Introduction

Myeloma is molecularly and phenotypically heterogeneous with some patients experiencing long periods of remission or functional cures while others relapse early or are refractory to current therapies. In order to improve outcomes, further information regarding the genetic abnormalities driving these differences is required. Technologies to analyze the molecular profile of myeloma have evolved significantly over the last few decades. Conventional cytogenetics identified translocations involving the immunoglobulin heavy chain (IGH) gene locus and hyperdiploidy as myeloma initiating events. These events occur in post germinal center B cells and underlie the development of MGUS, now understood to precede all cases of myeloma. Translocations including t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20) lead to the upregulation of MMSET/FGFR3, CCND3, CCND1, MAF and MAFB respectively, all of which ultimately drive G1/S checkpoint dysregulation. Hyperdiploidy, characterized by trisomies of odd numbered chromosomes also affects this checkpoint driving uncontrolled proliferation. Karyotyping also identified secondary lesions associated with disease progression including large segments of chromosomal loss or gain, such as del(13q). Such lesions, as well as smaller regions of loss or gain, are now routinely identified using interphase fluorescence in-situ hybridization (iFISH) and have been associated with outcome. t(4;14), t(14;16), t(14;20), del(17p), gain(1q) are considered to confer an adverse prognosis, with the presence of more than one lesion having a cumulative effect. 3–5

Gene expression profiling technologies also enabled the classification of myeloma into subgroups (Figure 1). This was initially based on the Translocation/Cyclin (TC) classification and University of Arkansas for Medical Sciences (UAMS) subgroups, which cluster cases with different mRNA expression profiles, largely driven by the underlying structural genetic events and abnormal expression of a D-group cyclin. Single nucleotide polymorphism (SNP) arrays and targeted sequencing studies added to these prior technologies and led to an increase in our understanding of myeloma pathogenesis. This has, however, been eclipsed in recent years by the wealth of information generated by next generation sequencing technologies. These technologies have led to a more detailed understanding of the molecular landscape of myeloma, with the potential for revolutionizing therapeutic approaches.

Current state-of-the-art

Next generation sequencing studies have highlighted the recurrent DNA mutations that characterize disease progression. They demonstrate that myeloma lies in the middle of all cancers in terms of genomic complexity with fewer mutations than genetically complex solid tumors, such as malignant melanoma, but more mutations than genetically ‘simple’ tumors such as childhood acute lymphoblastic leukemia. Several key pathways are recurrently mutated including the MAPK pathway (KRAS, NRAS, BRAF), NFKB pathway (CYLD, TRAF3, LTbeta), cell cycle pathway (RB1, TP53), DNA damage repair pathway (TP53, ATM, ATR) and genes involved in B cell differentiation (IRF4, PRDM1). Frequent mutations in the IGH translocation gene partners, e.g. FGFR3 and CCND1 have also been identified. Studies exploring the associations of mutations with outcome have identified an adverse outcome with mutations in DNA repair pathway and a favorable outcome with mutations in IRF4.9

By combining mutation, translocation and copy number data, further insights into myeloma risk have been highlighted. Biallelic inactivation events, by deletion and/or mutation, have been demonstrated to be more important in terms of adverse prognosis than single allele changes. Homozygous inactivation of TP53 (Chromosome 17p) is the most common but inactivation of RB1 (Ch 13), CYLD (Ch 16), FAM46C (Ch 1) and TRAF3 (Ch 14) also occur. Similarly, amplification of the 1q21 transcriptional unit has been identified as more adverse than a single gain. These adverse lesions, along with MYC dysregu-
tion events are increasingly common as disease relapses, suggesting they drive the disease to a more high-risk state. In order to inform clinical treatment strategies the proportion of clonal cells harboring a mutation needs to be considered. This is reported as the variant allele frequency and represents the influence of clonal heterogeneity on the myeloma clone. While tumor-initiating events are found in close to 100% of clonal cells, subsequent events may be present in only a subpopulation of cells. This suggests the presence of Darwinian evolution in which genetic features that impart a survival advantage are selected for and passed on to the next generation. Such selection takes place in the context of the bone marrow microenvironment, with the ‘survival of the fittest’ or cells best adapted in that context surviving. Clonal diversity is present at all stages of myeloma but becomes increasingly complex as disease progresses. Targeting a lesion present in all cells with the aim of clonal extinction has innate logic, whereas targeting a lesion present in only a subclone needs to have a conceptual rationale, such that the subclone is likely to be responsible for subsequent relapse or drive high-risk disease. Thus, identifying driver mutations that lead to a survival advantage through increasing cell proliferation or resistance to apoptosis is critical.

In addition to genetic evolution over time, spatial diversity in mutational spectrum has also been identified from biopsies of different anatomical sites. The localized evolution within focal lesions has important implications for risk prediction as it has been demonstrated that having a single focal lesion with high-risk molecular features may impart an adverse outcome even in the context of a low-risk bone marrow biopsy. This reinforces the importance of integrating molecular information with novel imaging techniques to fully understand the genomic landscape and spatio-temporal heterogeneity of a patient’s disease.

**Future perspectives**

Due to the complex heterogeneity between, as well as within, myeloma patients, large studies are needed to identify new drivers and to distinguish driver from passenger mutations. This approach is exemplified by two projects combining and studying large datasets of over 1,000 patients, the Myeloma Genome Project and the Multiple Myeloma Research Foundation CoMMpass study. The power afforded by such large studies will enable driver mutations to be distinguished from passenger mutations and the effect of outcome with different therapeutic regimens examined. The current whole exome sequencing studies are also being expanded to whole genome coverage to identify critical changes in non-coding regions and other interactions that may be key to myeloma initiation and evolution.

Newer technologies also have the potential to widen the clinical use of genomics. Circulating plasma cell and cell free DNA analysis offers the promise of a non-invasive test that may identify therapeutically actionable lesions, as well as having the theoretical capacity to capture information on all sites of disease, therefore overcoming the problem of spatial heterogeneity. To date, few clinicians use genetic information to inform treatment decisions. New clinical studies show that targeted therapy is possible with patients with MAPK pathway mutations being potentially sensitive to BRAF or MEK inhibitors and t(11;14) translocations being associated with response to venetoclax. Additional targeted therapeutics need to be developed and studied in representative models incorporating all elements of the bone marrow microenvironment, in order to predict their efficacy.

![Figure 1. Clinical application of molecular profiling. Bone marrow biopsies from iliac crest or other focal lesions are routinely analyzed using iFISH and increasingly using gene expression array or next generation sequencing technologies for mutation analysis. This information can be used to help predict outcomes for patients by identifying high-risk lesions or integrated into scores such as the R-ISS. As more targeted therapies become available this information can also be used for stratified or personalized medicine approaches. iFISH, interphase fluorescence in situ hybridization; R-ISS, revised international staging system.](images)
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