SOHO Supplement 2016

Clonal Evolution in Multiple Myeloma

Bita Fakhri, Ravi Vij

Abstract

Multiple myeloma (MM) is the second most common hematologic malignancy encountered among patients in the United States. The last decade has seen incremental improvements in the survival of patients with MM. These advances are, to a large extent, attributable to the addition of proteasome inhibitors and immunomodulatory drugs to the armamentarium of treatment options. The adoption of these drug classes was the result of an empiric research paradigm. However, with the application of next generation sequencing technologies, we are now starting to unravel the genomic landscape of MM. It is hoped that this will allow us to better disentangle the biology of the disease and allow for identification of new therapeutic targets. In this article, we review what we have learned to date about the mutational profile, clonal architecture, and evolution of the disease, and discuss the potential clinical implications of these findings.

Clinical Lymphoma, Myeloma & Leukemia, Vol. 16, No. S1, S130-4 Published by Elsevier Inc.
Keywords: Clonal evolution, Genomics, Multiple myeloma, Mutational landscape, Personalized medicine

The Mutational Landscape in Multiple Myeloma (MM)

Conventional karyotyping and fluorescent-in-situ hybridization (FISH) studies have shown that about 40% of cases of MM are characterized by chromosome translocations resulting in overexpression of genes translocated to the vicinity of the immunoglobulin heavy chain locus.[1] Another common finding on chromosomal analysis is hyperdiploidy. However, these are most likely not the critical events in the development of the malignant phenotype and progression to MM, as evidenced by their presence in the precursor steps in clinical evolution: monoclonal gammopathy of undetermined significance and smoldering myeloma.

Investigators have recently reported on results of whole-genome sequencing (WGS) and whole-exome sequencing (WES) using paired analyses of somatic tumor and genomic DNA to distinguish polymorphisms and characterize the mutational profile of MM (Table 1).

Chapman et al were the first to report on the results of next generation sequencing in samples of patients with MM. The investigators analyzed 38 multiple myeloma patients (WGS in 23 patients and WES in 15 patients).[2] They identified that the median number of mutations per genome was ~ 55 to 60 (range, 21-488 mutations). Apart from mutations in KRAS and NRAS, none of the 10 most commonly detected mutations were recurrent in more than 10% of patients. The spectrum of mutations included genes involved in protein translation, histone methylation, and homeostasis. Mutations were identified in 11 members of the NF-κB pathway. BRAF mutations were detected in 4% of patients, a finding with possible clinical implications, given the availability of BRAF inhibitors.[2]

Subsequently, Lohr et al reported on parallel sequencing of 203 tumor-normal pairs from patients with MM.[3] This study was designed to address some of the limitations of the report from Chapman et al by increasing the sample size and examining copy number alterations in addition to significantly mutated genes. Of 203 tumor-normal pairs, WES was performed on 177 and WGS on 26. Eleven significantly mutated genes were identified in this study, 5 of which had previously been described by Chapman et al. Similar to the pilot analysis of the MM genome study,[2] mutations of genes in the NF-κB pathway, histone modification, and the coagulation cascade retained significance across all 203 patients.[3]

Bolli et al analyzed 84 samples from 67 patients using WGS and single nucleotide polymorphism.[4] Seven genes were found to be recurrently mutated, including known KRAS and NRAS mutations present in 32 of 67 patients, BRAF mutations present in 10 of 67 patients, FAM46C found in 8 of 67 patients, and TP53 mutations present in 10 of 67 patients. Only 3 of 10 BRAF mutations were found to be the classic V600E, an atypical feature compared with melanoma and colorectal cancers. FAM46C mutations were statistically associated with a hyperdiploid karyotype. TP53 mutations were significantly enriched in patients with del(17p). In addition to these genes that have previously been described by other authors,[2,3]

Division of Hematology and Oncology, Washington University School of Medicine, St Louis, MO

Submitted: Feb 9, 2016; Accepted: Feb 9, 2016

Address for correspondence: Dr Ravi Vij, MD, MBA, Section of Stem Cell Transplant and Leukemia, Division of Medical Oncology, Campus Box 8007, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110
E-mail contact: rvij@im.wustl.edu

2152-2650/$ - see frontmatter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.clml.2016.02.025

Table 1 Summary of Landmark Studies Elaborating on the Mutational Landscape of MM

Author (Year)	Sample Size	Sequencing Method	Identified Genes	Clinical Implication	Study Summary
Chapman (2011)	38	WGS (23) WES (15)	NRAS KRAS FAM46C DIS3 TP53 CCND1 PNRC1 ALOX12B HLA-A MAGED1	MEK inhibitor MEK inhibitor Pablociclib	• Genes involved in: protein translation, histone methylation, and blood coagulation • A significant role for NF-κB signaling indicated. • Activating mutations of kinase BRAF in 4% of patients.
Bolli (2014)	84 samples from 67 patients	WES	NRAS KRAS FAM46C TP53 BRAF SP140 LTB	MEK inhibitor MEK inhibitor Vemurafenib	• WES, copy number profiling, and cytogenetics to analyze 84 myeloma samples. • Four patterns of clonal evolution identified: unchanged, linear, differential clonal response, and branching. • New candidate genes SP140, LTB, ROBO1, and EGR1 identified.
Lohr (2014)	203	WGS (26) WES (177)	KRAS (23%) NRAS (20%) TP53 (8%) DIS3 (11%) FAM46C (11%) BRAF (6%) TRAF3 (5%) RB1 (3%) CYLD (2%) PRDM1 (5%) ACTG1 (2%)	MEK inhibitor MEK inhibitor Vemurafenib	• Identified subclonal mutations • Estimated the cancer cell fraction that these mutations occur • Estimated the minimum number of subclones
Walker (2015)	463	WES	KRAS NRAS TP53 DIS3 FAM46C TRAF3 BRAF RB1 CYLD IRF4 MAX HIST1H1E EGR1 LTB FGFR3	MEK inhibitor MEK inhibitor Vemurafenib Masitinib	• The mutational spectrum dominated by RAS (43%) and NF-κB (17%) pathways • Mutations in CCND1 and DNA repair pathway alteration (TP53, ATM, ATR, ZNFHX4) associated with worse survival outcomes. • Mutations in IRF4 and EGR1 associated with favorable outcomes. • First study to combine mutation risk factors with molecular adverse features and ISS for more accurate prognostication

Abbreviations: ISS = International Staging System; MM = multiple myeloma; WES = whole exome sequencing; WGS = whole genome sequencing.

Bollie et al described additional genes mutated at a significantly recurrent rate, including SP140, a lymphoid-restricted homologue of SP100 that codes for a nuclear body protein implicated in antigen response of mature B cells,[5] and LTB, a type II membrane protein of the tumor necrosis factor family involved in lymphoid development.[6] These 2 new genes could be considered as novel candidate drivers in MM.[4]

Another study was conducted by Walker et al, in which WES was performed for 463 patients enrolled in the National Cancer Research Institute Myeloma XI trial.[7] Fifteen significantly mutated genes were identified, as summarized in Table 1. The mutational spectrum was dominated by mutations in RAS (43% [KRAS, 21.2%; NRAS, 19.4%; BRAF, 6.7%]) and NF-κB pathways (17%). This study also examined the association of mutated genes with cytogenetic subgroups. FGFR3 was found to be mutated solely in the t(4; 14) group, CCND1 in the t(11; 14) subgroup, and the transcriptional regulator EGR1 mutation in the hyperdiploid samples.[7]

Intraclonal Heterogeneity

MM has long been suspected to harbor intraclonal heterogeneity based on several observations: biclonal disease on protein electrophoresis; copy number and structural abnormalities detectable only in a subset of purified multiple myeloma cells; discordant therapeutic response between bone marrow and extramedullary disease; and the phenomenon of free light chain (FLC) escape. The clinical relevance of light chain escape was studied in a series of 647 patients enrolled on the Medical Research Council Myeloma IX trial. Among patients who relapsed on the trial, 35% had a measurable increase in both paraprotein and FLC levels (PLC), 50% had a significant increase in the level of paraprotein only (PO), and in 10%, the relapse was characterized by an increase in FLC only