Cytogenetics in Multiple Myeloma: R-ISS and Beyond – A Case Study.

Priya Rajakumar, MD; Agne Paner, MD
Rush University Medical Center

Case: A 63-year-old man with high-risk multiple myeloma.

Case History: A 63-year-old male was admitted to the hospital with shortness of breath attributed to an asthma exacerbation. He was incidentally found to have circulating plasma cells in his serum. His previous medical history includes severe asthma, hypertension, and prostate cancer (for which he received curative radiation therapy). Initial hematologic assessment revealed mild normocytic anemia (Hgb 11.0 g/dL) and several calvarial lucencies on skeletal survey. Serum protein electrophoresis revealed a monoclonal immunoglobulin A (IgA) kappa of 3.1 g/dL. Quantitative immunoglobulins revealed elevated IgA level (4730 mg/dL), and free light chain assay revealed elevated kappa light chain (52.71 mg/dL) with a kappa-to-lambda ratio of 61.291. He had an elevated beta-2-microglobulin (3.04 mg/dL), hypoalbuminemia (3.0 mg/dL), and normal lactate dehydrogenase (115 U/L). His renal function was preserved, and he had no electrolyte abnormalities.

Due to the presence of a monoclonal protein (IgA kappa) with evidence of end organ damage (calvarial lucencies), a bone marrow biopsy was performed. The biopsy demonstrated evidence of plasma cell myeloma with plasmablastic morphology, involving 70-80% of the marrow space. Fluorescence *in situ* hybridization (FISH) molecular analysis revealed deletion 17p and monosomy 13q. A clinicopathologic diagnosis of multiple myeloma (MM) with high risk cytogenetic features was confirmed.

Initial Treatment: The patient was started on induction chemotherapy with CyBorD (cyclophosphamide, bortezomib, and dexamethasone). Patient was deemed transplant-ineligible due to poor pulmonary function and lack of social support. He ultimately completed 12 months of therapy, after which he achieved a very good partial response (VGPR) based on International Myeloma Working Group (IMWG) response criteria. The patient was subsequently placed on bortezomib maintenance. After two years of maintenance therapy, patient began to develop peripheral neuropathy for which his proteasome inhibitor was discontinued. He eventually developed biochemical relapse, for which he was started on second-line therapy with elotuzumab, lenalidomide, and dexamethasone.

Discussion: The main presentation points by Drs. Rajakumar and Paner included: 1) Review of cytogenetic events in multiple myeloma; 2) Review of the Revised International Staging System (R-ISS); 3) Interpreting cytogenetics within a clinical context including aspects not included in the R-ISS, with specific attention to size of plasma cell clone, changes to chromosome 1, presence of multiple chromosome aberrations, and presence of t(11;14).

Review of Cytogenetic Events in Multiple Myeloma: Cytogenetic changes in multiple myeloma are characterized by primary and secondary genetic events.[1] Primary events are disease *defining*, and they generally include chromosomal translocations and trisomies. These events can occur in newly diagnosed multiple myeloma (NDMM) or its premalignant states, such as monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM). Secondary genetic events are *sub-clonal* and tend to develop upon disease progression. They include copy number abnormalities, secondary translocations, acquired genetic and epigenetic mutations, and DNA hypomethylation.[2]

Review of the Revised International Staging System (R-ISS): The International Staging System (ISS) was developed in 2005 in order to risk stratify patients with newly diagnosed multiple myeloma.[3] This system incorporated two parameters: $ß_2$-microglobulin (which represents disease volume and decreased renal function) and albumin (where decreased levels correlate to an inflammatory response induced by myeloma microenvironment).[3]

In 2015, the ISS was revised to incorporate high-risk cytogenetic aberrations (HRCA) and elevated lactate dehydrogenase (LDH) – as an attempt to include both disease volume AND disease biology when risk stratifying

patients with newly diagnosed multiple myeloma.[4] The Revised International Staging System (R-ISS) was validated by evaluating clinical and laboratory data of 3060 patients across 11 international, multicenter, clinical trials from 2005 to 2012. HRCA were defined as the presence of del(17p) and/or translocation t(4;14)(p16;q32) and/or t(14;16)(q32;q23). Three distinct risk groups were identified: R-ISS stage I (ISS stage I, no HRCA, normal LDH), R-ISS stage II (all patients who did not meet criteria for R-ISS Stage I nor III), and R-ISS stage III (ISS stage III and HRCA or high LDH). At a median follow-up of 46 months, the 5-year overall survival (OS) rate for R-ISS I, II, and III was 82%, 62%, and 40% respectively. The 5-year progression free survival (PFS) rate was 55%, 36%, and 24% respectively.[4] The majority of patients were treated with novel agents, and 60-65% of patients received an autologous stem cell transplant (ASCT).[4] While the R-ISS pioneered incorporation of high-risk cytogenetics into a validated prognostic tool, there are many factors not addressed by the staging system that should be taken into account.

***Relevant Factors NOT Included in the R-ISS**:*

Size of Clone: Interphase FISH has been the most widely used clinical test for detection of cytogenetic changes both at diagnosis and relapse. However, the size of the plasma cell clone harboring a specific genetic abnormality detected by FISH has not yet been standardized. While the European Myeloma Network (EMN) recommends conservative cut off values (10% for fusion or break apart probes, 20% for numerical abnormalities), no uniform thresholds have been identified.[5] For example, with deletion 17p (which may be considered the most significant prognostic marker), the optimal cutoff that has greatest prognostic significance remains to be unknown. The Intergroupe Francophone du Myelome (IFM) suggests prognostic significance only if ≥60% of plasma cells harbor this cytogenetic abnormality.[6] Other studies suggest anywhere between 10-50% of cells carrying deletion 17p is clinically relevant.[7-10]

Changes to Chromosome 1: Changes to chromosome 1 were not included in the R-ISS as only a few studies used to validate the staging system collected information on chromosome 1 at the time. Most common changes to chromosome 1 are amplification at 1q21 and/or deletion of 1p at several different loci.[11] Amplification or gain of 1q21 (+1q) is associated with myeloma cell proliferation and immortalization[12] and is present in approximately one-third of newly diagnosed patients.[13-15] Several studies have demonstrated that amplification of 1q21 is independently associated with worse outcomes,[11,13,16] though other studies did not validate such results.[14,17]

Moreover, the number of copy number gains that is associated with poorer outcomes remains controversial. One study found that patients with ≥3 copies of 1q21 carried a 5-year PFS/OS of 42%/49% vs. 73%/83% for those patients <3 copies of 1q21.[15] It is important to note, that majority of studies evaluating prognostic effects of +1q occurred in the pre-novel agent era. The few studies that have examined the impact of +1q with the use of novel agents (proteasome inhibitors, immunomodulatory agents) are limited by their small sample sizes. One study suggests bortezomib may be able to mitigate the negative effects of +1q[18], while other studies suggest that the negative prognostic influence is retained in spite of novel agents.[19,20,21]

Deletion of chromosome 1p at several different loci has also been recognized as a recurrent genetic event that may have prognostic significance, specifically for who received an autologous stem cell transplant.[22,23] The significance of this chromosomal abnormality outside of this clinical context remains to be unknown. Overall, this information emphasizes the value of assessing for genetic events at chromosome 1, though interpretation must be performed within clinical context.

Presence of Multiple HRCA: At the time the R-ISS was validated, few studies examined the cumulative effect of multiple high-risk cytogenetic aberrations. In the United Kingdom, investigators looked at a total of 1960 patients with NDMM who were enrolled in the MRC Myeloma IX Trial.[24] A total of 1180 bone marrow samples were available for FISH analysis. Investigators demonstrated a linear association between the number of adverse FISH lesions and worse overall survival. They defined several risk groups based on number of adverse lesions: low-risk (absence of adverse genetic lesions), intermediate group (one adverse lesion), high-risk group (co-segregation of >1 adverse lesion). They found median OS/PFS to be 60.6/23.5, 41.9/17.8, and 21.7/11.7 months for the low,

intermediate, and high-risk groups respectively.[24] On the contrary, the presence of concomitant trisomies may be protective.[25]

Translocation (11;14): Perhaps one of the most interesting cytogenetic abnormalities is t(11;14), which occurs in approximately 15% of patients with multiple myeloma.[26] Studies conducted in the pre-novel agent era classified t(11;14) as standard risk,[27] however in the context of novel agents, more recent retrospective reviews suggest that t(11;14) is associated with intermediate risk disease.[28-30] MM cells that harbor t(11;14) have a unique biology with increased expression of pro-apoptotic protein BCL-2, suggesting that t(11;14) may be the first predictive biomarker indicating susceptibility to BCL-2 inhibition. This is of particular relevance in the context of BCL-2 inhibitors, such as venetoclax. Multiple phase I/II studies incorporating venetoclax either as monotherapy or in combination with proteasome inhibitors demonstrate higher overall response rates in relapse/refractory patients who harbor t(11;14) when compared to those who do not carry this mutation.[31-33] Although the primary endpoint analysis of the BELLINI trial demonstrated a higher mortality rate when incorporating venetoclax for all relapsed/refractory patients, updated analysis of this phase III trial suggests that the t(11;14) subgroup demonstrated a higher overall response rate (ORR) and deeper response with addition of venetoclax to bortezomib/dexamethasone.[34]

Concluding Points: The R-ISS is a useful but simplified tool for risk stratifying patients with NDMM. While some institutions follow a risk-adaptive approach when it comes to therapeutic decision making, ultimately, validated prospective trials are needed to guide clinicians when incorporating high-risk cytogenetics into treatment paradigms. We must interpret cytogenetics within a clinical context that includes: additional chromosomal aberrations, size of plasma cell clone harboring HRCA, clinical features specific to the patient, the depth and duration of response to treatment. We should acknowledge the evolving role of t(11;14), specifically as a predictive marker for BCL inhibition. And lastly, as the concept of minimal residual disease (MRD) continues to evolve and gain relevance as a useful prognostic tool,[35] perhaps the role for cytogenetics in myeloma will continue to be redefined.

References:

1. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. *Nat Rev Cancer.* 2012;12(5):335-348.
2. Gonzalez D, van der Burg M, Garcia-Sanz R, et al. Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. *Blood.* 2007;110(9):3112-3121.
3. Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. *J Clin Oncol.* 2005;23(15):3412-3420.
4. Palumbo A, Avet-Loiseau H, Oliva S, et al. Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. *J Clin Oncol.* 2015;33(26):2863-2869.
5. Ross FM, Avet-Loiseau H, Ameye G, et al. Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. *Haematologica.* 2012;97(8):1272-1277.
6. Avet-Loiseau H, Leleu X, Roussel M, et al. Bortezomib plus dexamethasone induction improves outcome of patients with t(4;14) myeloma but not outcome of patients with del(17p). *J Clin Oncol.* 2010;28(30):4630-4634.
7. An G, Li Z, Tai YT, et al. The impact of clone size on the prognostic value of chromosome aberrations by fluorescence in situ hybridization in multiple myeloma. *Clin Cancer Res.* 2015;21(9):2148-2156.
8. Giri S, Huntington SF, Wang R, et al. Chromosome 1 abnormalities and clinical outcomes in multiple myeloma in the era of novel agents. *Journal of Clinical Oncology.* 2019;37(15_suppl):8044-8044.
9. Neben K, Jauch A, Bertsch U, et al. Combining information regarding chromosomal aberrations t(4;14) and del(17p13) with the International Staging System classification allows stratification of myeloma patients undergoing autologous stem cell transplantation. *Haematologica.* 2010;95(7):1150-1157.
10. Neben K, Jauch A, Hielscher T, et al. The Chromosomal Abnormalities Del(17p), t(4;14), and +1q21 Predict Progression From Smoldering to Symptomatic Multiple Myeloma. *Blood.* 2012;120(21):1806-1806.
11. Shaughnessy JD, Jr., Zhan F, Burington BE, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. *Blood.* 2007;109(6):2276-2284.
12. Sawyer JR, Tricot G, Mattox S, Jagannath S, Barlogie B. Jumping translocations of chromosome 1q in multiple myeloma: evidence for a mechanism involving decondensation of pericentromeric heterochromatin. *Blood.* 1998;91(5):1732-1741.
13. Avet-Loiseau H, Attal M, Campion L, et al. Long-term analysis of the IFM 99 trials for myeloma: cytogenetic abnormalities [t(4;14), del(17p), 1q gains] play a major role in defining long-term survival. *J Clin Oncol.* 2012;30(16):1949-1952.
14. Fonseca R, Van Wier SA, Chng WJ, et al. Prognostic value of chromosome 1q21 gain by fluorescent in situ hybridization and increase CKS1B expression in myeloma. *Leukemia.* 2006;20(11):2034-2040.
15. Hanamura I, Stewart JP, Huang Y, et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. *Blood.* 2006;108(5):1724-1732.
16. Cremer FW, Bila J, Buck I, et al. Delineation of distinct subgroups of multiple myeloma and a model for clonal evolution based on interphase cytogenetics. *Genes Chromosomes Cancer.* 2005;44(2):194-203.
17. Avet-Loiseau H, Li C, Magrangeas F, et al. Prognostic significance of copy-number alterations in multiple myeloma. *J Clin Oncol.* 2009;27(27):4585-4590.
18. Smetana J, Berankova K, Zaoralova R, et al. Gain(1)(q21) is an unfavorable genetic prognostic factor for patients with relapsed multiple myeloma treated with thalidomide but not for those treated with bortezomib. *Clin Lymphoma Myeloma Leuk.* 2013;13(2):123-130.
19. An G, Xu Y, Shi L, et al. Chromosome 1q21 gains confer inferior outcomes in multiple myeloma treated with bortezomib but copy number variation and percentage of plasma cells involved have no additional prognostic value. *Haematologica.* 2014;99(2):353-359.

20. Chang H, Trieu Y, Qi X, Jiang NN, Xu W, Reece D. Impact of cytogenetics in patients with relapsed or refractory multiple myeloma treated with bortezomib: Adverse effect of 1q21 gains. *Leuk Res.* 2011;35(1):95-98.
21. Schmidt TM, Barwick BG, Joseph N, et al. Gain of Chromosome 1q is associated with early progression in multiple myeloma patients treated with lenalidomide, bortezomib, and dexamethasone. *Blood Cancer J.* 2019;9(12):94.
22. Qazilbash MH, Saliba RM, Ahmed B, et al. Deletion of the short arm of chromosome 1 (del 1p) is a strong predictor of poor outcome in myeloma patients undergoing an autotransplant. *Biol Blood Marrow Transplant.* 2007;13(9):1066-1072.
23. Wu KL, Beverloo B, Lokhorst HM, et al. Abnormalities of chromosome 1p/q are highly associated with chromosome 13/13q deletions and are an adverse prognostic factor for the outcome of high-dose chemotherapy in patients with multiple myeloma. *Br J Haematol.* 2007;136(4):615-623.
24. Boyd KD, Ross FM, Chiecchio L, et al. A novel prognostic model in myeloma based on co-segregating adverse FISH lesions and the ISS: analysis of patients treated in the MRC Myeloma IX trial. *Leukemia.* 2012;26(2):349-355.
25. Kumar S, Fonseca R, Ketterling RP, et al. Trisomies in multiple myeloma: impact on survival in patients with high-risk cytogenetics. *Blood.* 2012;119(9):2100-2105.
26. Lakshman A, Alhaj Moustafa M, Rajkumar SV, et al. Natural history of t(11;14) multiple myeloma. *Leukemia.* 2018;32(1):131-138.
27. Fonseca R, Blood EA, Oken MM, et al. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood.* 2002;99(10):3735-3741.
28. Sasaki K, Lu G, Saliba RM, et al. Impact of t(11;14)(q13;q32) on the outcome of autologous hematopoietic cell transplantation in multiple myeloma. *Biol Blood Marrow Transplant.* 2013;19(8):1227-1232.
29. An G, Xu Y, Shi L, et al. t(11;14) multiple myeloma: a subtype associated with distinct immunological features, immunophenotypic characteristics but divergent outcome. *Leuk Res.* 2013;37(10):1251-1257.
30. Leiba M, Duek A, Amariglio N, et al. Translocation t(11;14) in newly diagnosed patients with multiple myeloma: Is it always favorable? *Genes Chromosomes Cancer.* 2016;55(9):710-718.
31. Kaufman JL, Gasparetto CJ, Mikhael J, et al. Phase 1 Study of Venetoclax in Combination with Dexamethasone As Targeted Therapy for t(11;14) Relapsed/Refractory Multiple Myeloma. *Blood.* 2017;130(Suppl 1):3131-3131.
32. Kumar S, Kaufman JL, Gasparetto C, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. *Blood.* 2017;130(22):2401-2409.
33. Moreau P, Chanan-Khan A, Roberts AW, et al. Promising efficacy and acceptable safety of venetoclax plus bortezomib and dexamethasone in relapsed/refractory MM. *Blood.* 2017;130(22):2392-2400.
34. Philippe Moreau SH, Michele Cavo, Javier De La Rubia, Rakesh Popat, Cristina Gasparetto, Vania T.M. Hungria, Hans Salwender, Kenshi Suzuki, Inho Kim, Francesca Gay, Gábor Mikala, Elizabeth A Punnoose, Wan-Jen Hong, Anjla Sood, Muhammad Jalaluddin, Jeremy A. Ross, James E. Ward, Paulo C. Maciag, Shaji K. Kumar. Updated Analysis of Bellini, a Phase 3 Study of Venetoclax or Placebo in Combination with Bortezomib and Dexamethasone in Patients with Relapsed/Refractory Multiple Myeloma. *Blood.* 2019;134:1888.
35. Goicoechea I PN, Cedena MT, et al. Clinical significance and transcriptional profiling of persistent minimal residual disease (MRD) in multiple myeloma (MM) patients with standard-risk (SR) and high-risk (HR) cytogenetics. *Blood.* 2018;132:112.