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MULTIPLE MYELOMA GENOMICS: A SYSTEMATIC REVIEW

CASEY J. WEAVER AND JOSEPH D. TARIMAN

OBJECTIVES: *This integrative review describes the genomic variants that have been found to be associated with poor prognosis in patients diagnosed with multiple myeloma (MM). Second, it identifies MM genetic and genomic changes using next-generation sequencing, specifically whole-genome sequencing or exome sequencing.*

DATA SOURCE: *A search for peer-reviewed articles through PubMed, EBSCOhost, and DePaul WorldCat Libraries Worldwide yielded 33 articles that were included in the final analysis.*

CONCLUSION: *The most commonly reported genetic changes were KRAS, NRAS, TP53, FAM46C, BRAF, DIS3, ATM, and CCND1. These genetic changes play a role in the pathogenesis of MM, prognostication, and therapeutic targets for novel therapies.*

IMPLICATIONS FOR NURSING PRACTICE: *MM genetics and genomics are expanding rapidly; oncology nurse clinicians must have basic competencies in genetics and genomics to help patients understand the complexities of genetic and genomic alterations and be able to refer patients to appropriate genomic professionals if needed.*

KEY WORDS: *multiple myeloma, genomics, next-generation sequencing, translation research.*

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Multiple myeloma (MM) is a hematologic malignancy characterized by the monoclonal expansion of bone marrow plasma cells (BMPCs).¹⁻³ MM is the third most common hematologic malignancy in the United States with an estimated 30,330 new diagnoses and 12,650 deaths in 2016.⁴ Although MM remains an incurable disease, improvement in survival over the past decade has been widely attributed to advances in the diagnosis, risk stratification, and available treatment options.^{2,5,6}

While research of the genomic components of MM has greatly contributed to the understanding of the MM pathogenesis, it has also revealed a further complexity of the disease.⁷ Next-generation sequencing (NGS) has given researchers the ability to view hundreds of thousands of genetic variants in a single run.⁸ The use of NGS in MM has made way for the discovery of many genomic variants specific to the disease but, similar to most genomic discoveries, has not made its way into evidence-based practice.⁹ The purpose of this systematic review was to identify the current state and knowledge of MM genomics and to analyze the use of NGS in identifying MM genomic data. Implications for practice are also discussed.

BACKGROUND AND SIGNIFICANCE

Before recognizing the genetic transformations that cause plasma cells to become malignant, it is important to understand the basic pathophysiology of MM. All immune cells begin as stem cells with the ability to mature into either a lymphoid or myeloid cell. Lymphoid cells are the main cells of the immune system that differentiate into T lymphocytes and B lymphocytes. The B lymphocytes that respond to an infection mature into plasma cells. These plasma cells make and release immunoglobulins, also called antibodies, that attack and kill germs. One of the characterizations of MM is the abnormal production of immunoglobulins, also known as the M proteins.¹⁰

MM exists on a spectrum of plasma cell disorders beginning with monoclonal gammopathy of undetermined significance (MGUS), which advances to asymptomatic MM then to symptomatic

MM, and finally to extramedullary or plasma cell leukemia.^{1,2,6} The evolution of MGUS occurs through branching evolution evidenced by numerous mutations and genomic aberrations at both the clonal and subclonal levels, thus contributing to the genomic heterogeneity of MM.¹¹

MM can be generally characterized as either hyperdiploid, having more than the diploid number of chromosomes, or non-hyperdiploid. Using fluorescent in situ hybridization (FISH) and cytogenetics, approximately 50%–60% of MM cases have been identified as non-hyperdiploid and involving an early immunoglobulin heavy chain (IgH) translocation on chromosomes 4, 8, 11, and 16.¹² The remaining percentage of MM cases are characterized as hyperdiploid and most commonly affect chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 as trisomies and chromosomes 13, 14, 16, and 22 as monosomies. Hyperdiploid MM is generally associated with a more favorable outcome.¹³ This genetic information has been incorporated into the Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) and is used in stratifying risk and guiding treatment decisions in patients with MM.⁶

As knowledge of the human genome has continued to grow, methods for identifying and using genes of interest have also evolved. Previously, genetic alterations of interest were identified by the use of cytogenetics, FISH, DNA microarrays, multiparameter flow cytometry (MFC), or gene expression profiling (GEP) (3). A detailed evolution of genomic progress in myeloma genetics and genomics are outlined in Figure 1.

NGS, also phrased high-throughput nucleotide sequencing, has emerged over the past 5 years as a new method for identifying genomic abnormalities. NGS is a collective term for novel technologies

FIGURE 1. Timeline of technological advances in myeloma biomarkers.³

including long-range polymerase chain reaction (PCR), multiplex PCR, microdroplet PCR, whole-exome sequencing (ES), targeted capture, and whole-genome sequencing (WGS). These novel technologies have the capability to selectively enrich regions of interest from the whole genome and identify sequence variants of interest.¹⁴ Even with the hundreds of mutations in MM that have been identified using NGS, identification of a consistent single gene across all occurrences of the disease has yet to occur.³ While NGS has introduced a greater understanding of the genomic alterations associated with MM, it has also provided knowledge of the extensive complexity of the disease. As researchers begin to associate more genomic changes with MM, translation into evidence-based practice remains a challenge. There is currently a large gap in the literature regarding the application of MM genomics into the day-to-day clinical practice.

CONCEPTUAL FRAMEWORK

A framework for the continuum of translation research in genomics was developed to serve as an agenda for moving genomic data from the laboratory to evidence-based practice.⁹ In this framework there are four phases of research, T1 through T4. T0 is an unofficial phase of translation research that exists prior to gene discovery. From the time of gene discovery to health application is considered T1 research. T1 research entails both observational and phase I and II clinical trials. As health application of the findings moves to become an evidence-based practice guideline, phase III clinical trials occur, observational studies continue, and evidence is synthesized to develop guidelines. The T2 phase of research involves the implementation of this evidence-based practice. The next phase in the continuum, T3, takes the evidence from practice guidelines to utilization in health practice. T3 research is characterized by dissemination and implementation research, diffusion research, and phase IV clinical trials. Finally, the T4 phase of translation research aims to advance practice to have an overall population health impact. T4 research uses outcomes research and monitoring of population morbidity, mortality, benefits, and risks. Figure 2 shows a visual representation of the model.^{9,15}

The purpose of this model is to provide a framework to assist in the transitioning of genomic data to public and clinical health application. A mere 3% of research published in the field of genomics was estimated to have reached beyond the T2 phase

FIGURE 2. Continuum of translational (T) research in genomics. Adapted with permission from Khoury et al.⁹

of the continuum.⁹ With the introduction of NGS, the identification of genomic alterations of interest, T0 phase research, has grown significantly. As genes of interest become more readily available, it will become increasingly important for all members of the interdisciplinary healthcare team to assist in the translation of this data from the bench to the bedside. Advanced practice oncology nurses and nurses should have the knowledge to interpret genomic data as well as incorporate their understanding into the care of patients with MM.¹⁶ In addition, advanced practice nurses play a key role in educating patients and empowering them to make informed decisions regarding treatment and involvement in clinical trials.¹⁷ Knowledge and incorporation of genomic data to the day-to-day clinical practice are vital to the nursing profession's role in advancing MM genomic research to application to population health.

A review of the literature was performed to identify where the current research in MM genomics, specifically that using NGS, falls in the continuum of translation research. The objective of this review was to answer the following research questions (1) what is the current state and knowledge of MM genomics, and (2) how is NGS being used to identify genomic data in MM?

METHODS

A systematic review of the literature was conducted, guided by the methods outlined by the

Preferred Reporting Items for Systematic Review and Meta-Analyses: The PRISMA Statement.¹⁸

Search Strategy

The literature was searched using PubMed, Cumulative Index of Nursing and Allied Health Literature (CINAHL), and Web of Science Core Collection. Each database was searched using the medical subject heading term “multiple myeloma” plus the Boolean operator AND combined individually with the following: “next generation sequencing”, “whole genome sequencing”, and “exome sequencing”. Initially 244 articles were retrieved. The results were limited to articles published in the English language and in peer-reviewed journals from 2011–2016, yielding 230 articles.

Articles that reported MM genomic data were eligible for inclusion. In addition to original research, case studies were also included due to the limited amount of randomized control trials (RCTs) on the topic. Articles that had a main focus on other plasma cell abnormalities including plasma cell leukemia (PCL), mantle cell lymphoma (MCL), chronic myeloid leukemia (CML), chronic lymphocytic leu-

kemia (CLL), and MGUS were excluded. Articles that focused on database management of genomic data were also excluded. Although literature reviews were not directly included in the results, ancestry searching was applied and references from the reviews that met all inclusion and exclusion criteria were included. After full text review, a final total of 33 articles were included in the review. Figure 3 provides an overview of the search strategy and corresponding results.

Data Evaluation and Analysis

Data for this review were extracted from the selected articles regarding genomic characteristics of MM as seen in Table 1 by independent reviewers and the authors.¹⁹⁻⁵⁰ A modified version of the Critical Appraisal Skills Programme (CASP) checklist was used in the extraction and evaluation of data from each article. In using CASP, each study was reviewed for its validity, clinical importance of results, and relevance to the question at hand, as well as the framework used to outline the review.

Through the process of data reduction, relevant genomic data were grouped together by genes

FIGURE 3. Search strategy for systematic review.

TABLE 1.
Evidence-based table on studies related to genomic biomarkers for myeloma

Authors	Sample size	Techniques use	Genomic and genetic data	Stage in translation research	Implications for practice
Agirre et al. ¹⁹	n = 158	NGS WGS	SLC15A4, PVT1, STAT5, PAX5, NFATC1, BTF	T0	Discovery of DNA hypermethylation of developmentally regulated enhancers as a mechanism in the pathogenesis of MM.
Barbieri et al. ²⁰	n = 162	NGS FISH	<i>FAM46C</i> , <i>DIS3</i>	T0	<i>FAM46C</i> is involved in the pathogenesis of MM as a potential tumor suppressor.
Amend et al. ²¹	n = 669 (11 mice; 658 human)	WGS WES GWAS NGS	2p23.3, 3p22.1, 7p15.3, SAMS1, Tnfrsf22, Tnfrsf23, Tnfrsf26	T0	Components of SAMS1 pathway appear likely to be involved in myeloma susceptibility and/or disease progression. Multiple cell lineages are involved in MM pathogenesis prior to disease manifestation, independent of tumor cell somatic mutations.
Boneva et al. ²²	n = 50	WGS FISH	Monosomy 13, del X, 1.q21.3, CKS1B, 5p14.3, CDH12, CDH10, 8q24.3, MAPK15, TOP1MT, CYP11B11, ZNF41, ZNF616, ZNF707, ZNF517, 9q34.3, ZNF79, CDK9, SET, 11q13.1, SCYL1, MAP3K11, <i>CCND1</i> , FGF4, FGF3, NUMA, RELT, 19p13.3, TYK2, IL2, DNMT, 21q22, SIK1, 1p13.2, MAGI3, BCL2, 2p11.2, IGL, 6q25.3, IGFR3, 8p21.3, TNFRSF10B, DOCK5, 13q14.11, TNFSF11, RBL, 14q32.33, IGH, VDJ, 16q.24.3, CBFA2T3, 22q11.22, ZNF, PRAME, IGL, Xp22.11, ZBED1, C99, <i>TP53</i> , MAP2K4	T0	aCGH, in combination with IGH/FGFR3 assay can replace FISH as a first line test to confirm MM and could be used cost effectively as a front line assay for MM diagnosis.
Chretien et al. ²³	n = 965	NGS SNP array	t(4;14), del (17p), trisomy 9, trisomy 15, trisomy 19, trisomy 5, trisomy 3, trisomy 11, trisomy 7, trisomy 21, trisomy 18, trisomy 17	T0	Trisomy 3 improved time to progression and trisomies 3 and/or 5 improved overall survival. Trisomy 21 significantly worsened survival. del (17p) and t(4;14) are the 2 major chromosomal changes that impair patient survival. High hyperdiploidy or the presence of at least one trisomy can be regarded as a risk indicator. Patients presenting with high-risk features should have SNP array performed to identify presence of trisomies.

(Continued)

TABLE 1.
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Authors	Sample size	Techniques use	Genomic and genetic data	Stage in translation research	Implications for practice
Gupta et al. ²⁴	n = 1	WGS	t(2;10)(q33.3;p12.2), t(5;11)(q35.3;q23.3), del 1(p34.1;p13.1), FAF1, CDKN2C, <i>CCND1</i> , t(11;14)(q13;q32), <i>TP53</i> , PIK3R1, IGF2BP2, CAMK2D, ASXL3, CCNG2, ZWILCH, MYBL1	T0	Increase in copy number changes associated with tumor progression. Structural variation plays a large role in MM pathogenesis and drug resistance. Disease progression and/or acquisition of drug resistance may be associated with plasmacytic maturation arrest or dedifferentiation to earlier stages of B-cell oncogenesis. Effective management of end-stage MM may necessitate approaches that promote mitotic quiescence or cell cycle exit and terminal plasmacytic differentiation.
Hansmann et al. ²⁵	n = 27	NGS	CD27, CD24, CD38	T0	CD24, CD38, CD27 B cells found in peripheral blood are uniquely expanded in MM patients and associated with active disease. Potential to drive use of peripheral blood to determine status of cancer.
Ikeda et al. ²⁶	n = 1	NGS	SYNE1, EGFR, IKBKB, ERBB3, MYH11, CYLD, <i>TP53</i> , CDH2, t(11;14)(q13;q32), <i>CCND1</i> , IKBKE, CD79B, SYK, 13q14.2	T0	<i>CCND1</i> and RB1 may play roles in the pathobiology of the disease. NGS can allow for prediction of drug resistance and facilitate improvements in the treatment of MM.
Koh et al. ²⁷	n = 1	WES	NEK2, IARS2, OR2T33, PPIG, AOX1, TMEM129, UNC5CL, ARHGEF10, H1FNT, ZIC2, TMX1, PANK2, RBM15, OPTC, OR5H1, MRPL23, FRY, KIAA1199 AP3B2, IL27, KIF1C, ZNF486, ARSE	T0	Only a small subset of nonsynonymous SNV's in cancer are affected by selection. SNV trends can be interpreted as a reflection of the underlying mutational processes.
Lionetti et al. ²⁸	n = 164	NGS	D487V, D487H, T93, H764, F775, R789, R820, R108, R467, S550, A751D, L48_A56el, R467Q, R689Q	T0	<i>DIS3</i> mutations were identified in 18.5% of newly diagnosed MM patients and is considered to be a potential tumor suppressor in MM.
Lionetti et al. ²⁹	n = 167	NGS	<i>BRAF</i> V600E, E586K, G596R, K601N, K601T, D594N, D594G, D594E, <i>NRAS</i> , <i>KRAS</i>	T0	<i>BRAF</i> mutations were identified in 12% of the study population. Genetic alterations in ERK signaling was identified in 57% of the study population. Provides rationale for <i>BRAF</i> -targeted therapeutic strategies.
Lionetti et al. ³⁰	n = 151	NGS	I195, R273, P278, R248, E285, <i>DIS3</i> , <i>NRAS</i> , <i>FAM46C</i>	T0	Confirmation that <i>TP53</i> mutations are rare at MM diagnosis and represent tumor progression and deletion of the locus.

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TABLE 1.
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Authors	Sample size	Techniques use	Genomic and genetic data	Stage in translation research	Implications for practice
Melchor et al. ¹¹	n = 6	ES	<i>ATM</i> , <i>KLK8</i> , <i>GMEB1</i> , <i>POLE</i> , <i>KRAS</i> , <i>BRAF</i> , <i>STK24</i> , <i>ACAD10</i> , <i>NRAS</i> , <i>ABCA4</i> , <i>FAT</i> , <i>IRF4</i> , <i>EGR1</i> , <i>TP53</i> , <i>DNAH14</i> , <i>FAM47C</i>	T0	Clonal remnants from the earliest stage of MM may persist late in the myeloma natural history. Evidence for parallel evolution at the single cell level: <i>RAS</i> /MAPK genetic pathway was altered more than once in the same tumor but divergent clones. Suggests <i>RAS</i> mutations as true driver mutations in myeloma, thus warranting targeted treatment. Recurrent <i>IRF4</i> mutations significantly display <i>KRAS</i> / <i>NRAS</i> -activating mutations. Provide evidence to support biopsy-based prognosis and targeted therapy strategies.
Peterson et al. ³¹	n = 1	ES	<i>ARHGAP26</i> , <i>ARID1A</i> , <i>ER884</i> , <i>PARP1</i> , <i>ATM</i> , <i>NRAS</i> , <i>ESR1</i> , <i>FOXO3</i> , <i>JAK3</i> , <i>NTRK3</i> , <i>PMS2P3</i> , <i>POLK</i> , <i>CD36</i> , <i>KRAS</i> , <i>POLE</i>	T0	The use of CloneViz as a novel bioinformatics approach to visualize, quantify, and analyze variant and mutational dynamics from ES data. In each serial MM sample from this case, a <i>RAS</i> gene species (<i>NRAS</i> , <i>KRAS</i>) with a hotspot mutation was present. This can drive potential treatment options as new drugs have been developed to target the MAPK pathway.
Peterson et al. ³²	n = 1	ES	<i>MYC</i> , <i>MTOR</i>	T0	Indication for targeted therapy for <i>MYC</i> and <i>MTOR</i> . <i>MYC</i> oncogene species with VUS missense mutation was present in all serial MM samples. Implication to collect tissue sample at diagnosis and relapse to guide therapy.
Thiele et al. ³³	n = 12	NGS	n/a	T0	Clonotypic B cells are consistently absent in blood of myeloma patients. Malignant plasma cells circulate in the peripheral blood and provide evidence for clonal evolution possibly contributing to spread of the disease.
Vij et al. ³⁴	n = 60	NGS	<i>CD45</i> , <i>CD38</i>	T0	MM clones were detected at 98% in the peripheral blood mononuclear cells and cell-free compartments using DNA and RNA assays. Presents a case for monitoring minimal residual disease using peripheral blood samples.

(Continued)

TABLE 1.
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Authors	Sample size	Techniques use	Genomic and genetic data	Stage in translation research	Implications for practice
Walker et al. ³⁵	n = 5	FISH WGS	RUNX2, BRCA2, UNC5D	T0	Intraclonal heterogeneity is present at early stage of the disease development. Hypothesize that adaptation of the myeloma cells is driven by genetic variation and can experience selective pressure naturally, via the bone marrow environment, or from exposure to treatment.
Walker et al. ³⁶	n = 463	ES	APOBEC, MAF, MAFB, IGH, IGL, <i>FAM46C</i> , IGK, FOXO3, BMP6, RB1, XBP1, TXNDC5, CCND3, <i>CCND1</i> , FGFR3	T0	Translocations involving MYC are the most common structural chromosomal abnormalities in MM. APOBEC mutations are associated with a poor prognosis linked to a high mutation load and t(14;16) and t(14;20) translocation subgroups.
Walker et al. ³⁷	n = 22	ES	<i>KRAS</i> , <i>NRAS</i> , MYC, CYLD, <i>FAM46C</i> , PTPRG, FGFR3, ICAM1, LGMN, BMP2K, BCL6, ROBO1, FLT3, <i>DIS3</i> , R750K, ABCA13, DNAH5, PRKD2, ACTG1, EFNB2, F8, LRR1Q1, PCLO, PLD1, SEC31A, SSPO, KDM6A, ACTL7B, ASXL1, CHAF1B, ESCO1, KDM2A, KDM4B, KDM6A, MLL, MLL2, MLL5, MYST1, PCGF5, SETD1B, SETD6, TOX, DNAH2, DNAH3, DNAH10, DNAH11, DYNC2H1, TXNDC2, CCDC154, WWC1, TRPS1, RPRG, ODF1, ARHGEF10, ARHGEF6, ARL2BP, MAP3K4, MAPKBP1, RALGAPA2, RANGAP1, RASA4, RASGRF2, SPRED2, PRDM1, PAX2, PAX7IL6R, IRF4, IL10RA, BCL6, MAX, MALT1, BMP2K, CARD6, TNIP1, IRF5, MK167, LTB, ATR, CCAR1, CDC14B, PTPLAD1, PTPN21, PTPRU, <i>TP53</i> , TACC2, FAT1, FAT4	T0	t(4;14) samples at presentation have an increased mutational load compared to t(11;14) samples. t(11;14) group showed enrichment for protein phosphorylation and Ras signaling. RAS pathway mechanisms were present in both t(4;14) and t(11;14) samples.
Walker et al. ³⁸	n = 1	FISH NGS	t(7;14), EGFR	T0	Treatment of MM can target EGFR using HER-inhibiting antibodies.

(Continued)

TABLE 1.
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Authors	Sample size	Techniques use	Genomic and genetic data	Stage in translation research	Implications for practice
Weissbach et al. ³⁹	n = 81	NGS FISH	<i>DIS3</i> , D488N, E665K, R780K, T131I, P412L, D784H, t(11;14), t(4;14), t(14;16), t(8;14), t(14;20), del 13q14, del 17p13, gain 1q21, gain 9q34	T0	<p>DI53 mutations are equally distributed along PIN, CSD2, RNB, and S1 domains.</p> <p>Slight trend towards shorter median OS of MM patients carrying <i>DIS3</i> mutations.</p> <p><i>DIS3</i> mutations in minor subclones were significantly associated with an inferior response to chemotherapy as compared to <i>DIS3</i> mutations in major subclones.</p>
Weston-Bell et al. ⁴⁰	n = 1	NGS	CSMD3, FAT1, CELF2, KIAA1274, CSMD3, AKAP9, PCDHGA6, C6orf81, NPR3, WDR73, ELMOD1, ASB15, COL4A3, GAS2, SUB1, SPATA6	T0	<p>Tumor clone should be carefully analyzed after each phase of treatment.</p> <p>Multi-target combination therapy should be used to eradicate genetically variant subclones.</p>
Andrulis et al. ⁴¹	n = 379 (n = 251 specific to MM)	NGS multiplex PCR	<i>BRAF</i> V600E, <i>KRAS</i> , SF3B1	T1	<p>Activation of <i>BRAF</i> V600E association with more aggressive form of MM and decreased overall survival.</p> <p>Pharmacologic inhibition via the mutation specific <i>BRAF</i> inhibitor, vemurafenib, can result in disease remission and impressive clinical benefit for patients with refractory MM.</p>
Bolli et al. ⁴²	n = 84	WES FISH	t(4;14), t(11;14), t(14;16), del (1p), (+1q), del (12p), del (13), del (14q), del (16q), del (17p), FGFR3, MMSET, <i>KRAS</i> , <i>BRAF</i> , PD, <i>CCND1</i> , <i>NRAS</i> , NF1, RASA2, <i>FAM46C</i> , <i>TP53</i> , SP140, LTB, ROBO1, ERG1, FAT3, TGDS, SNX7, 1P21.3, PEG3, USP29, NFKB1, NFKBIA, CYLD, TRAF3, RAG2, CD79A, SF3B1, ARID2, PIK3CA, PTEN, KDM6A, CDK4, CDKN2C, SETD2	T1	<p>Confirmation of previous reports showing subclonal <i>KRAS</i>, <i>NRAS</i> and <i>BRAF</i> mutations are observed in nearly one third of patients. This is relevant for the consideration of treatment options targeting the MAPK pathway.</p> <p>First finding of concurrent <i>BRAF</i>, <i>NRAS</i>, or <i>KRAS</i> mutations in a single patient.</p> <p>Confirmation that <i>FAM46C</i> is recurrently inactivated in MM and is associated with the hyperdiploid subgroup of MM.</p> <p>Suggests that SP140 mutations may have prognostic features for MM.</p> <p>Heterogeneous subclonal structure and mutational repertoire of MM is generated by a minimum of two mutational processes.</p> <p>The ability of MM tumors to grow independently of the bone marrow environment is gained through significant mutations of the plasma cells.</p> <p>Cases with a significantly higher number of mutations had worse relapse-free and overall survival.</p> <p>Therapeutic interventions should target more than one single genomic anomaly.</p>

(Continued)

TABLE 1.
(Continued)

Authors	Sample size	Techniques use	Genomic and genetic data	Stage in translation research	Implications for practice
Egan et al. ⁴³	n = 26	WGS aCGH RNA seq.	aCGH del 1(p13:2-34-2), monosomy 13, monosomy X WGS/WES/RNA t(14;16), <i>KRAS</i> , <i>PIK3CA</i> , <i>ATM</i> , <i>NFKB2</i> , <i>CRBN</i> : (Q99* and R283K), <i>PSMG2</i> (E171K), <i>NR3C1</i> (G369A), <i>KRAS</i> (G12C), <i>ATM</i> (T19851)	T1	Mutations in <i>CRBN</i> , <i>PSMG2</i> , and <i>NR3C1</i> were identified and have been shown to suggest causality of drug-refractory disease. <i>KRAS</i> and <i>ATM</i> mutations affect downstream signaling of MEK pathway; MEK inhibitors as therapeutic option.
Egan et al. ⁴⁴	n = 1	WGS aCGH FISH	<i>AFF1</i> , <i>ATXN1</i> , <i>CNGA3</i> , <i>COL2A1</i> , <i>CSMD3</i> , <i>KRT9</i> , <i>LRRRC4C</i> , <i>MAG11</i> , <i>MYPN</i> , <i>RNF145</i> , <i>C12orf42</i> , <i>PCDH7</i> , <i>PTPRD</i> , <i>PPFIBP1</i> , <i>RB1</i> , <i>ZSCAN3</i> , <i>TNN</i> , <i>TUBB8</i> , <i>ZNF521</i> , <i>PDE4DIP</i> , <i>DOK5</i> , <i>PARD3B</i> , <i>ZNF557</i> , <i>CACNA1S</i> , <i>DSC1</i> , <i>TLR9</i> , <i>MLLT11</i> , <i>NOTCH2NL</i> , <i>HOXA9</i> , del13	T1	Damaging mutation in <i>AFF1</i> could result in dysregulation of <i>HOXA9</i> , contributing to myelomagenesis. Absence of <i>ZKSCAN3</i> protein and loss of <i>RB1</i> shown to be associated with dysregulation of the G _i /S cell-cycle checkpoint. Combination of mutations in <i>ZKSCAN</i> , <i>RB1</i> , deletion of <i>TP53</i> , and mutation in <i>AFF1</i> could lead to transformation of myeloma to sPCL. First research to study myeloma over time – demonstrates clonal heterogeneity.
Kortum et al. ⁴⁵	n = 25	NGS FISH Targeted Sequencing	<i>KRAS</i> , <i>TP53</i> , <i>DIS3</i> , <i>FAM46C</i> , <i>SP140</i> , <i>FAT1</i> , <i>SPEN</i> , <i>PRDM1</i> , <i>NEB</i> , <i>ANK2</i> , <i>ATM</i> , <i>XBP1</i>	T1	Use of M ³ P as a targeted sequencing panel for MM is able to characterize mutational tumor profiles, identify clonal heterogeneity, and track successful clonal evolution over time.
Leich et al. ⁴⁶	n = 49	WES Microarray Analysis	<i>KRAS</i> , <i>FAM46C</i> , <i>WHSC1</i> , <i>DIS3</i> , <i>LRP1B</i> , <i>PKHD1</i> , <i>NRAS</i> , <i>ALOX12B</i> , <i>IGF1R</i> , <i>EGFR</i> , <i>ERBB3</i> , <i>NRTK1</i> , <i>NRTK2</i> , <i>BRAF</i> , <i>MAP2K2</i> , <i>AKAP1</i> , <i>IKBKE</i> , <i>STAM2</i> , <i>GRK2</i> , <i>ADRBK1</i> , <i>SDK1</i> , <i>LRFN5</i> , <i>THBS1</i> , <i>SLAMF1</i> , <i>VCAN</i> , <i>ALOX12B</i> , <i>NCAM2</i> , <i>TGFBR2</i> , <i>EPHB2</i> , <i>EPHA8</i> , <i>HK3</i> , <i>STAM2</i> , <i>ITGA6</i> , <i>ITGA7</i> , <i>ITGB5</i> , <i>CDH23</i> , <i>CDH18</i> , <i>CDH9</i> , <i>PCDH9</i> , <i>PCDHGA3</i>	T1	Neoplastic cells in MM are affected by multiple and heterogeneous somatic mutations in adhesion and RTK associated signaling molecules. Evidence for the development of therapeutic efforts to more selectively target the interaction between MM cells and their surrounding stroma. Receptor tyrosine kinase (RTK-) associated molecules have a role in MM pathogenesis and may represent targets for potential novel therapies.

(Continued)

TABLE 1.
(Continued)

Authors	Sample size	Techniques use	Genomic and genetic data	Stage in translation research	Implications for practice
O'Donnell et al. ⁴⁷	n = 67	NGS multiplex PCR	<i>KRAS</i> , <i>NRAS</i> , <i>BRAF</i> , <i>TP53</i> , <i>HRAS</i>	T1	Evidence for the use of SNaPshot, a mutation detection protocol that uses multiplex PCR, as a way to integrate rapid genomic analysis into clinical practice.
Rustad et al. ⁴⁸	n = 209	allele-specific RT-PCR ES	<i>BRAF</i> V600E, <i>NRAS</i> , <i>KRAS</i> , <i>ATXN1</i> , <i>HCFC2</i> , <i>IQSEQ3</i> , <i>PCMTD1</i> , <i>PNRC2</i>	T1	<i>BRAF</i> V600E was detected by AS-PCR in 5.3% of the population. Creatinine was significantly higher in patients with the <i>BRAF</i> V600E group. No significant difference in progression free survival or outcome survival between <i>BRAF</i> V600E positive and negative groups. V600E-positive cells are sensitive to broad acting drugs in most cases.
Walker et al. ⁴⁹	n = 463	ES	<i>IRF4</i> , <i>KRAS</i> , <i>NRAS</i> , <i>MAX</i> , <i>HIST1H1E</i> , <i>RB1</i> , <i>EGR1</i> , <i>TP53</i> , <i>TRAF3</i> , <i>FAM46C</i> , <i>DIS3</i> , <i>BRAF</i> , <i>LTB</i> , <i>CYLD</i> , <i>FGFR3</i> , <i>CCND1</i> , <i>ATM</i> , <i>ATR</i> , <i>ZNFHX4</i>	T1	Establish that MM is driven by RAS pathway mutations and by MYC translocations. Provide data that suggest DNA repair pathway alterations may not benefit from alkylating agents for treatment. The greater the number of adverse copy number and structural abnormalities including t(4;14), del (17p), MYC, translocations, and amp (1q), the worse the outcome. Combined novel mutation risk factors with the recurrent molecular adverse features and international staging system to generate an international staging system mutation score.
Yang et al. ⁵⁰	n = 3	WGS	t(4;14), t(14;16), <i>MAF</i> , <i>CDKN2A</i> , <i>TRAF3</i> , <i>KDM6A</i> , <i>KCTD8</i> , <i>ABHD17B</i>	T1	SVfinder can be applied to MM WGS data to recover novel structural variations.

of interest and the technique used for identification. A data display matrix was also created to organize the results into a table containing the categories of: author and year, sample size, genomic data, techniques used, stage of translation research, and implications for practice. The data matrix was then carefully analyzed to identify comparisons, contrasts, and trends in the results. Visual charts were created to assist in providing clarification and easy identification of the patterns and relationships between genomic data, the sequencing technique used, and the stage of translation research. Conclusions were explicitly drawn from these findings. Interpretation of the results was reviewed for any bias or confounding influences. Finally, overall results and conclusions were grouped together to form an integrated summation of MM genomics in regards to both the sequencing techniques used and phase of translation research.

RESULTS

Genomic data presented in the review addressed the pathogenesis, diagnosis, prognosis, and treatment of MM. While a majority of the studies were classified as T0 research, several showed application of genomic data from NGS and were classified as T1 research. Overall, the state of MM genomics remains heavily in the T0 phase of research in identifying driving genomic alterations in the disease process. Further research is necessary to confirm or rule out the hundreds of identified genomic alterations and their role in the pathogenesis, development, and treatment of MM.

MM Genomic Data

Unanimously, all of the articles provided verification of the clonal and subclonal heterogeneity of MM. Table 2 shows the most commonly reported genomic changes in this review. Among those most commonly reported are: *KRAS*, *NRAS*, *TP53*, *FAM46C*, *BRAF*, *DIS3*, *ATM*, and *CCND1*.

Pathogenesis and Diagnosis of MM

With the introduction of NGS, many questioned its use as an effective tool in identifying already known genetic and genomic alterations related to MM. WGS was shown to be an effective front line assay for MM diagnosis in comparison to the use of FISH alone.²² Single-nucleotide polymorphism array was also used as a tool for identifying the presence of trisomies in MM.²³ While no other studies specifically looked at a comparison between older sequencing techniques, many reported confirmations of the commonly known genomic alterations such as *KRAS*, *NRAS*, *BRAF*, *TP53*, *DIS3*, and *FAM46C*.

Another proposed method for diagnosis and management of MM is the use of peripheral blood samples for NGS. Peripheral blood samples from patients with MM were used in NGS to identify alterations in B cells, providing support for this form of monitoring patients with MM.^{25,34} In a much smaller study, clonotypic B cells were reported as consistently absent in peripheral blood and this method should not be used to monitor disease progression of MM.³³ The use of peripheral blood samples for monitoring MM currently does not have significant evidence to be applied to health practice.

Trends in genomic changes found in MM can be interpreted as a reflection of the underlying

TABLE 2.
Frequency and cumulative percentages of the most commonly reported genomic changes in myeloma

Frequency of detection	No. of genomic changes	Genomic changes	Cumulative percent
3.00	5	EGFR, IGL, IRF4, LTB, TRAF3	0.91%
4.00	9	CSMD3, CYLD, FAT1, FGFR3, KDM6A, RB1, t(11;14), t(14;16), t(4;14)	1.6%
6.00	2	ATM, CCND1	0.36%
7.00	1	DIS3	0.18%
8.00	1	BRAF	0.18%
9.00	2	TP53, FAM46C	0.36%
11.00	1	NRAS	0.18%
13.00	1	KRAS	0.18%
Total	22/548		4.0%/100%

mutational processes.²⁷ Multiple studies found associations between genomic changes and the pathogenesis of MM including DNA hypermethylation of developmentally regulated enhancers,¹⁹ *FAM46C* as a potential tumor suppressor,²⁰ components of the SAMSN1 pathway,²¹ and mutations affecting *CCND1* and *RB1*.²⁶

Prognosis of MM

Various genomic alterations have also been associated with prognosis in MM. A combination of NGS and FISH to analyze the *DIS3* mutation in MM revealed patients carrying the mutation had a slightly shorter median of outcome survival. More specifically, patients with *DIS3* mutations in minor subclones were associated with an inferior response to chemotherapy as opposed to those with *DIS3* mutations in major subclones.³⁹ *DIS3* has also been identified as a potential tumor suppressor in MM.²⁸ APOBEC mutations have also been associated with a poor prognosis as these mutations are often linked to the t(14; 16) and t(14; 20) subgroups.³⁷ Some genomic changes are even isolated to progressed forms of MM such as *TP53*.³⁰

The role of *BRAF* V600E as a prognostic biomarker for MM remains controversial. A 2013 study reported an association between activation of this gene to a more aggressive form of MM and ultimately a poorer overall survival.⁴¹ A more recent study used a combination of allele-specific PCR and ES to determine that only 5.3% of their study population harbored the *BRAF* V600E mutation. This study reported no significant difference in the progression-free or outcome survival for patients with this mutation.⁴⁸ These results warrant further investigation into the role of *BRAF* and the mitogen-activated protein kinase (MAPK) signaling pathway in MM and how the genomic heterogeneity of the disease can affect prognosis and eventually treatment.

Treatment of MM

Many pathways and specific mutations were also identified as potential areas for targeted novel therapies. Mutations in epidermal growth factor receptor (EGFR) can be targeted with therapy using HER-inhibiting antibodies.³⁶ Genomic alterations such as *MYC* and *MOTOR* are also indications for targeted therapy.³²

BRAF V600E was also reported to have potential therapeutic indications with the use of *BRAF* inhibitors, such as vemurafenib, resulting in both

disease remission and clinical benefits for patients with the mutation.⁴¹ The rationale for the use of *BRAF*-targeted therapeutic strategies was confirmed in another study as 12% of their subjects harbored a *BRAF* mutation, and 57% of the study population had a broader genetic alteration in the MAPK, specifically ERK, signaling pathway.²⁹ Multiple other studies provided genomic support for the development and use of novel therapeutics targeting the MAPK pathway.^{11,31,42,49} In contrast, it has also been reported that MM cells that are positive for the *BRAF* V600E mutation were sensitive to broad-acting drugs in most cases, not warranting a targeted therapeutic.⁴⁸

NGS can be used to identify genomic alterations associated with the acquisition of drug resistance and to facilitate improvements in the overall treatment of MM.^{24,26}

PHASES OF TRANSLATION RESEARCH IN GENOMICS

Nearly 70% (23/33) of the studies in this analysis were classified in the T0 phase of translation research. A majority of genomics research remains in the T0 stage with the primary goal of identifying genes and/or genomic data of interest. Many of these studies provided possible implications for practice in their conclusions, but did not show application of the genomic data to practice. Figure 4 shows a comparison of the phases of translation research for each study using NGS, WGS, and ES individually and collectively. For the purposes of this review, studies that exclusively used ES or WGS were separately grouped. Studies that used either a mixture of WGS, ES, and other NGS techniques or simply used other NGS techniques were labeled NGS. Forty percent of studies using ES, 20% of studies using NGS, and 37.5% of studies using WGS were classified in the T1 phase of research.

Exome sequencing has been used to identify RAS pathway mutations and *MYC* translocations as driving variants in MM and concluded that structural abnormalities such as t(4; 14), Del (17p), *MYC* translocations, and amp (1q) are associated with a poor outcome in MM.³⁸ UK researchers in collaboration with US, French, and Italian researchers among others used their findings in combination with the recurrent molecular adverse features in MM and the international staging system to create

FIGURE 4. (A) Phase of translation research related to sequencing methods of studies. (B) Comparison of phase of translation research among sequencing methods. ES = exome sequencing; NGS = next-generation sequencing; WGS = whole-genome sequencing.

an international staging system mutation score. Another application of findings from ES confirmed previous reports showing subclonal *KRAS*, *NRAS*, and *BRAF* mutations are present in nearly one third of patients with MM.⁴² British researchers were the first group to discover concurrent mutations of *BRAF* and *NRAS* or *KRAS* in a single patient. Additionally, the researchers identified *FAM46C* and *SP140* genomic alterations to have prognostic features for MM. They concluded that cases with a higher number of genomic mutations had a worse relapse-free and overall survival.⁴²

ES has also provided evidence for targeted novel therapeutics. One study identified mutations in the adhesion- and receptor tyrosine kinase (RTK)-associated signaling molecules in patients with MM. This finding provides implications for therapeutic efforts to be targeted towards the interaction of MM

cells and their surrounding stroma as nearly 50% of the patients in the study had mutations in these networks.⁴⁶

Application of genomic knowledge gained from NGS to practice was used in the creation of SNaPshot, a mutation detection protocol that uses multiplex PCR. The goal of SNaPshot is to provide a protocol that allows for rapid integration of genomic analysis into practice. In this study, the protocol used was able to identify key genomic alterations such as *KRAS*, *NRAS*, *BRAF*, *TP53*, and *HRAS*.⁴⁷ Application of NGS was also seen in the use of M³P, a targeted sequencing panel, used to characterize mutational tumor profiles, identify clonal heterogeneity, and track clonal evolution over time.⁴⁵ The use of protocols and the development of sequencing profiles such as these suggest further application in clinical practice.

Application of WGS was seen in the use of SVfinder, a method for identifying key driver mutations in MM. SVfinder and WGS were used to identify the following novel structural variations: t(4;14), t(14;16), MAF, *CDKN2A*, and *TRAF3*.⁵⁰ Another example of WGS health application was seen in the first research studying myeloma over time. This case study identified that combinations in mutations of *ZKSCAN*, *RB1*, *AFF1*, and deletion of *TP53* could lead to the transformation of MM to plasma cell leukemia.⁴⁴ This was one of the first studies to confirm the presence of clonal heterogeneity in MM. The genomic knowledge from WGS can also be applied to therapeutic options in MM. Mutations in *CRBN*, *PSMG2*, and *NR3C1* have been associated with potential causality of drug-refractory disease,⁴³ thus proposing a possible biomarker for progression or relapse of MM. In addition, the researchers suggested MEK inhibitors as therapeutic options for patients with MM due to the common mutations seen in *KRAS* and *ATM*, which affect the downstream signaling of the MEK pathway. T1 research was seen in application to risk stratification, development of sequencing protocol, identification of progression markers, and the use of novel therapeutic agents.

DISCUSSION

NGS, WGS, and ES have given new insight to the complexity of the intraclonal heterogeneity of MM. Hundreds of new genes have been identified through the use of NGS, yet only a handful have been established as markers to be used in the diagnosis, prognosis, and treatment for MM. With the

enormous output of genomic information, scientists and clinicians will need to work together to identify the significant driver mutations among the thousands of passenger mutations. As the number of known driver mutations continues to grow, clinicians should be aware of the implications for prognosis and treatment. The results discussed in this review provide multiple implications for further research involving larger sample sizes focused on the genomic variation in MM.

It is now well understood that a higher number of genomic mutations is associated with poorer relapse-free and overall survival. Sampling of the bone marrow should occur throughout therapy as the intracлонаl heterogeneity has been shown to be present at each stage in MM.^{11,31,32} For this reason, many researchers have suggested the use of multi-targeted treatments to eradicate the variant clones and subclones present in MM.⁴⁰

Certain genomic changes have already been associated with progression-free survival, outcome survival, and as potential targets for novel therapeutic agents. As research in MM genomics continues to grow, integration into diagnostic criteria, risk stratification guidelines, and the development and use of novel therapeutic agents, will assist in the continued movement of knowledge from the bench to the bedside.

Due to the extensive nature of this topic, there is a possibility for missing data. Additionally, the search strategy used for this review could have limited the

amount of available research on the topic. Inclusion of case studies in the review provided data that is neither high quality or generalizable to populations. Finally, inclusion of and interpretation of articles for this review may be skewed as the process was completed by a single reviewer.

There is still much to be learned about the genomic nature of MM. It is important for clinicians to not only stay current on the advances in MM genomics but also integrate them into day-to-day clinical practice. Clinicians should also provide education to patients on the recent advances in MM genomics and discuss how the data are being used in treatment decision-making. Finally, clinicians can assist patients in gathering genomics-related information so that they can become active participants in the shared decision-making process.

The translation of myeloma genomic research findings into current practice remains a major challenge in contemporary hematology practice. Genomic testing of myeloma cells is not accessible and widely available to some community-based and rural hematology practice. Genomic testing is not considered a standard of care for myeloma for this reason.⁵¹ Moreover, oncology nurses still need to develop competencies (knowledge, skills, and attitudes) on myeloma genomics in order to provide accurate interpretation of the prognostic significance of poor genomic profiles (eg, deletion 17p, t(14;16), t(14;20), hypodiploidy, etc) to patients and their caregivers.

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