling cascades implicated in MM and Table 2 lists the various miRNAs involved in the drug resistance and/or the pathogenesis for the benefit of the reader.

# Role of microRNAs (miRNAs) in MM

miRNA deregulation (up/downregulated) impacts the initiation, progression, and metastasis of cancers [156]. We have previously described the architecture of signature miRNA networks in cancer chemoprevention and chemoresistance [157]. Recently, we have also elucidated the regulatory miRNA interactome in the pathogenesis of neuropathic pain of various etiologies including chemotherapy-induced peripheral neuropathy [158]. In MM, miR-29b overexpression leads to downregulation of MCL-1 and apoptosis via caspase 3 activation [159] while miR-19a and miR-19b are correlated with downregulation of SOCS-1 (suppressor of cytokine signaling protein-1) that plays a key role

in inhibiting IL-6 growth cascade [160]. Additionally, miR-29b was found to exert its anti-proliferative effects by causing downregulation of CDK6 (cyclin-dependent kinase 6) and MCL-1 mRNAs. Furthermore, Sp1 (specific protein 1, oncogenic transcription factor) negatively regulated miR-29b expression in MM and this miR-29a-Sp1 regulatory loop sensitised MM cells to bortezomib-induced apoptosis [161]. However, lncRNA NEAT1 expression sponged miR-29b-3p to upregulate Sp1 and promoted resistance to bortezomib [162]. Interestingly, enforced expression of miR-29b overcame osteoclast activation and impaired their differentiation which is triggered by MM cells [163]. miR-181a, miR181-b, miR106b-5 and miR-32 are reported to target PCAF (p300-CBP-associated factor) gene which is involved in p53 regulation [160]. Stat3 dependent miR-21 induction by IL-6 is observed in MM cells. Interestingly, inducing miR-21 expression ectopically in absence of IL-6 decreased apoptosis levels, suggesting that miR-21 is an important contributor to the oncogenic potential of Stat3 [164]. Alternatively, PIAS3 (protein inhibitor of activated STAT3) downregulation contributes to the oncogenic role of the miR-21-STAT3 axis [165]. Also, miR-21 expression is higher in MM cells when compared to normal plasma cells. Therefore, coupling cytotoxic drugs like doxorubicin or dexamethasone with miR-21 inhibition provides more enhanced anti-myeloma effect than either when used alone [166]. This rationale for inhibiting miR-21 for anti-myeloma effect has also been reported in vivo [167]. Recently, it was also shown that antagonising miR-21 activity inhibited the tumorigenic functions of Th17 (IL-17 producing CD4+ cells) in MM [168].

Tumorigenicity in myeloma is also partly driven by the Mycinducible miR-17-92 [169]. Interestingly, miRNAs such as miR-20a, miR-99b, miR-148a, miR-181a, miR-221 and miR-625 can be used as clinical biomarkers. It is important to note that amongst them, high



**Fig. 2.** Correlation between ncRNA and signal transduction: The set of miR-181a, miR-181b, miR-106b-5 and miR-32 inhibit PCAF which regulates p53 activity. STAT-dependent miR-21 is induced by IL-6. PTEN is upregulated by using miR-221/222 inhibitors. Further, miR-29b induction leads to activation of caspase 3. Additionally, miR-15a/16-1 overexpression downregulates VEGF and AKT3 expressions. Similarly, miR-15/16 affects NF-κB activation. miR-34a downregulated NOTCH1. IL-6 expression inhibits miR-15a/16. miR-202 expression downregulated JNK/SAPK. IncRNA MEG3 overexpression enhances osteogenic markers such as RUNX2 and osterix. In contrast, ANRIL overexpression causes PTEN silencing. STAiRs 1, 2 6, 15, 18 are induced by Il-6 dependent STAT3 activation and STAiR 18 interacts with H3K27Me3.

## Table 2

List of miRNAs involved in the pathogenesis and/or drug resistance of MM

miRNAs	Dysregulation	Outcome(s)	Refs.
miR-126	Downregulation	Enhanced proliferation	[145]
miR-29b	Downregulation	Resistance to bortezomib	[162]
miR-17	Upregulation	Poor prognosis and PFS	[169]
miR-20			
miR-90			
miR-29b	Upregulation	Apoptosis via caspase activation	[159]
miR-21	Downregulation	Inhibits proliferation	[164,167]
	Upregulation	Drug resistance	
miR-221/222	Upregulation	Cell growth and cell survival	[171]
miR-106b~25	Upregulation	p53 suppression	[160]
miR-181a			
miR-32			
miR-202	Upregulation	Sensitization to bortezomib>thalidomide>dexamethasone	[176]
miR-15a	Downregulation	Tumor proliferation	[172]
miR-16	-		
miR-631	Upregulation	Confers sensitivity to bortezomib	[175]

levels of miR-148a and miR-20a is correlated with shorter relapse free survival [170]. Silencing the miR-221/222 cluster by miR-221/222 inhibitors exerts anti-myeloma effects by upregulating PTEN, PUMA, p27Kip1 and p57Kip2 [171]. Roccaro et al. [172] established the roles of miR-15/16 in regulating growth and proliferation by downregulation of cyclin CDC25A, D1 and D2 along with inhibition of MAPK, AKT3 (AKT serine/threonine protein kinase), NF-kB activator MAP3KIP3 and ribosomal-protein-S6. They also reported that miR-16-1 and miR15-a exert their anti-angiogenic activity by regulating VEGF both *in vitro* and *in vivo*.

Synthetic miR-34a mimics or transient expression of miR-34a downregulates BCL2, NOTCH1 and CDK6 at the protein and mRNA levels to exert anti-myeloma effect. This is also validated in vivo using SCID mice. IL-6 secreted by bone marrow stromal cells suppresses miR-15a and miR-16 expressions (miR-15a being more significant), which inturn promotes drug resistance in MM [173]. Leotta et al. indicated miR-125a-5p antagonism activated p53 pathway in myeloma cells and provided a basis for a combination of miR-125a inhibitors and miR-194 for [174]. or miR-192 mimics MM treatment The miR-631/UbcH10/MDR1 axis is a key driver of bortezomib resistance in MM and overexpression of miR-631 is found to resensitize cells to bortezomib [175]. Finally, Shen et al. [176] reported that miR-202 overexpression sensitizes myeloma cells to bortezomib more profoundly when compared to dexamethasone and thalidomide. Furthermore, JNK/SAPK cascade was identified to be a part of the effect of miR-202 on drug resistance in MM cells.

# Role of long non-coding RNAs (lncRNAs) in MM

We have previously described the role of lncRNA(s) and the molecular systems pharmacology of lncRNA-miRNA interactions in cancers [156]. As reported by Ronchetti et al. [177], lncRNAs which are deregulated have a strong impact on MM progression. They identified 31 deregulated lncRNAs; among these MALAT1 upregulation was associated with signal transduction that regulated mRNA maturation processes, p-53-mediated DNA damage response and cell cycle regulation. Furthermore, 21 lncRNAs were identified whose expression progressively deregulated through more aggressive stages, which highlighted their possible in disease progression. Studies show that MALAT1 is overexpressed in MM patients [178] and MALAT1 is an inducible stress response gene which is upregulated by chemotherapy (doxorubicin, bortezomib) and is associated with poor prognosis and extramedullary spread of MM [179]. Furthermore, levels of MALAT1 are also associated with TGF-β1 (transforming growth factor beta-1) which is regulated by LTBP3 gene [180]. MALAT1 downregulation abrogated invasion, glycolysis and tumorigenesis by upregulating miR-1271-5p and downregulating its target SOX13 in vivo [181]. Interestingly, miR-188-5p was reported to bind to MALAT1 directly and it acted as a tumor suppression

in MALAT1-induced myelomagenesis [182].

MEG3 upregulation enhanced osteogenic differentiation (upregulation of osteogenic markers RUNX2, osteocalcin and osterix) of mesenchymal cells from MM patients by activating BMP4 transcription [183]. MEG3 upregulation also elevated the levels of p53 (tumor suppressor) and treatment with a methylation inhibitor (5-Aza-CdR) led to a decrease in aberrant MEG3 hypermethylation which was coupled with apoptosis and inhibited proliferation [184]. lncRNA ANRIL is overexpressed in tumor samples and is often associated with myeloma relapse. ANRIL has been reported to upregulate in HIF-1 $\alpha$  by sponging miR-411-3p. However, miR-411-3p mimics when used in vivo (U266 xenograft model) decreased tumor volume, inhibited proliferation and improved the survival [185]. Reportedly, ANRIL overexpression increased the IC<sub>50</sub> of bortezomib, suggesting its role in chemoresistance and this was facilitated by epigenetic PTEN silencing by EZH2 [186]. This suggests that restoring PTEN or inhibiting EZH2 could improve outcomes. Poi et al. [187] identified that rs2151280 (CàT) SNP in ANRIL had worst progression free survival while the TT variant upregulated ANRIL and downregulated p15, p16 and p14ARF when compared to TC/CC genotype. They concluded that ANRIL SNP can serve as a prognostic biomarker for relapse in MM patients treated with melphalan.

Among the 5 lncRNAs identified and named by Binder et al. [188], STAiRs 1, 2, 6 showed myeloma specific expression and were unprocessed while STAiRs 15 and 18 are broadly expressed and spliced. More importantly, STAiR 18 was overexpressed in all samples, thus indicating a global role in tumor pathogenesis. In addition, it was also found that STAiR18 was associated with Histone H3 lysine 27 tri-methylation, thus indicating role of lncRNA in chromatin silencing. STAiRs are induced by IL-6-induced STAT3 signalling suggesting a STAT-3 driven tumor development in MM.

## Drug resistance and toxicity in MM

Drug resistance is a major impediment to treatment of MM. Interestingly, overexpression of ABCC2 promotes efflux of anti-cancer agents, which contributes to resistance. The IMMEnSE (International Multiple Myeloma Research) study conducted by Macauda et al. [189] included 1365 patients from Portugal, Italy, Spain, Poland and Denmark (ethnicity not mentioned). Different genes along with their SNPs involved in metabolic pathways were investigated and it was reported that ABCB1 SNP rs2235013 and ABCC2 SNP rs4148388 were significantly associated with the survival of MM patients and were functional. A characteristic feature of myeloma cells is CD38 overexpression. The first monoclonal antibody to be approved by US FDA for treatment of myeloma is Daratumumab, which is a CD38 directed monoclonal antibody [190]. In SIRIUS, a phase 2 trial (NCT01985126), 124 patients previously treated with atleast 3 lines of therapy (immunomodulatory drugs and proteasomal inhibitors) or refractory to them from USA, Canada and Spain (ethnicity not mentioned) were administered Daratumumab (16 mg/kg and 8 mg/kg). Overall, it was reported that Daratumumab was well tolerated with anaemia (33%, 35 patients) and fatigue (40%, 42 patients) were most common adverse event and no drug-related adverse event led to discontinuation of the treatment [191]. Similarly, in another phase 1-2 trial (NCT00574288), 104 patients refractory to two or more lines of treatment were administered Daratumumab (0.005-24 mg/kg). Thrombocytopenia and pneumonia were the most common grade 3 or 4 adverse events and were seen in  $\geq$  5% of patients. However, this study too concluded a favourable safety profile of Daratumumab monotherapy.

Co-existence of high or low chromosomal instability (CIN) and multiple subclone in myeloma causes drug resistance and heterogeneity, consequently causing relapse. Franqui-Machin et al. [192] reported that NEK2 (a CIN gene) binds to deubiquitinase USP7 and stabilises itself. This induced the NF- $\kappa$ B (canonical) cascade *via* the PP1 $\alpha$ /AKT axis which promoted bone destruction in addition to enhancing the tumorigenicity of cancerous cells as discussed earlier. Importantly, USP7 and NEK2 inhibitors greatly inhibited MM cell growth and overcame NEK2-induced and acquired resistance *in vivo*. Similarly, in a study with 19 patients (ethnicity not known), Zhou et al. [193] also reported that NEK2 promotes CIN overexpression, drug resistance and cell proliferation by promoting efflux pumps. Furthermore, NEK2 expression also upregulated AKT and Wnt (canonical) cascade. Interestingly, NEK knockdown by shRNA [1–4] was seen in ARP1 cells which increased the sensitivity to bortezomib with shRNA-3 being the most potent.

Ramani et al. [194] showed that levels of heparanase, which is a potent promoter of myeloma progression and growth were elevated in MM cells that survived prior chemotherapy. This high heparanase expression improved resistance to chemotherapy. Mechanistically, this was mediated by activation of the ERK signalling by heparanase. Importantly, Roneparstat (heparanase inhibitor, either before or after chemotherapy) when administered in vivo prevented growth of disseminated myeloma tumors in vivo. These encouraging results provide the rationale for development of heparanase inhibitors to treat myeloma patients. Turner et al. [195] studied if the acquired drug resistance can be overcome with a combination of Selinexor (XPO1 (exportin1) inhibitor) and doxorubicin hydrochloride using ex vivo samples obtained from patients with refractory/relapsed myeloma, multidrug-resistant in vitro models, in vivo xenograft tumors. They found that Selinexor improved the sensitivity of multidrug-resistant human MM cell lines 8226Dox6, 8226Dox40, 8226B25 and U266PSR to doxorubicin levels. The combination treatment showed that tumor growth had significantly reduced in NOD/SCID –  $\gamma$  mice. Importantly, inhibition of XPO1 by Selinexor prevents nuclear export of TOP2A (topoisomerase II alpha). This eventually increases the efficiency of doxorubicin to bind with TOP2A in the nucleus which leads to double strand break in the DNA and subsequent apoptotic cell death.

Myeloma treatment is often accompanied by severe infections. Proteasomal inhibitors are often used to treat myeloma clinically. However, their use as anti-myeloma agents should be judicious as they have the potential to cause toxicity. Iannaccone et al. [196] studied cardiovascular damage associated with use proteasomal inhibitors for the treatment of MM in 28 patients with relapsed/refractory disease. They found that bortezomib or carfilzomib treatment decreased GLS (global longitudinal strain) when compared to controls. Moreover, carfilzomib treated patients had increased left ventricle mass index, which is suggestive of a cardiotoxic effect of proteasomal inhibitors.

In another study, 2607 patients (from 8 trials) with relapsed/refractory disease were recruited by Zhao et al. [197] to determine cardiac toxicity associated with carfilzomib by measuring the incidence of cardiac dysfunction in patients. It was reported that pooled incidence of carfilzomib related all grade CHF (congestive heart failure) and IHD (ischemic heart disease) was 5.5% and 2.7% respectively. Furthermore, the incidence of high grade and all grade CHF was significant, while that of IHD was insignificant in response to use of carfilzomib. Overall, this study highlights the risk of CHF in response to carfilzomib treatment and serves as a caution to clinicians to minimize toxicities and maximize benefits of the therapy. Mølle et al. [198] studied 113 treatment courses of autologous stem cell transplantation and high-dose melphalan in MM patients from Denmark (ethnicity not known) and found that homozygous carriers of wild-type MBL2 (Mannose binding lectin 2, a component of innate immune system) showed a significant decline in the septicaemia risk during autologous stem cell transplantation when compared with carriers of variant MBL2 (odd ratio 0.19). However, risk of grade 3-4 infections according to Common Toxicity Criteria was not influenced by wild-type MBL2 (odds ratio 1.20). This means that MBL partially protects against severe infections during autologous stem cell transplantation. Similarly, SNP of SLC7A5 (encodes for LAT1, an amino acid transporter), rs4240803 (first intron of SLC7A5) is associated with the need of total parenteral nutrition and is correlated with gastrointestinal toxicity in MM patients (n=135) receiving autologous stem cell transplantation and high dose melphalan therapy [199].

Using data from 9 studies (genome wide association-studies), the frequency of CIPN (chemotherapy induced peripheral neuropathy) was identified in 148 patients (102, 17, 29 patients treated with bortezomib, thalidomide, vincristine respectively) from a cohort of 1082 German patients (ethnicity not known). In particular, SNPs rs1903216 (near BCL6 gene), rs8014839 (near FBXO33 gene), rs4618330 (near INTU gene) and rs4687753 (near IL17RB gene), relevant to nerve function were put forth as strong candidates to predict CIPN risk in MM [200]. Furthermore, the set of SNPs in CETP (rs289747), CINP (rs7011), GAN (rs2608555), VEGF (rs699947), CDKN1A (rs3829963), ALDH1A (rs168351), ALDH1A1 (rs610529), which are genes involved in DNA repair, cytokine balance and drug metabolism/transport, identified thalidomide-induced venous thromboembolism correctly in MM patients with a sensitivity of 81% and specificity of 59% [201].

# Summary and future perspectives

Multiple myeloma is an aggressive cancer with a median age of 69 years and its diagnosis is based on  $\geq 10\%$  plasma cells in bone marrow (BM) coupled with clinical history. In the recent years, this definition has changed to include patients with either  $\geq 60\%$  clonal plasma cells in BM when examined conventionally or presence of > 1 lytic lesion in MRI or involved/uninvolved serum free light chain ratio  $\geq 100$  [202]. The goal of therapy in an ideal patient is to achieve quick and profound response by employing aggressive chemotherapeutic regimens and followed by ASCT. Unfortunately, the frail and elderly constitute the majority of the patients. This leads to several complications in the proposed course of therapy, chemotherapy-induced toxicity, off-tumor toxicity, and ineligibility to participate in trials as these populations are often excluded from them.

By applying the principles of pharmacogenetics in MM, it is understood that there is an ethnic component that acts as a driver of variable response to chemotherapy in different sub-populations globally. Notably, this variability exists in populations of different regions; however, the reason for such ethnic variability is not fully understood. Indeed, the greater challenge is to improve the global accessibility to the most recent advancements in MM treatment [51] and limit failure of therapy due to resistance. Elucidating the genetic basis of multiple myeloma along with identification of ethnic variabilities in different patient sub-populations will provide insights for exploring new therapeutic regimens targeted to individuals. The emerging single cell technologies hold great promise for enhancing our understanding of MM tumor heterogeneity and clonal diversity. The concerted use of high-powered molecular expertise with advances in pharmacogenetic profiling will help to improve clinical decisions and thus enhance clinical success by the application of precision medicine [203].

A particular challenge of pharmacogenomic profiling of study

populations is that, often, the exact race/ethnicity being studied is not delineated accurately. This is of particular significance in immigrant populations. In future, clinical trials should ideally document the race/ ethnicity of the patients accurately before including them in the trials to ensure better classifications of study populations. This will take us a step closer to using precision medicine for MM treatment. Another major challenge to determine interethnic variability is the lack of data from underdeveloped and developing nations. Needless to say, extrapolating the data from developed world to the underdeveloped and developing world will lead to misadventures with life-threatening consequences. Nonetheless, knowledge of genetic variability has paved the way for rapid strides in the use of precision medicine in cancer, as we have discussed earlier [204,205].

Recent years have seen extensive use of single cell technologies to determine the clonal heterogeneity, trajectory analysis of disease progression, and analyse gene expression at single cell resolution, amongst others. This has greatly revolutionized our understanding of disease biology. Despite these advantages, scRNAseq is associated with distinct disadvantages. Indeed, the major limitation of single cell technologies, despite the availability of several algorithms for the analysis of raw data, is reproducibility and accuracy of the results. Furthermore, biological factors and technical limitations render the scRNAseq data susceptible to more noise compared to bulk RNA-seq data. This is a confounding variable while developing newer algorithms to analyse the raw data. Various aspects of scRNAseq including quality control, batch effect correction, normalisation, trajectory analysis, cell clustering, amongst others have been lucidly discussed elsewhere [206]. Concurrent development of more efficient bioinformatic algorithms and further advances in single cell sequencing technologies will provide researchers with the capability to delve deeper into understanding clonal heterogeneity, key resistance mechanisms and genetic drivers of multiple myeloma. A synergy of epistemological evidence derived from single cell technologies coupled with a better understanding of interethnic variability in population pharmacogenetics will likely facilitate research towards the laudable goal of precision medicine for MM. The current review collates our understanding of the molecular underpinnings of multiple myeloma and seeks to promote future pharmacogenetic research of this aggressive cancer. This will ultimately translate into more informed strategies for precision medicine and stratify the multiple myeloma population into non-responders and responders that will likely improve outcomes of patient care in the clinical setting.

## Authors' contributions

MG, VB, and AM collected the data and performed analyses. FDA, AL, RS, JT, and SN drafted and revised the manuscript. SN supervised and coordinated. All authors read and approved the final version of the manuscript.

#### Ethical statement

This is a review manuscript and does not involves the use of human subjects. Therefore, the ethical approval statement and informed consent are not required.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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