MYC dysregulation in the progression of multiple myeloma

Running Title: *MYC* in the progression of MM

K Misund[1,2*], N Keane[1,3*], CK Stein[1], YW Asmann[4], G Day[1], S Welsh[1], SV Wier[1], D Riggs[1], G Ahmann[1], M Chesi[1], D Viswanatha[5], SK Kumar[5], A Dispenzieri[5], V Gonzalez-Calle[1], RA Kyle[5], M O'Dwyer[3], SV Rajkumar[5], KM Kortüm[6], J Keats[7], MMRF CoMMpass Network[8], R Fonseca[1], AK Stewart[1], WM Kuehl,[9] E Braggio[1], PL Bergsagel[1]

* shared first co-authors

[1]Comprehensive Cancer Center, Mayo Clinic, Scottsdale, Arizona

[2]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

[3]National University of Ireland Galway, Ireland

[4]Department of Health Sciences Research, Mayo Clinic, Jacksonville, Florida

[5]Department of Medicine, Mayo Clinic, Rochester, Minnesota

[6]Department of Hematology and Oncology, University Hospital Würzburg, Würzburg, Germany

[7]Integrated Cancer Genomics Division, Translational Genomics Research Institute, Phoenix, Arizona, USA

[8]Multiple Myeloma Research Foundation, Norwalk, CT

[9]Center for Cancer Research, National Institute of Health, Bethesda, Maryland

Multiple myeloma (MM) is a plasma cell malignancy preceded by a premalignant stage, named monoclonal gammopathy of undetermined significance (MGUS), and often a smoldering phase (SMM).[1, 2] Primary events, which include recurrent translocations of the IgH locus and hyperdiploidy, occur early in pathogenesis, and are followed by the acquisition of secondary genetic events such as *MYC* structural variants (SV), mutations that activate the RAS or NFkB pathways, mutations of *DIS3* or *FAM46C* that drive precursor stages of disease toward MM.[3-6] Whole exome sequencing (WES) studies comparing serial MGUS/SMM and MM samples indicate clonal stability, and no significant increase in mutational load in patients that progress rapidly to MM.[7] In contrast, in 33 unselected MGUS patients single-nucleotide variants (SNVs) were less frequent, and no *MYC* translocations identified.[8]

To study the role of *MYC* in myeloma we performed an integrated genomic analysis of 612 newly diagnosed myeloma (NDMM) patients enrolled in the CoMMpass study, as well as targeted sequencing of 23 patients with MGUS and 90 patients with SMM. We identified *MYC* SV in 42% of NDMM, including the majority of HRD (57%), and a quarter of MM with primary IgH translocations. The majority of these rearrangements resulted in juxtaposition of a super-enhancer (SE) and/or stretch enhancer adjacent to *MYC*, with one third involving an *Ig* super-enhancer, one third involving another recurrent super/stretch enhancer and the remaining third split between non-recurrent super/stretch enhancers, no identified super/stretch enhancer, or rearrangements wholly confined to the region telomeric to *MYC*, frequently duplications with no exogenous sequences present (**Table S1A-B**, **Table S2-4).** The *IgH MYC* rearrangements often were complex - sometimes involving duplications and 3 or more chromosomes - and the *IgH* breakpoints were often within or near the 3' SE regions, suggesting a different timing or mechanism than the primary *IgH* translocations.

By using an informative group of patients in which we were able to identify germline polymorphisms within the exons of *MYC* (n=147), we found 66/69 (96%) NDMM with elevated mono-allelic *MYC* expression have a MYC SV, whereas in 69/77 (90%) with variable levels of biallelic *MYC* expression no *MYC* SV was identified (**Table S5**). This highlights the functional significance of *MYC* SV, suggesting that our analysis is neither missing nor overcalling the presence of many *MYC* SVs, and that the primary mechanism of cis-dysregulation of *MYC* is by

SV. The level of expression of *MYC* is higher in samples with rearrangements compared to those without (**Figure 1A, B**), with similar levels whether an Ig or non-Ig enhancer is involved (p-value > 0.10), but intermediate levels for samples with a wholly confined telomeric rearrangement.

While in patients with *MYC* SV there was no correlation between *MYC* expression and NFkB index (**Figure 1C**), in patients lacking *MYC* SV, there was a strong linear correlation (**Figure 1D**), identifying coordinate dysregulation of *MYC* associated with both constitutive and ligand-dependent NFkB pathway activation. Unlike many cancers, we did not find a correlation between the presence of *MYC* SV, or the level of MYC expression, and proliferation, as measured using a gene expression index (data not shown). As recently noted[9] *MAX* mutations or inactivation correlate with extremely low levels of *MYC* expression, and we found that aberrations in these genes rarely occur together (**Figure 1B**). (**Table S6**). This data suggests that as reported for small cell lung cancer[10] and oligodendroglial tumors[11], aberrancies of *MYC* and its heterodimeric partner *MAX*[12], operate in a mutually exclusive fashion.

Taken altogether, *MYC* SV, *MAX* inactivation and NFκB pathway mutation, identify a genetic mutation associated with *MYC/MAX* pathway dysregulation in two-thirds of NDMM (261+22+127/612, **Table S1A**). In 86 of the remaining patients (14% of the total) there is ligand dependent NFκB activation associated with increased *MYC* expression. The overwhelming majority of the remaining patients, representing 14% of the total (86/612), have a mutation activating the MAPK pathway (RAS/*BRAF*/*FGFR3*). Only one in twenty (30/612) tumors lacks evidence of dysregulation of the *MYC/MAX*, NFkB or MAPK pathway. In contrast, there is no correlation between *MYC* dysregulation and mutations of the MAPK pathway, which are instead inversely correlated with NFκB activation, particularly in patients without *MYC* SV (**Figure 1E** and **1F**, **Table S7, S8**).

We further expanded our genomic analysis and included premalignant stages in MM development. First, we established a sequencing panel targeting regions surrounding *IgH* (500 Kb), *IgL* (100 Kb), *IgK* (50 Kb) and *MYC* (2 Mb) loci, in addition to detecting SNVs in 88 important MM genes[13]. The robustness of the approach was tested by comparing with FISH data

from 90 primary samples, and for 60 of these also with Mate Pair whole genome sequencing (WGS). Across all 90 samples, the Custom Capture approach was able to detect 93% (39/42) of *IgH* translocations and 86% (19/22) of *MYC* SVs previously detected by FISH and Mate Pair WGS, respectively (details in Supplementary Methods). and as such we slightly underestimate the incidence of *MYC* SV in MGUS and SMM compared to MM patients in the CoMMpass study.

We analyzed 23 unselected MGUS cases using the sequencing panel. Three patients had an N and/or K-RAS, two had NFKB pathway mutations (TRAF2 and CYLD) and none had rearrangements in the *MYC* locus. However, canonical initiating events (HRD and *IgH* translocations with *MAF*, *MAFA*, *MMSET*, *CCND1*, and *CCND3*) were observed in all but four samples, three with no clear initiating event and the other an *IgH* rearrangement with *UPK2* (**Table S9**, **Table S10-S12**). When analyzing 90 SMM samples with the sequencing panel, 22 cases were observed with *MYC* SVs (24%), including 5 *IgH-MYC* and 1 *IgL-MYC* SV (**Table S9**). The time to progression (TTP) for SMM cases with non-Ig *MYC* SVs was not significantly different than cases without any *MYC* SV (median TTP of 45 versus 61 months, p-value >0.10). However, the SMM cases with Ig *MYC* SVs progressed rapidly to MM (all 6 cases progressed within 23 months of observation, **Figure 2A**). On multivariate analysis performed using Mayo Clinic criteria for high risk of progression Ig *MYC* SVs retained significance (HR 4.59, p 0.003) as an independent prognostic marker for rapid progression to MM (**Table S13**).

In an expanded analysis of potentially relevant genomic features within this SMM cohort, *DIS3* mutations associated with rapid progression to MM along with Ig *MYC* SVs (**Table S14**). Notably, many CNAs that are commonly aberrant and often associated with adverse prognosis in NDMM, such as gain of 1q or deletion of 13q, bore no significant association with progression to MM despite increased frequency with advancing disease stage (**Figure 2C, Table S15, S16**).

While *DIS3* mutation and Ig *MYC* SVs were significantly associated with rapid progression to MM in SMM, Ig *MYC* SVs alone only bordered on significance in NDMM (PFS p-value = 0.055) but did achieve significance when paired with *DIS3* mutation (PFS p-value < 0.05, **Table S17**). In contrast to SMM, we observed that IgL, rather than IgH or IgK *MYC* SV, were

associated with more rapid disease progression in NDMM (**Figure S1**)[14]. In a more focused analysis comparing prognostic associations of *MYC* SV types (Ig, non-Ig, or none), we observed that HRD cases with a non-Ig *MYC* SV had uniquely beneficial prognosis with a significantly reduced rate of progression (82% cases without PFS events at 2-years compared to 59% in remaining cases, **Figure 2B**) while no difference in outcome was noted across *MYC* SV type in non-HRD MM (**Figure S2, S3**). Both the combination of non-Ig *MYC* SVs with HRD positivity and IgL *MYC* SV retained significant association with PFS in multivariate models including covariates for key genomic features (*MMSET* or *MAF* translocations, 1q gain, 13q loss, 17p loss, *DIS3* mutation), treatment strategy, i.e. use of combined therapy with Immunomodulatory drugs (Imids), and International Staging System (ISS) stage (**Table S18**). Whereas previous studies in lymphoma, and MM have shown *MYC* SVs to be an adverse prognostic factor [6, 15] we did not observe this in our studies of SMM or MM. This suggests that *MYC* may serve a somewhat different role in MM, less focused on proliferation and instead driving protein translation and metabolism to meet the demands of highly secretory plasma cells. Our findings require further investigation but provides preliminary evidence that outcome, and likely function, of *MYC* rearrangements is dependent upon partnered enhancer and genetic context. It is supported by the parallel observation of a much more rapid progression from MGUS through SMM to MM for patients with Ig *MYC* SV, but not non-Ig *MYC* SV.

Our analyses demonstrate that MM tumors rely for progression on a few signaling pathways (*MYC*, RAS, NFkB) that show functional redundancy and complementary activation, with at least one pathway activated in 95% of NDMM. In contrast to previous studies of serial samples,[7] our analysis of MGUS cases showed a lack of key progression features, e.g., 0/23 with MYC SV and only 2/23 with a clonal NFkB or KRAS mutation. This discrepancy is likely due to not selecting samples known to progress to MM. Focusing on the same 3 progression pathways for SMM vs MM, *MYC* SV are 24 vs 43%, NFkB mutations 12 vs 32%, and RAS pathway mutations 46 vs 53%. Rapid progression of SMM to MM appears to be independently associated with Ig *MYC* SV and *DIS3* mutations, and possibly with NFkB mutations.

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Authorship Contributions

PLB, WMK and KM originated concept and design of investigation, KMK EB developed custom capture panel, SVW FISH analysis, GA primary samples, YA GD WMK PLB developed additional bioinformatics methods, NK KM CKS PLB WMK performed analyses, and NK KM CKS WMK PLB composed manuscript. We thank JK and MMRF research network for their work on CoMMpass. All authors read and approved of final manuscript.

Conflict of Interest Disclosures

None.

Figure Legends

Figure 1. Location of *MYC* breakpoints and variation in *MYC* expression

The exact location of breakpoints (black dots) at the *MYC* locus for the 260 NDMM cases with *MYC* SV in the CoMMpass cohort, shows that the breakpoints clustered within an approximately 2Mb region around *MYC*, with three breakpoint cluster regions: one centered on *MYC*, a less frequent one centromeric, and more common one telomeric to *MYC*. The level of *MYC* expression (log transform of Salmon TPM) is shown on the Y-axis, and shows that the breakpoints were associated with an increased expression of *MYC*, (**A)**. The level of *MYC* expression is highest in cases with IG or Non-IG *MYC* SVs (median TPM 79; non-significant one-sided Wilcoxon test between IG and Non-IG MYC SVs, p-value > 0.05) according to data from 612 NDMM CoMMpass cases. Cases with IG or non-IG *MYC* SVs have significantly higher *MYC* expression than those with wholly confined telomeric *MYC* SVs (median TPM 38, p-value < 0.001), who in turn have significantly higher expression than cases with NFkB aberrations (median TPM 26, p-value < 0.05), and cases with RAS or FGFR3 mutations (median TPM 15) have low expression of *MYC*, even lower than cases with NFkB aberrations (p-value < 0.001). *MYC* expression is lowest in cases harboring *MAX* aberrations (median TPM 1, **B**). Across patients with *MYC* SVs, there was no correlation between the level of expression of *MYC* and NFkB aberrations or index (**C** and **E**). However, in patients without *MYC* SV, there is a significant correlation between the level of *MYC* TPM and the NFkB index (**D** and **F**). Vertical line in plots **C** and **D** denotes the median NFkBi. Correlation triangles report Spearman correlations between variables when highly significant (p-value < 0.001) with negative correlation in blue, positive correlation in red, and size of circle associated with absolute value of correlation.

Figure 2. Progression in SMM and NDMM and genomic copy number comparison of MGUS, SMM, and NDMM

An analysis of *MYC* SVs in SMM cohort revealed that *MYC* rearrangements that juxtaposed any of the Ig regions (five *IgH*, one *IgL*) had a rapid progression to MM (**B**). However, in NDMM, only cases with *IgL MYC* SVs had inferior outcomes (see also Supplementary Figure S1)

Additionally, HRD cases harboring a Non-Ig *MYC* SV had a significant association with improved performance (**B**) not observed for Non-HRD (NHRD) cases (Supplementary Figures S2 and S3). Across 23 MGUS, 90 SMM, and 612 NDMM cases, the percent of samples with a gain and loss were determined at equal 30 Kb intervals across the entire genome. A gain was denoted if copy number segment values at given location was greater than log2(2.25/2) and loss if segment value was below log2(1.30/2). Across entire chromosomes, many of the copy number gains and losses are similarly prevalent across disease stages, however gain of 1q and loss of 13q significantly increase in frequency with disease stage, more so than any other chromosomes. (**C**)

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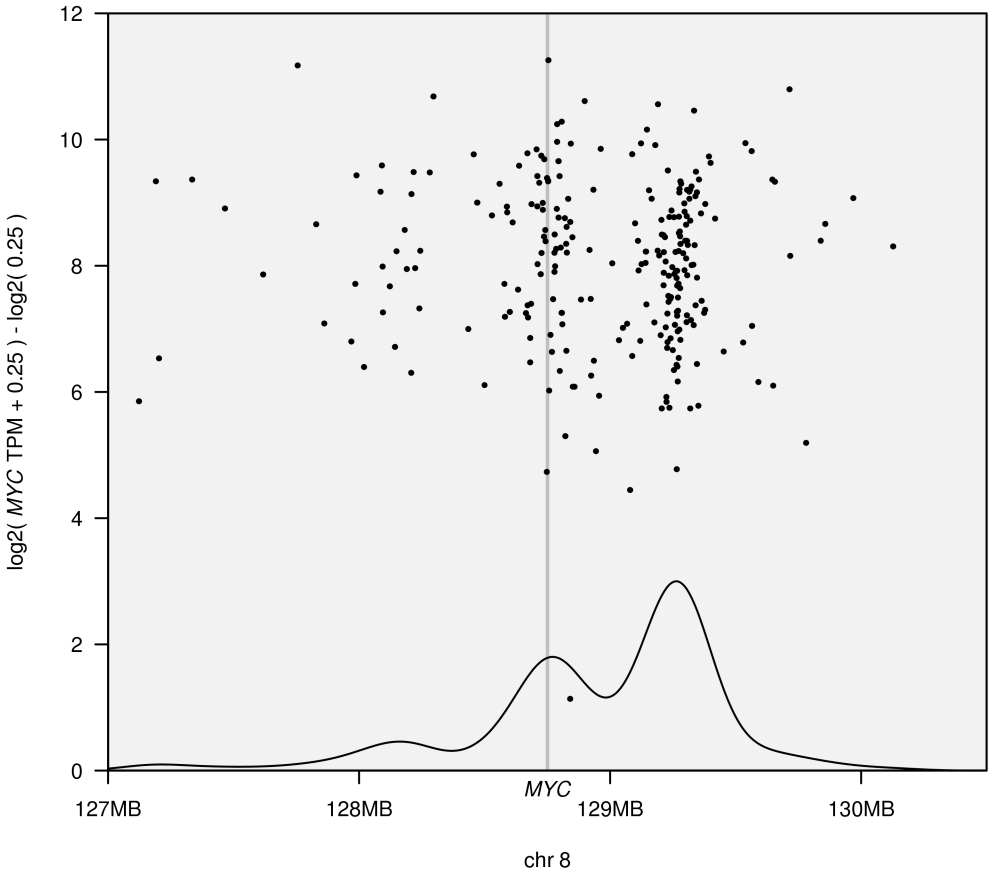
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MYC
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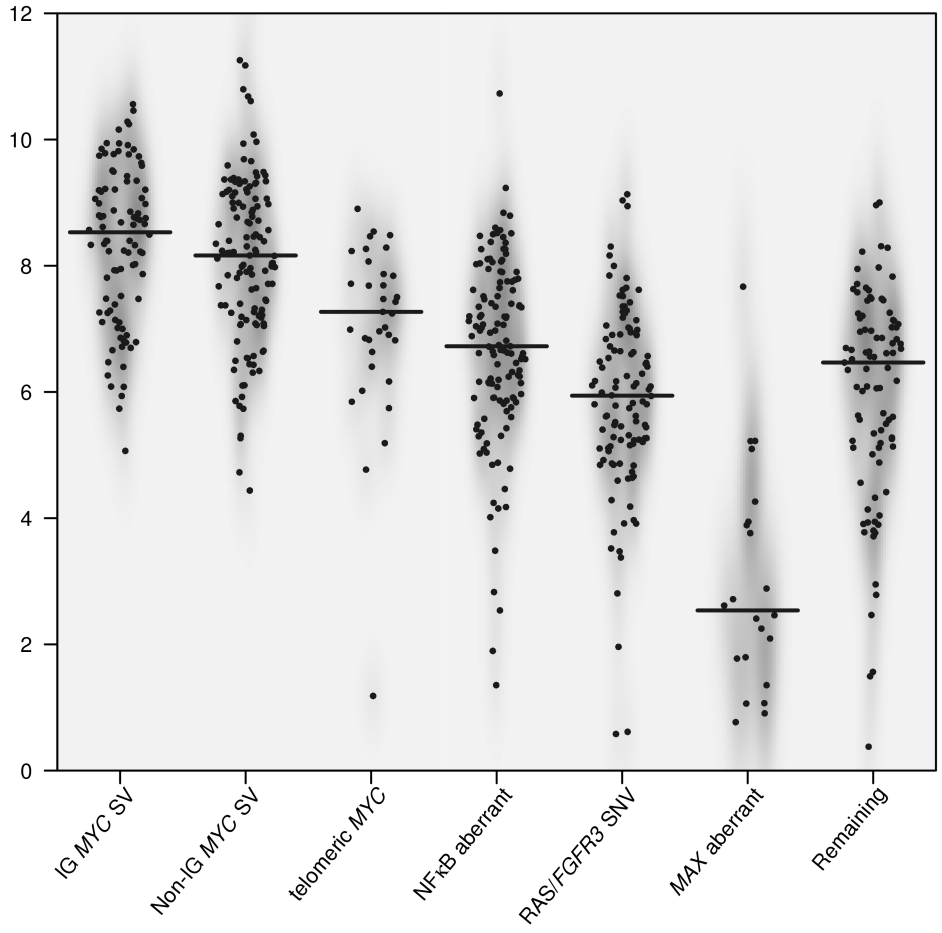
128MB
129MB
130MB
chr 8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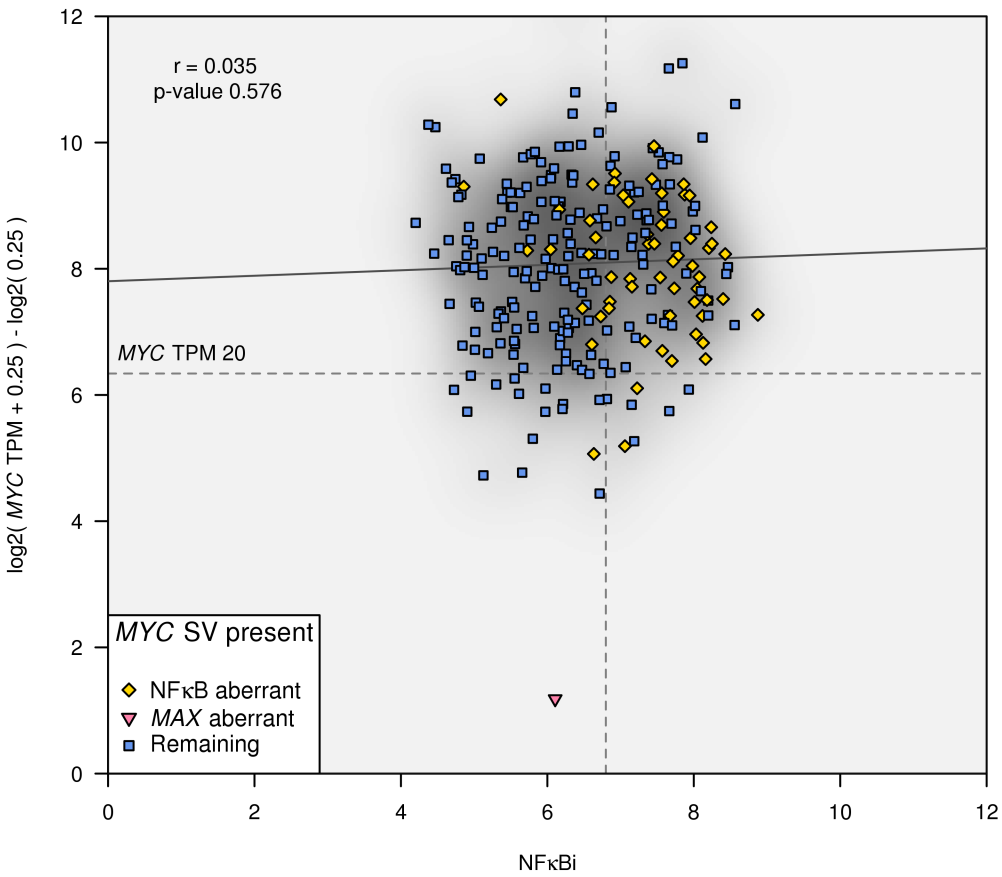
Non-IG MYC SV
telomeric MYC
NFκB aberrant
RAS/FGFR3 SNV
MAX aberrant
Remaining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MAX aberrant
Remaining
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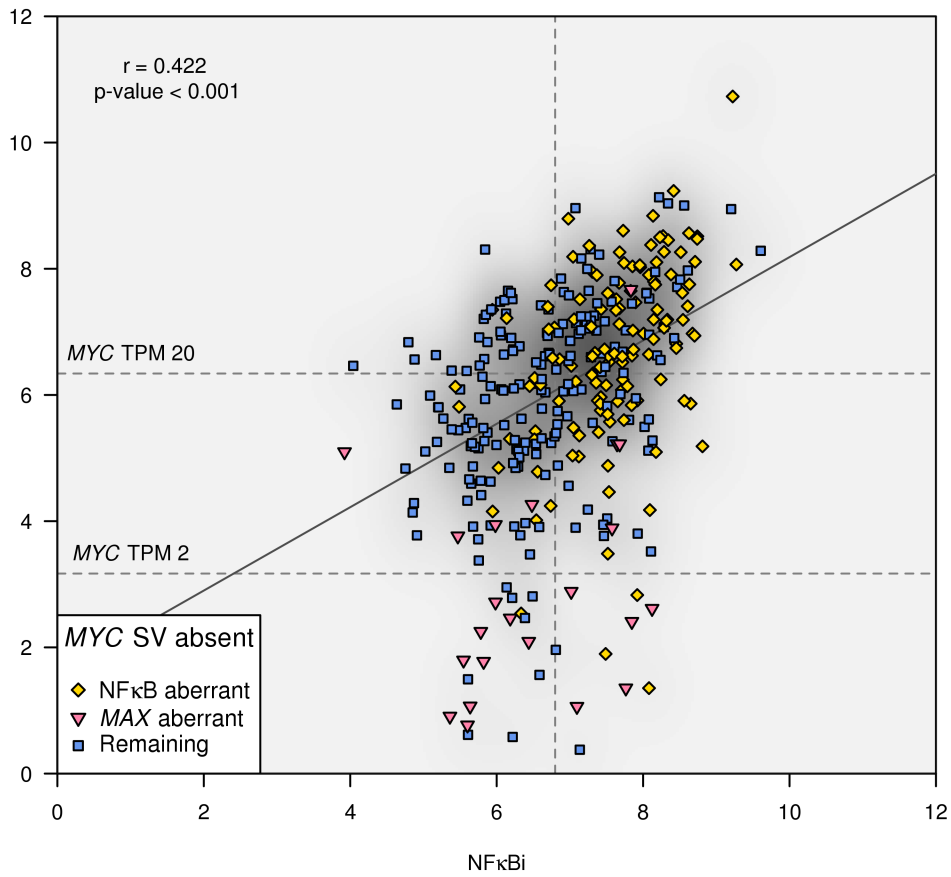
NFκBi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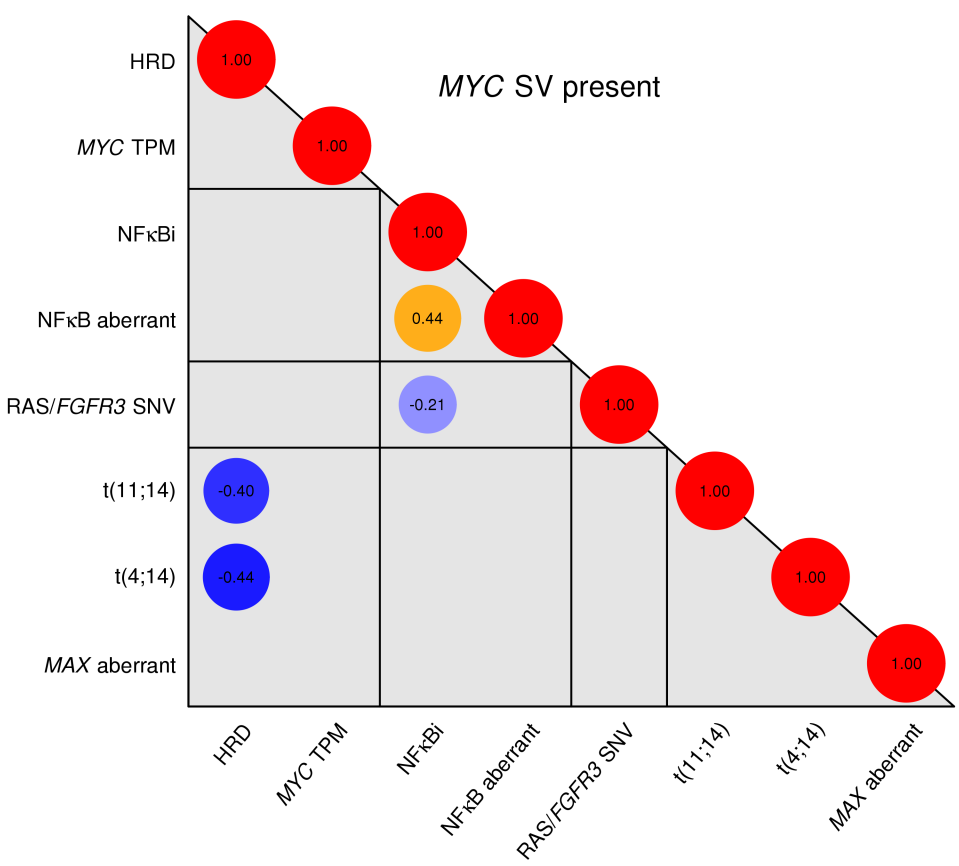
MAX aberrant
Remaining
NFκBi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HRD
MYC TPM
NFκBi
NFκB aberrant
RAS/FGFR3 SNV
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t(4;14)
MAX aberrant
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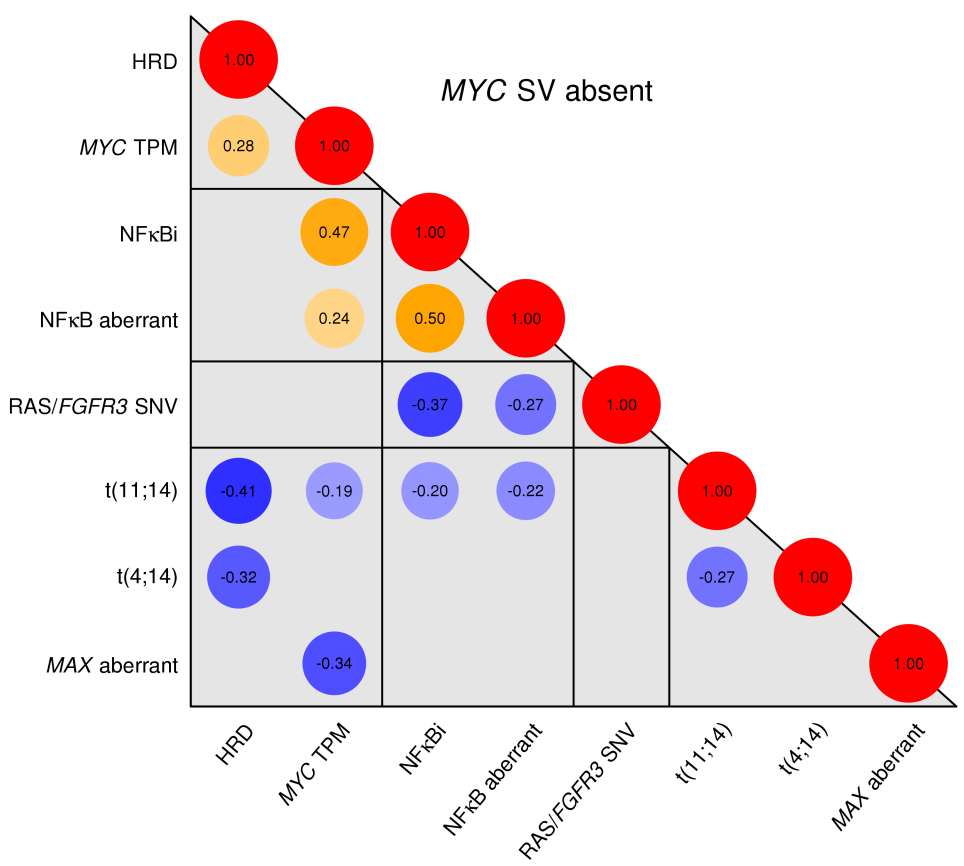
-0.44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HRD
MYC TPM
NFκBi
NFκB aberrant
RAS/FGFR3 SNV
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t(4;14)
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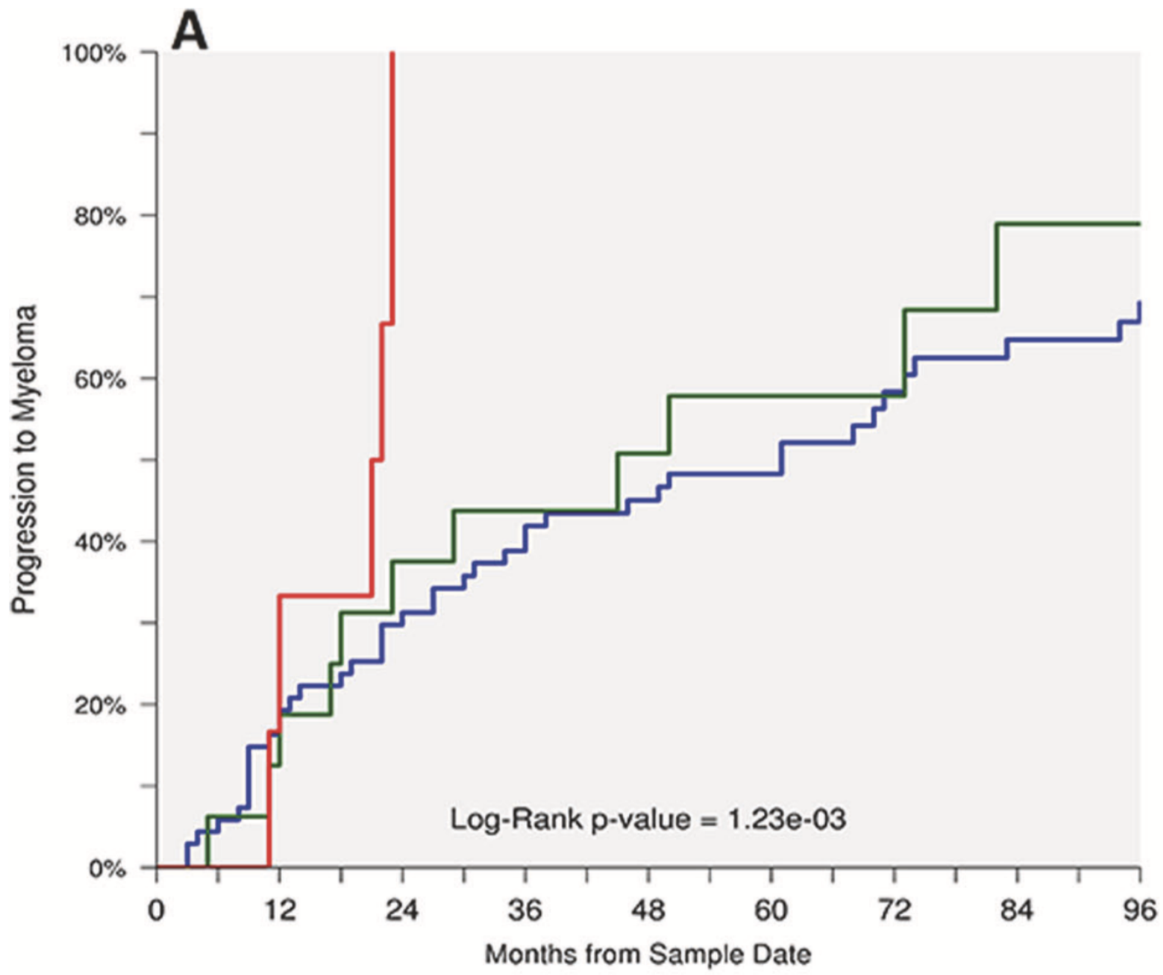
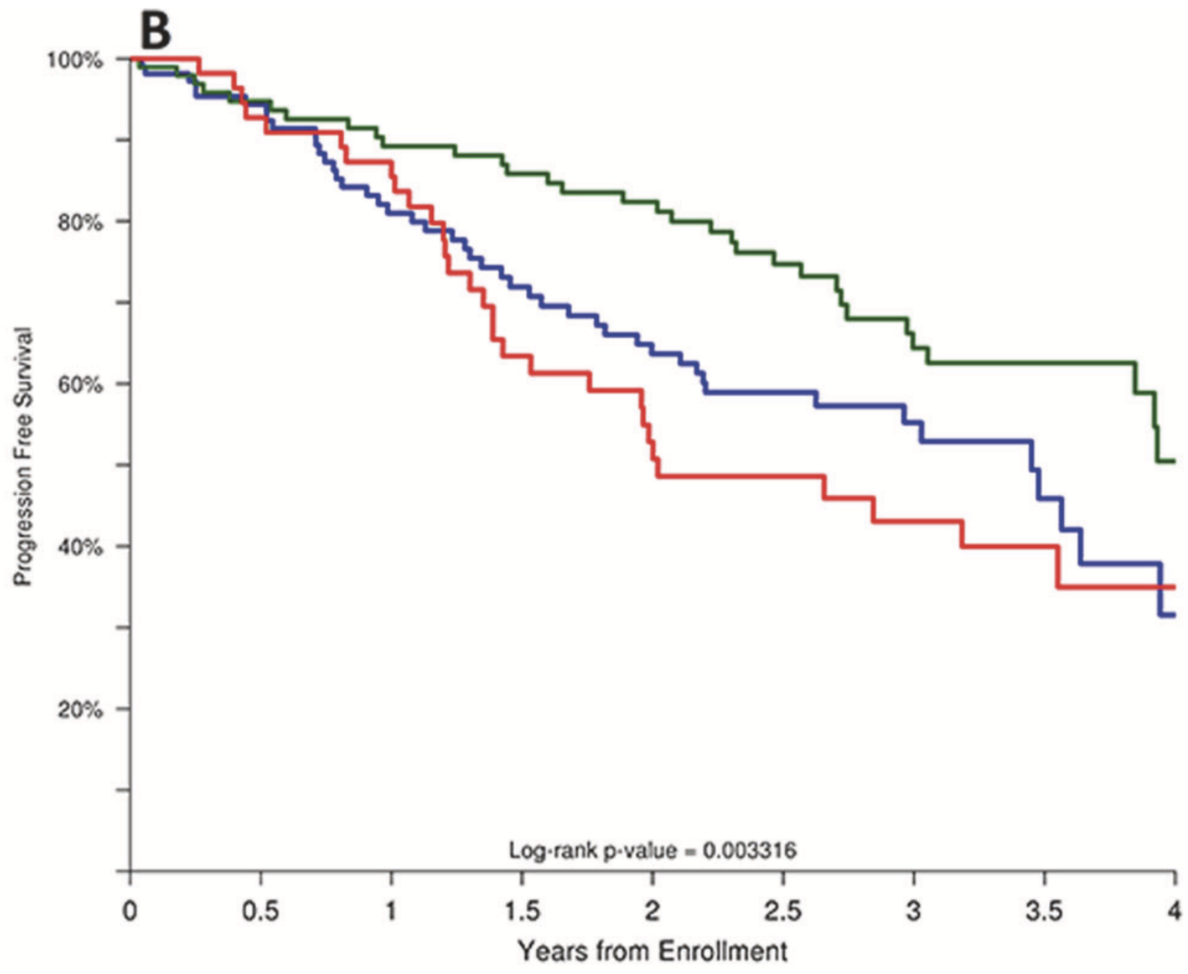


		Events / N	Median TTP (Months)
	IG MYC SV	6 / 6	22
	Non-IG MYC SV	13 / 16	45
	None	45 / 68	61



		Events / N	2-Year Survival
	HRD, MYC IG SV	33 / 56	52.8 (40.6 , 68.7)
	HRD, MYC Non-IG SV	35 / 97	82.4 (74.9 , 90.6)
	HRD, No MYC SV	46 / 109	63.7 (54.5 , 74.4)