Genomic profiling of smoldering multiple myeloma identifies patients at a high risk of disease progression.

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Context Summary:

Key objective: Identifying the Smoldering Multiple Myeloma (SMM) patients who will eventually progress to overt malignancy can allow for early intervention to prevent end-organ damage and potentially achieve long-term remission. Current risk models are based on solely clinical markers and often lack precision and accuracy to predict progression. We hypothesized that genomics can improve the prediction of progression from SMM to overt Multiple Myeloma (MM).

Knowledge generated: Most genetic alterations have already occurred by the time of SMM diagnosis, suggesting that next-generation sequencing (NGS) can be used at that stage for reliable prognostication. Alterations in the MAPK and DNA repair pathways or MYC are independent risk factors of progression, whose addition to current clinical risk models significantly improves prediction of progression.

Relevance: Clinical-grade NGS at the SMM stage can be utilized to identify patients at high risk of progression to MM, who might benefit from early intervention approaches for better outcomes.

Abstract

Background Smoldering multiple myeloma (SMM) is a precursor condition of multiple myeloma (MM) with a 10% annual risk of progression. Various prognostic models exist for risk stratification; however, those are based on solely clinical metrics. The discovery of genomic alterations underlying disease progression to MM could improve current risk models.

Methods We used next-generation sequencing to study 214 SMM patients. We performed whole exome sequencing (WES) on 166 tumors, including 5 with serial samples, and deep targeted sequencing on 48 tumors.

Results We observed that most of the genetic alterations necessary for progression have already been acquired by the diagnosis of SMM. Particularly, we found that alterations of the MAPK pathway (KRAS and NRAS SNVs), the DNA repair pathway (deletion 17p, *TP53* and *ATM* SNVs), and *MYC* (translocations or CNVs) were all independent risk factors of progression after accounting for clinical risk staging. We validated these findings in an external SMM cohort, showing that indeed patients who have any of these three features have higher risk of progressing to MM. Moreover, APOBEC-associated mutations were enriched in patients who progressed and associated with a shorter time to progression in our cohort.

Conclusions SMM is a genetically mature entity whereby most driver genetic alterations have already occurred, suggesting the existence of a right-skewed model of genetic evolution from MGUS to MM. We identified and externally validated genomic predictors of progression that could identify patients at high risk of progression to MM, and thus improve on the precision of current clinical models.

Introduction:

Multiple Myeloma (MM) is an incurable plasma cell malignancy with significant inter- and intra-patient heterogeneity. It is almost always preceded by asymptomatic precursor stages, namely monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) [1, 2]. SMM patients have a higher risk of progression to MM (10%/year), compared to MGUS (1%/year) [3], although some patients progress rapidly, others remain in an MGUS-like state for years.

Current prognostic models do not fully capture SMM progression risk, as patients who are considered to be intermediate or low risk by those criteria can still progress. This might be because these models are based mainly on tumor burden markers and may not adequately reflect the underlying biology that may be critical for disease progression. Thus, there is an urgent need for novel prognostic markers that can more accurately identify SMM patients who are at risk of progression and could benefit from early treatment.

Herein, we studied 214 SMM patient samples to comprehensively characterize the genomic landscape of SMM and identify biomarkers of progression MM.

Methods

We used next-generation sequencing technologies to study 214 patients with SMM at time of diagnosis, including 5 serial samples. We performed whole exome sequencing (WES) on 72 matched tumor-normal samples and 94 tumor-only samples, and targeted deep sequencing on 48 samples. Patients who presented at diagnosis with MM symptoms, including hypercalcemia, renal impairment, anemia, or bone lytic lesions (CRAB), or had any myeloma-defining event were excluded from the analysis[4]. Light-chain and non-secretory SMM patients were included, as they are understudied subtypes and their biology needs to be understood more. All samples were obtained after written informed consent, according to the Declaration of Helsinki. Time-to-event endpoints are estimated using the method of Kaplan and Meier. Differences in survival curves were assessed using log-rank tests. Median follow-up was calculated using the reverse Kaplan-Meier method. Time to progression (TTP) was measured from date of diagnosis to date of documented progression to MM. Cox modeling was performed to assess the impact of a MM driver on clinical outcome measures. Statistical analysis is described in detail in Supplemental methods.

Results

The genomic landscape of SMM

The median age in our cohort was 62 (range: 34 - 85) years. Patients were stratified into Low, Intermediate, and High risk groups based on the Mayo 2008 criteria [5] as well as the revised Mayo 2018 criteria [6]. **(Supp. Table 1)**.

The genomic landscape of the entire cohort is illustrated according to single nucleotide variants (SNVs), somatic copy number alterations (SCNAs), and translocations, and the patients were divided according to their clinical risk stage in **Figure 1.** Immunoglobulin heavy chain (IgH) translocations commonly seen in MM were present in 76 patients (36%), as identified by Fluorescence in Situ Hybridization (FISH), while SCNAs were the most common genomic alterations, and were present in 189 patients (88%). Hyperdiploidy (HRD), i.e. with 48 or more chromosomes in the genome, was found in 55% of patients; hypodiploidy, defined as less than 45 chromosomes, was found in only 10 patients (4.6%), and whole genome doubling (ploidy > 2.5) in six (2.8%). The median mutation density in SMM patients was 1.4 mutation/Mb, and single nucleotide variations (SNVs) in genes significantly mutated in MM were present in 118 patient samples (55%). Forty-six percent of those had alterations in the MAPK pathway (*KRAS, NRAS*, *BRAF*, and *PTPN11*). DNA repair pathway alterations (*TP53* and *ATM* SNVs and deletion 17p) were found in 21 (10%). SNVs in genes of NFkB, protein processing, and cell cycle pathways were found in 22%, 21%, and 6.7% patients, respectively. Bi-allelic inactivation events affecting *TP53*, *RB1*, *CDKN2C*, *ZNF292*, *DIS3*, or *FAM46C* were present in only 6% patients. Copy number neutral loss of heterozygosity (LOH) was observed in 44 patients (27%), with chromosome 16 being most frequently affected (20%), followed by chromosomes 1 (16%) and 6 (11%). Of note, 36 LOH events were clonal, and 31 events were subclonal. No significant focal amplifications were discovered in our dataset (n = 166), by GISTIC2. However, four focal deletions were considered significant: del 1p22.1, del 6q27, del 14q24.3, and del 14q32.31, all containing putative tumor suppressors **(Supp. Figure 1)**. In fact, patients with del 1p22.1 and del 14q24.3 had a shorter TTP (p = 0.009 and 0.02, respectively) **(Supp. Figure 2)**. Of note, the most common MM whole

chromosome and arm level CNAs, including gains of 1q and chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, as well as deletions of 13q and 16q were significant events **(Supp. Figure 1)**. In terms of alteration co-occurrence, we observed that HRD often significantly co-occurred with mutations in *KRAS* and *NRAS*, while t(11;14) was significantly mutually exclusive with HRD and gain of 1q **(Figure 4A)**. On the other hand, t(4;14) co-occurred with mutations in *DIS3*, *BRAF*, del 13q and gain of 1q. Deletion of 13q was shown to co-occur infrequently with HRD and rarely with *NRAS*; however, it co-occurred frequently with del 16q and gain 1q. Our analysis also showed that deletion 1p frequently co-occurred with deletions 8p and 17p.

The clonal architecture and phylogeny of SMM

We observed heterogeneity in the clonality of mutations affecting recurrently mutated genes in MM. Certain genes (*TP53, KLHL6, DIS3*, *MAX*, *NFKBIA*, and *CCND1*) carried clonal alterations more frequently, while other genes were more frequently subclonal (*FAM46C, NFKB2, LTB,* and *TRAF3*), likely representing later events in disease course **(Figure 2C)**. Interestingly, mutations in *KRAS*, *NRAS,* and *BRAF* were subclonal in 64%, 70%, and 86% of patients, respectively. In contrast, our analysis showed that SCNAs were clonal in most of the cases, especially trisomies of odd-numbered chromosomes that were clonal in 98%, with the exception of trisomies 9, 19 and 21 which were found to be subclonal in a few cases **(Figure 2B)**. Moreover, Del 13q, 14q, 16q, and gain 1q were also clonal in 78, 76, and 72, and 68%, respectively. This data suggests that SCNAs are founder events in SMM, while SNVs in the MAPK pathway participate in tumor progression.

We next analyzed serial samples from five patients, sampled at two timepoints of at least one year apart (range: 1-8 years). We observed evidence of clonal heterogeneity in all five of them, indicating that clonal branching has already happened at the smoldering stage. While copy number abnormalities were mostly clonal in all five samples, we observed evidence of subclonal copy number events in three patients (SMM_060: Del 13q/Del 1p, SMM_002: Del 17p, SMM_093: Del 14q, Del 20p) **(Figure 3A-C)**; all these events were associated with significant clonal expansion at the late timepoints, suggesting that copy number events have strong driving

potential and eventually become clonal, even when acquired during progression. The majority of alterations present at progression were already present at the SMM stage; however, in one out of five patients (SMM_060) sampled with a 5-year interval, there was evidence of a newly acquired subclone carrying a *KRAS* mutation, as well as Del13q/Del1p, during progression. In another case (SMM_064), a subclone carrying mutations in *KRAS*, *TP53*, *CDKN2C,* and *DIS3* appeared to have occurred during progression; however, both timepoints had low tumor purity precluding such conclusion. In one case that has not progressed (SMM_077) and was sampled twice at the SMM stage with an 8-year interval, no new alterations or increase in tumor burden was observed **(Figure 3D**), suggesting that the presence of clonal branching and heterogeneity is not enough to lead to disease progression; instead, either the functional impact of genomic alterations or tumor cell-extrinsic factors. In all five cases, we observed changes in the cancer cell fraction (CCF) of subclones.

Identifying genomic predictors of progression from SMM to MM

To define biomarkers of progression, we used a subset of patients (n = 85) who did not receive any treatment before progression to MM. Their baseline characteristics are reported in **Supp. Table 2**. Median follow-up time for all patients was 6.2 years. Median TTP was 3.9 years. In this cohort, 53 patients (62%) have progressed, while 32 (38%) remained asymptomatic. The genomic landscape is illustrated according to progression status in **Supp. Figure 3.**

Patients harboring *MYC* aberrations (translocations or amplifications) had the shortest median TTP (8.4 vs. 51.6 months, $p < 0.001$), followed by those with MAPK pathway mutations (14.4 vs. 60 months, $p < 0.001$), and DNA repair pathway alterations (15.6 vs. 50.4 months, $p = 0.004$) **(Figure 4).** Moreover, t(4;14), as well as del(1p), del(8p) and biallelic deletions events (including *TP53*, *RB1*, *DIS3*, *MAX*, and *CDKN2A*), were associated with shorter TTP **(Supp. Figure 4)**.

MAPK pathway mutations and *MYC* alterations were associated with higher bone marrow infiltration at time of diagnosis ($p = 0.001$ and < 0.001, respectively). MAPK pathway mutations were the only alteration that significantly correlated with M protein levels ($p < 0.001$), while none of these high-risk genomic alterations correlated with FLCr **(Supp Figure 5)**. Interestingly, although not a high-risk feature, t(11;14) was associated with the light-chain SMM subtype (p =

0.002) and lower M-protein levels compared to other primary events **(Supp. Table 3A, 3B and Supp. Figure 6)**.

Developing a genomic model for prediction of progression to MM

We searched for independent risk factors of progression from SMM to MM to develop a genomic model for prediction of progression **(Supp. Table 4)**. Four genomic features were independent predictors of progression: *MYC* aberrations, alterations in the DNA repair and MAPK (*KRAS*, *NRAS* SNVs) pathways, and t(4;14) translocation (**Supp. Figure 7C**). Of note, in a multivariable model accounting for the Mayo 2018 clinical risk stratification, all but t(4;14) were independent risk factors of progression **(Figure 4D)**. Thus, our model is predicated upon these three genomic alterations (GA).

Patients with one or more of the high-risk GA had significantly shorter TTP (1.2 vs. 7.2 years, respectively, $p < 0.001$) **(Figure 5C)**. Moreover, patients with 0, 1, or ≥2 high-risk factors, had a significantly different TTP **(Supp. Figure 7A)**. Of note, in our cohort, patients with any of these high-risk alterations, regardless of their clinical risk group, progressed even faster than patients who are considered high-risk by Mayo 2018 model (1.3 vs. 3.4 years, $p = 0.006$). Moreover, clinically intermediate and high-risk patients with these genomic alterations had significantly shorter TTP (2.6 vs 9.3 years, $p = 0.004$ and 1.2 vs 3.5 years, $p = 0.001$, respectively). **(Figure 5A, 5B)**. Of note, these results are independent of the clinical model used, Mayo 2008 or 2018 **(Supp. Figure 8A, 8B)**. Interestingly, high-risk GA were found in patients described as low risk by both models, in whom they conferred a significantly increased risk of progression (**Supp. Figure 8C, 8D**). Importantly, our genomic model improved the prediction of progression when added to the Mayo 2008 or 2018 models ($p < 0.001$, C-statistic: 0.66 vs 0.75 and 0.72 vs 0.77, respectively) (Table 1 and Supp. Table 5).

External validation of the genomic prediction model

To test the robustness and generalizability of our model, we validated it in an external cohort of 72 patients with SMM, whose tumor DNA had been previously sequenced [7]. Forty-seven

patients in this cohort progressed to MM with a median TTP of 5 years. The cohort's characteristics are summarized in **Supp Table 5**. Again, we found that patients with any of the high-risk GA (*n* = 47) had a higher risk of progression (2.5 vs. 10 years, *p* = 0.001) **(Figure 5D)**. Similarly, patients with 0, 1, or ≥2 high-risk factors had different risks of progression **(Supp. Figure 7B)**. Moreover, low, intermediate and high-risk patients, as defined by the Mayo 2018 model, with high-risk GA had shorter TTP (9.7 vs. not reached, *p* = 0.028, and 2.3 vs. 6.9 years, *p* = 0.01, respectively) **(Supp. Figure 9A, 9B)**. These findings held true, when we used the Mayo 2008 model as well **(Supp. Figure 9C, 9D)**. Importantly, in a multivariate analysis accounting for clinical risk group in this cohort, the genomic model was an independent risk factor of progression; when combined with the 2008 or 2018 clinical models, the genomic model performed better than the clinical model alone (*p* < 0.001 and 0.001, C-statistic: 0.57 vs 0.66 and 0.61 vs 0.67, respectively) (Table 1 and Supp. Table 6).

Analysis of mutational signatures in SMM and their impact on progression:

We analyzed our WES data for specific mutational signatures that play an important role in myeloma pathogenesis and development: aging (COSMIC SBS1 and SBS5), adenosine-induced deaminase (AID), and APOBEC signatures. As expected, the majority of mutations were associated with aging signatures, while there was roughly equal contribution of AID (n = 217) and APOBEC (n = 250) signatures. Among myeloma recurrently mutated genes, the majority of events were attributable to aging signatures. However, we observed APOBEC-associated mutations in fourteen MM driver genes, and AID-associated mutations in eleven **(Supp. Figure 10E)**. Of note, all three mutations in *ZNF292* were attributed to APOBEC, and t(14;16) had a significantly higher number of APOBEC mutations compared to the rest of the cohort (*p* = 0.005).

AID-associated mutations were observed in most of the patients with no significant difference between progressors and non-progressors (*p* > 0.99) **(Supp. Figure 10A)**. However, we found APOBEC to be significantly enriched in patients who progressed, after accounting for total mutation burden (*p* = 0.029) **(Supp. Figure 10B)**. Patients with versus those without APOBEC-associated mutations and those with more than the median number of APOBEC mutations (2.5)

had shorter TTP **(Supp. Figures 10C, 10D)**. Interestingly, although approximately 40% of APOBEC-associated mutations were clonal, patients with more than 50% subclonal APOBEC-associated mutations were enriched for progressors (p = 0.046). Although APOBEC can be an early event in certain patients, this data indicate that continuous activity may underlie disease progression.

Discussion:

In this study, we leveraged next-generation sequencing technology to analyze diagnostic tumor samples of 214 SMM patients. We observed that SMM has similar genetic make-up to newly diagnosed MM, with similar mutation density and clonal heterogeneity. Although most of the SCNAs were clonal, del 1p and del 17p were mainly subclonal, suggesting that not all SCNAs are acquired early in a single catastrophic event, and that in some cases the clonal CNA profile could reflect the serial acquisition of certain SCNAs that eventually grew into clonal status. Indeed, in three of our patients with serial samples available, some clonal SCNAs at progression were subclonal at SMM diagnosis. On the other hand, SNVs affecting the most common MM drivers were mainly subclonal, suggesting they are acquired later during disease progression. Nevertheless, mutations in *KLHL6*, *TP53, DIS3, and MAX* were mostly clonal, indicating that in certain instances driver mutations can perhaps be acquired early and drive clonal expansion.

Taken together, these findings indicate that MM disease progression, from tumorigenesis all the way to smoldering and overt MM, is likely governed by the serial acquisition of genetic alterations, most of which will have been acquired by the time of SMM diagnosis. It should however be noted that in all five patients with serial samples, we found evidence of clonal evolution, with subclone CCFs changing over time, which argues against the existence of a static model of progression in which the exact same clonal composition is observed in both the SMM and the MM stage [8]. Based on our findings, we can expect some new driver alterations to be acquired between SMM diagnosis and progression to MM in a few patients; however, for the majority of patients, the tumor's genetic make-up will have been largely shaped by the time of SMM diagnosis. This "right-skewed" model of MM genetic evolution, whereby the bulk of genetic alterations have already occurred by the time of SMM diagnosis, allows for reliable prognostication based on genetic biomarkers discovered at SMM diagnosis.

Given these observations, we asked whether we could leverage diagnostic SMM samples to identify genomic predictors of progression that reflect an aggressive underlying biology and act independently of tumor burden. Specifically, we analyzed 85 untreated patients, excluding those

who had been treated on SMM clinical trials. We found that mutations affecting genes in the MAPK and DNA repair pathway, as well as *MYC* translocations or amplifications, were all associated with a higher risk of progression to MM, even after accounting for the two currently used clinical risk stratification models. Namely, SMM patients who carried any of the above alterations were at higher risk of progression, compared to those in the same clinical risk group without them. Furthermore, we successfully validated our model in an external cohort of SMM patients. In both cohorts, the genetic model improved the prediction of progression when added to the current clinical models, as assessed by both a likelihood ratio test and the C-statistic, suggesting that these genetic biomarkers are robust and can be reliably used for improved prognostication.

In an era where clinical-grade NGS is available in many centers, we could envision the stratification of SMM patients being based on both clinical and genomic biomarkers. One of the limitations of the current clinical models is dichotomizing continuous variables, such as M protein, FLC ratio and others, which decreases the accuracy and predictability of such models. Using our genomic model helped to better stratify by identifying biologically aggressive group that is as important a predictor as increased tumor burden. And while MAPK/DNA repair pathway alterations and *MYC* aberrations should certainly be part of the prognostic genomic features, based on the results from this and other studies [7], there are more features that appear to be significant predictors of progression suggesting that larger cohorts are necessary to assess their impact. Such candidates include gene expression signatures in SMM [9], Del 1p, Del 8p and APOBEC activity.

In conclusion, sequencing a large cohort of SMM patients has allowed us to understand that SMM is a genetically mature tumor with slight differences from overt myeloma, suggesting a right-skewed model of genetic evolution from MGUS to MM, whereby most driver genetic alterations have already occurred by the SMM stage. Therefore, we believe that sequencing patients' tumors at the time of SMM diagnosis represents an improved strategy for identifying patients at high risk of progression who could benefit from early intervention.

Authors Contribution:

M.B, S.M, R.S.P, K.S, I.M.G and G.G conceived the study. M.B, R.S.P, R.R, E.K, J.P, C.J.N, S.C, and L.B collected the data. M.B, K.S, T.H.M, M.R, C.J.N, A.D, and C.J.B performed the experiments and prepared DNA libraries. M.B, R.S.P, J.P, R.R, B.Z, J.P, S.A, A.J.D, L.E, J.C, I.L, L.T, F.A, C.S, P.L.B, I.M.G and G.G analyzed the data. M.B, R.S.P, R.R, S.M, G.G and I.M.G wrote the manuscript. All the authors have contributed in the scientific discussion, reviewed and edited the manuscript and agreed on its content.

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Conflict of Interests:

There was no commercial funding for this study. M.B has advisory role and received Honoraria from Takeda and has received honoraria Dava Oncology. E.K has received honoraria and research funding from Amgen, Genesis Pharma, Janssen, Takeda, and Prothena. R.J.S is on the Data and Safety Monitoring Board of Juno and Celgene, has consulting role with Gilead, Merck, and Astellas, and is on the Board of Directors of Kiadis. M.A.D has received honoraria from Amgen, Celgene, Janssen, Takeda. I.M.G has a consulting and advisory role with Celgene, Takeda, Bristol-Myers Squibb, Genentech, Janssen Pharmaceuticals, and Amgen, and has received research funding/ honoraria from Celgene, Takeda, Bristol-Myers Squibb, Janssen Pharmaceuticals, and Amgen.

Tables and Figures Legends:

Figure 1: The genomic profile of the 214 SMM patients divided into SNVs (upper 6 panels), SCNAs (yellow panel), and translocations (bottom panel). The risk stratification according to Mayo 2018 risk model (low, intermediate, and high-risk colored as red, green and blue, respectively).

Figure 2: A) Correlation matrix of the 214 patients demonstrating the significant associations and co-occurrence of different MM drivers (adjusted $p < 0.05$). The size of the bubble corresponds to the odds ratio. The blue color indicates negative correlations, while the red color depicts positive ones. **(B)** The clonal proportions of recurrent SCNAs and **(C)** SNVs across the samples.

Figure 3: Fish plots of 4 SMM cases with two serial samples at different timepoints. In figures 2A-C, timepoints correspond to time of SMM diagnosis and time of progression to MM. In figure 2D, both timepoints are at the SMM stage, as the patient has not progressed to date.

Figure 4: Kaplan-Meier curves for analysis of TTP in patients with**: A)** MAPK pathway mutations (*KRAS* and *NRAS*). **B)** MYC alterations, including translocation and amplifications. **C)** DNA repair pathway alterations (deletion 17p, *TP53* and *ATM* SNVs). **D)** Forest plots of multivariate cox-regression of the genomic alterations and clinical the clinical risk model.

Figure 5: A) Kaplan-Meier curves of clinically high-risk patients with or without the high-risk genomic alterations **B)** Kaplan-Meier curves of clinically intermediate-risk patients with or without the high-risk genomic alterations. **C**) Kaplan-Meier curves of patients with or without the high-risk genomic alterations in the Dana-Farber multicenter cohort **D)** Kaplan-Meier curves of patients with or without the high-risk genomic alterations in the Mayo Clinic validation cohort.

Table 1: Performance of the clinical models with and without the genetic model. Improvement in goodness of fit was assessed with a likelihood ratio test. The genetic model significantly improved the fit of the clinical-only models. A global assessment of each model was also assessed

using a C-statistic for censored survival data[10]. The statistic for each time-to-event model is reported with a 95% confidence interval. Values range between 0.5 to 1 indicating a poor to perfect model.

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Figure 1. The genomic landscape of the full SMM cohort (n=214).

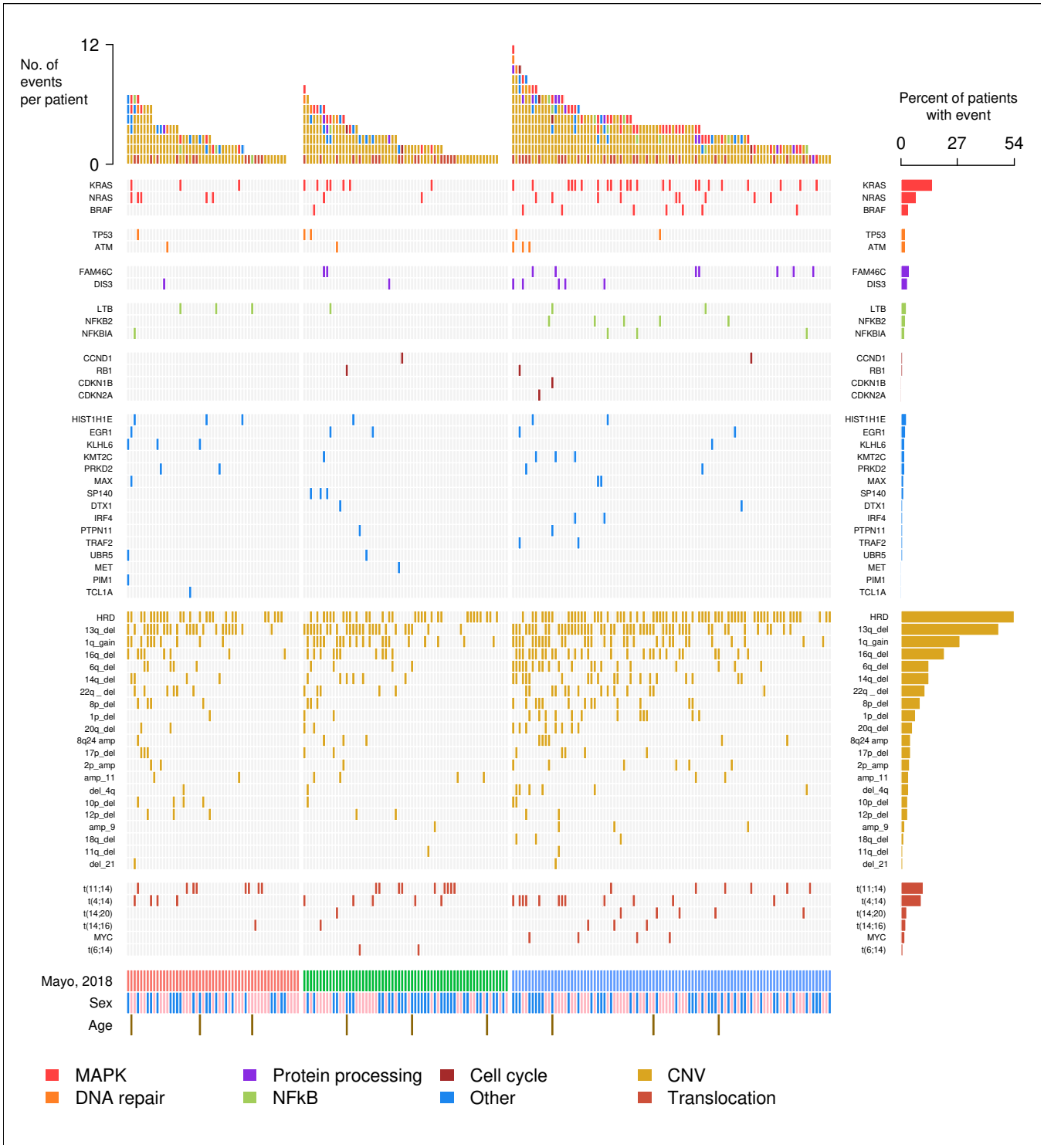
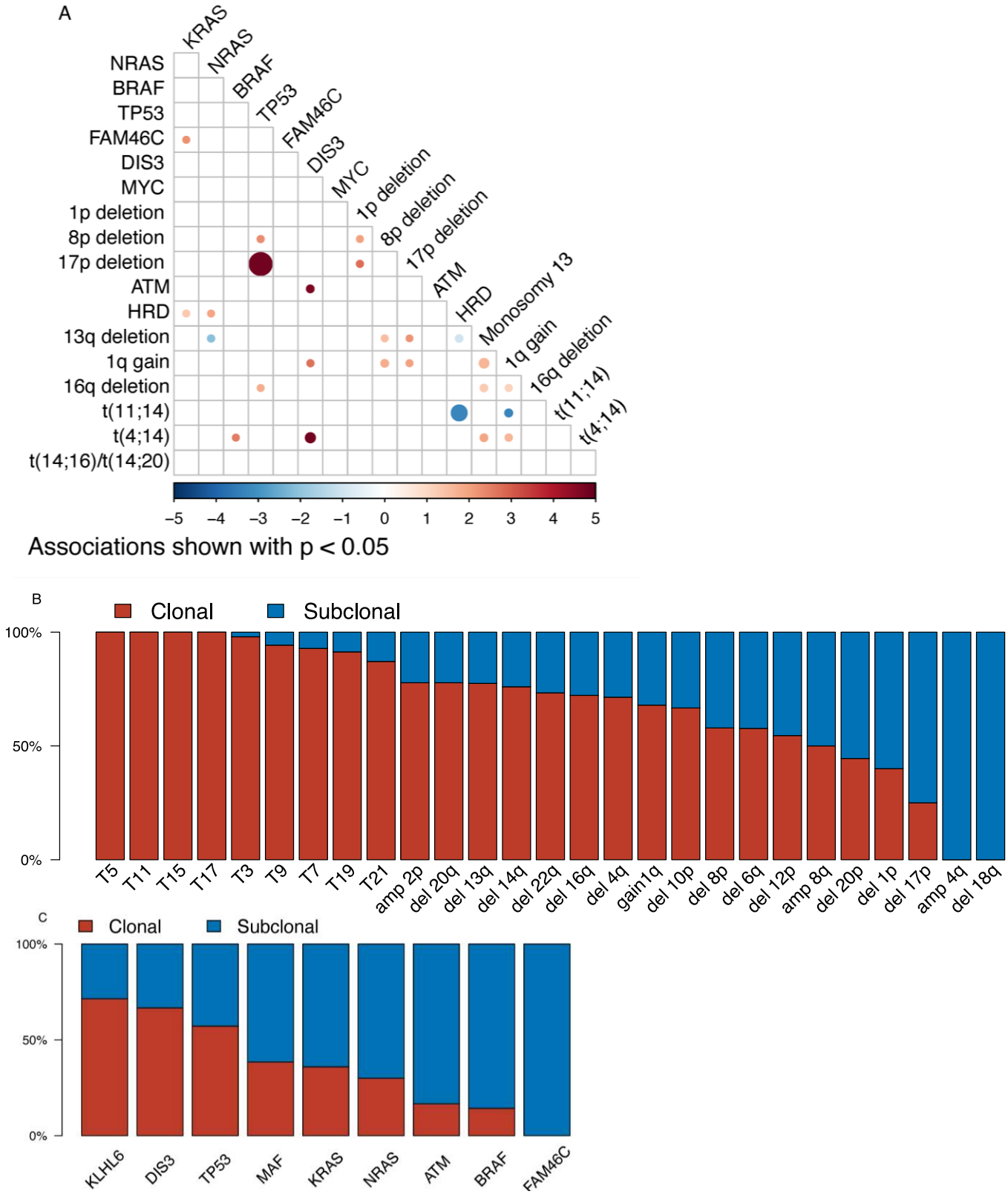


Figure 2:


A
KRAS
NRAS
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FAM46C
DIS3
MYC
1p deletion
8p deletion
17p deletion
ATM
HRD
Monosomy 13
1q gain
16q deletion
t(11;14)
t(4;14)
NRAS
BRAF
TP53
FAM46C
DIS3
MYC
1p deletion
8p deletion
17p deletion
ATM
HRD
13q deletion
1q gain
16q deletion
t(11;14)
t(4;14)
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−5 −4 −3 −2 −1 0 1 2 3 4 5
Associations shown with p < 0.05
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Clonal
Subclonal
100%
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T5 T11 T15 T17 T3 T9 T7 T19 T21 amp 2p del 20q del 13q del 14q del 22q del 16q del 4q gain1q del 10p del 8p del 6q del 12p amp 8q del 20p del 1p del 17p amp 4q del 18q
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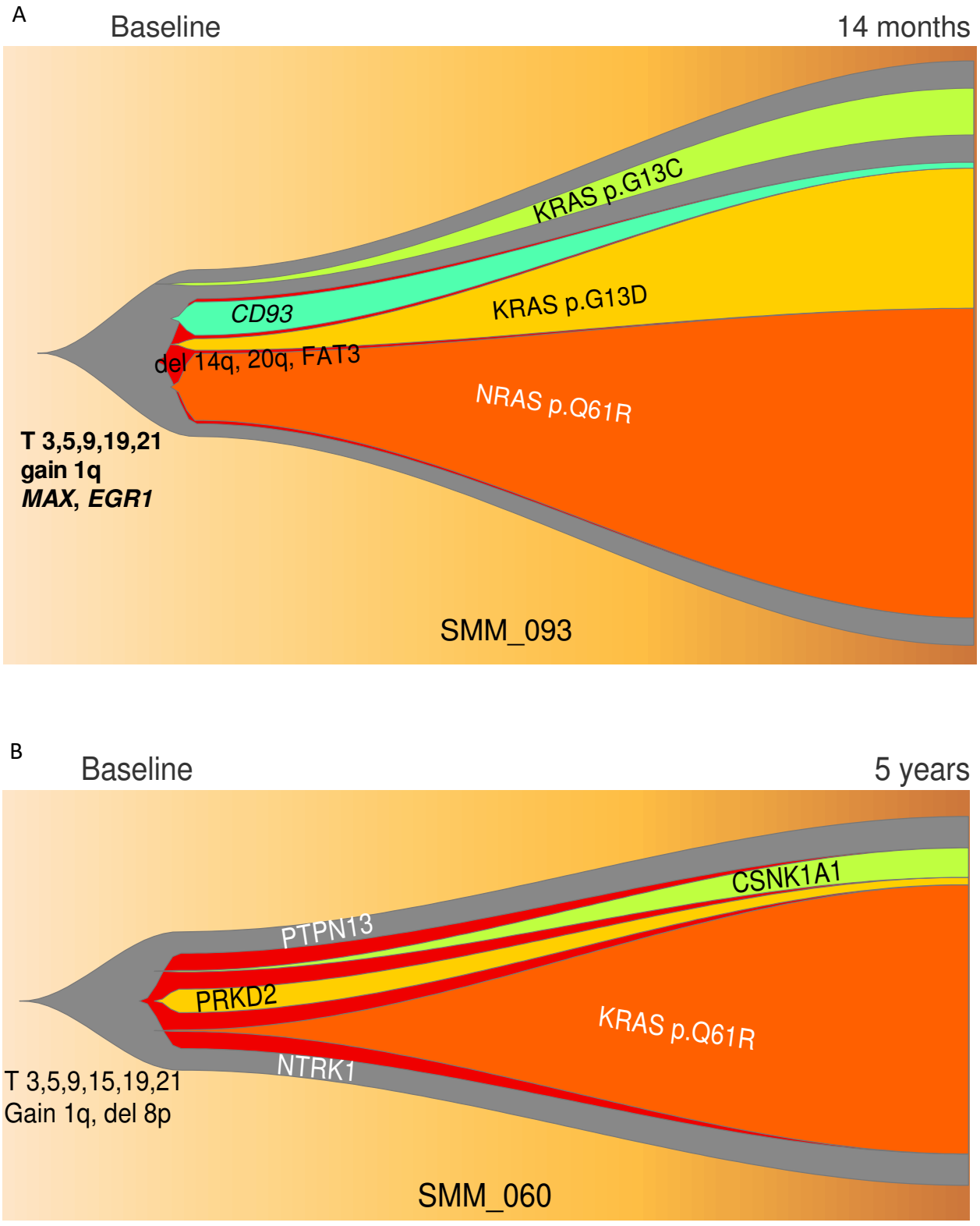
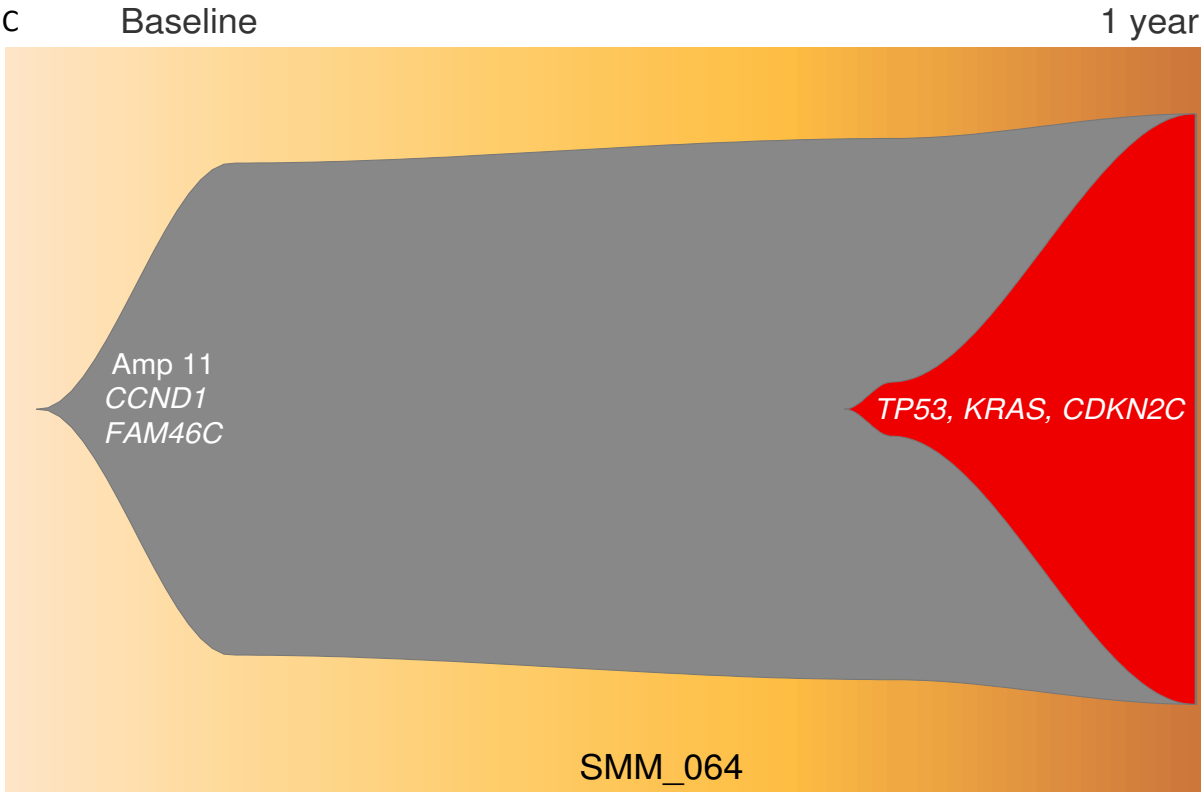
Clonal
Subclonal
100%
50%
0%
KLHL6 DIS3 TP53 MAF KRAS NRAS ATM BRAF FAM46C

Figure 3:


A
Baseline
14 months
KRAS p.G13C
CD93
KRAS p.G13D
del 14q, 20q, FAT3
NRAS p.Q61R
T 3,5,9,19,21
gain 1q
MAX, EGR1
SMM_093
B
Baseline
5 years
CSNK1A1
PTPN13
PRKD2
KRAS p.Q61R
NTRK1
T 3,5,9,15,19,21
Gain 1q, del 8p
SMM_060

C


Baseline
1 year
Amp 11
CCND1
FAM46C
TP53, KRAS, CDKN2C
SMM_064

D

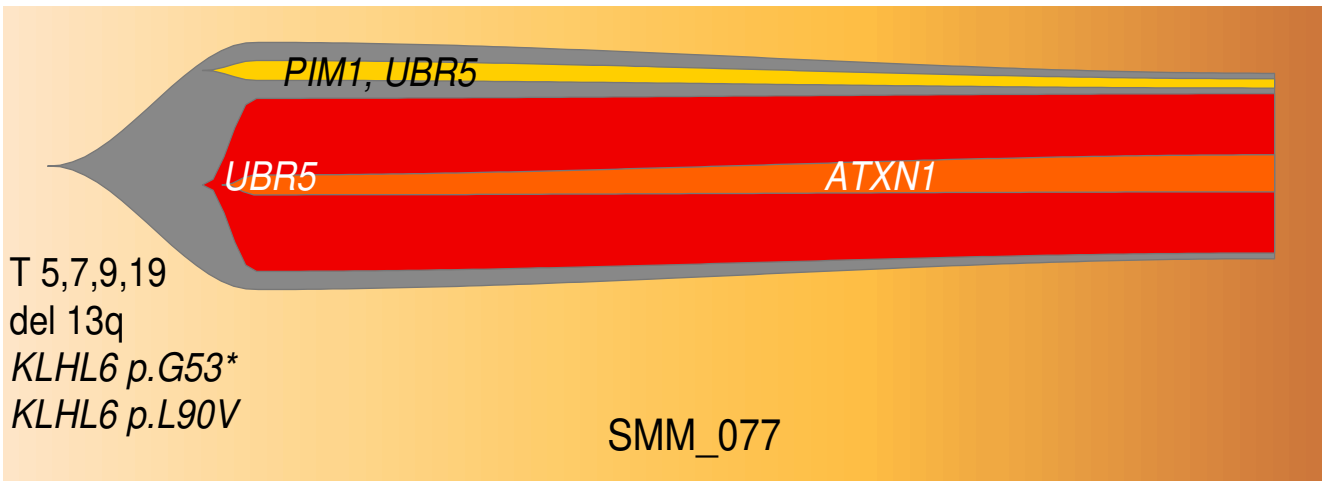

Baseline
8 years
PIM1, UBR5
UBR5
ATXN1
T 5,7,9,19
del 13q
KLHL6 p.G53*
KLHL6 p.L90V
SMM_077

Figure 4:

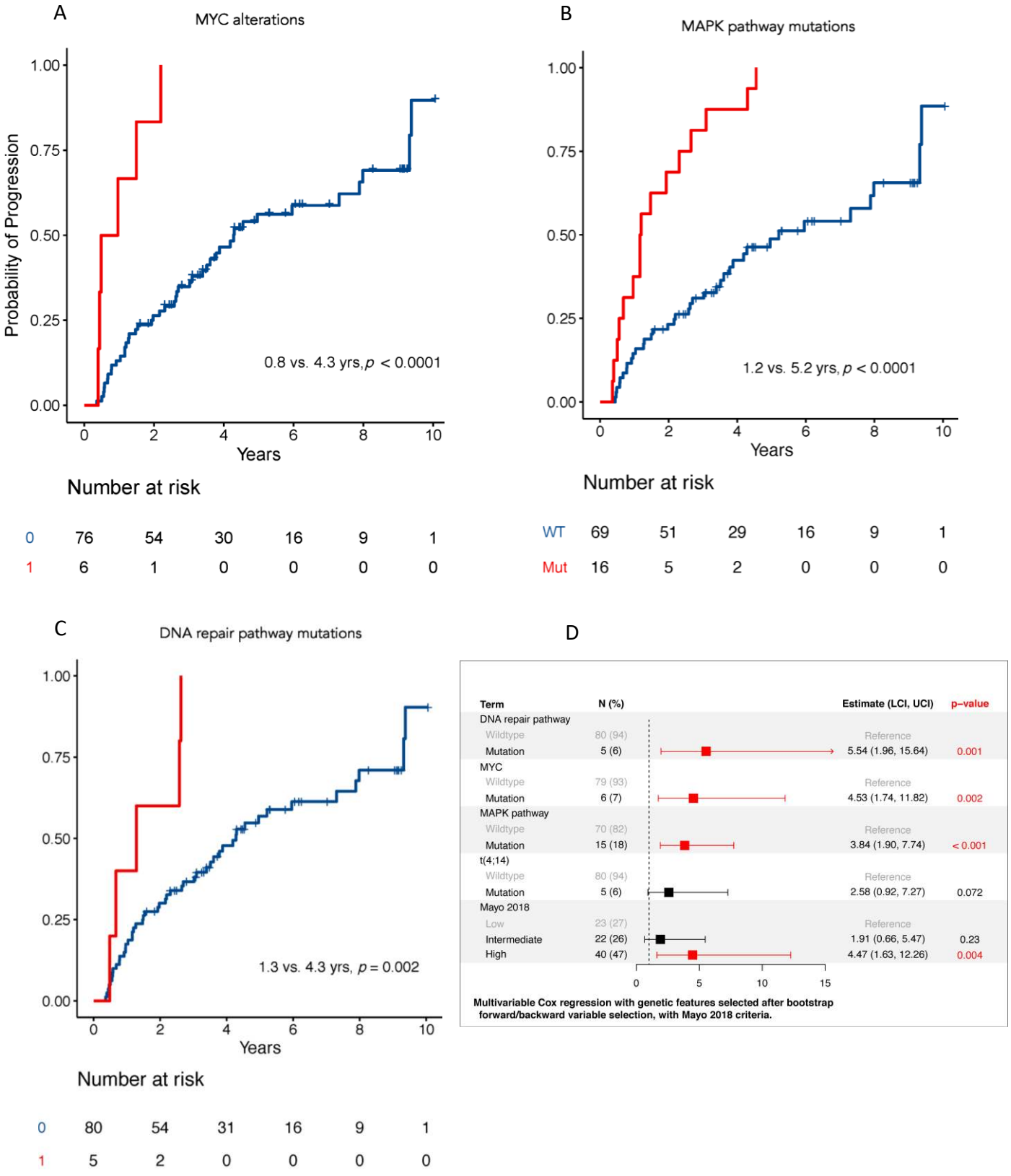

A
MYC alterations
Probability of Progression
1.00
0.75
0.50
0.25
0.00
0.8 vs. 4.3 yrs, p < 0.0001
0 2 4 6 8 10
Years
Number at risk
0 76 54 30 16 9 1
1 6 1 0 0 0 0
B
MAPK pathway mutations
1.00
0.75
0.50
0.25
0.00
1.2 vs. 5.2 yrs, p < 0.0001
0 2 4 6 8 10
Years
Number at risk
WT 69 51 29 16 9 1
Mut 16 5 2 0 0 0
C
DNA repair pathway mutations
1.00
0.75
0.50
0.25
0.00
1.3 vs. 4.3 yrs, p = 0.002
0 2 4 6 8 10
Years
Number at risk
0 80 54 31 16 9 1
1 5 2 0 0 0 0
D
Term
N (%)
Estimate (LCI, UCI)
p-value
DNA repair pathway
Wildtype 80 (94) Reference
Mutation 5 (6) 5.54 (1.96, 15.64) 0.001
MYC
Wildtype 79 (93) Reference
Mutation 6 (7) 4.53 (1.74, 11.82) 0.002
MAPK pathway
Wildtype 70 (82) Reference
Mutation 15 (18) 3.84 (1.90, 7.74) < 0.001
t(4;14)
Wildtype 80 (94) Reference
Mutation 5 (6) 2.58 (0.92, 7.27) 0.072
Mayo 2018
Low 23 (27) Reference
Intermediate 22 (26) 1.91 (0.66, 5.47) 0.23
High 40 (47) 4.47 (1.63, 12.26) 0.004
0 5 10 15
Multivariable Cox regression with genetic features selected after bootstrap forward/backward variable selection, with Mayo 2018 criteria.

Figure 5:

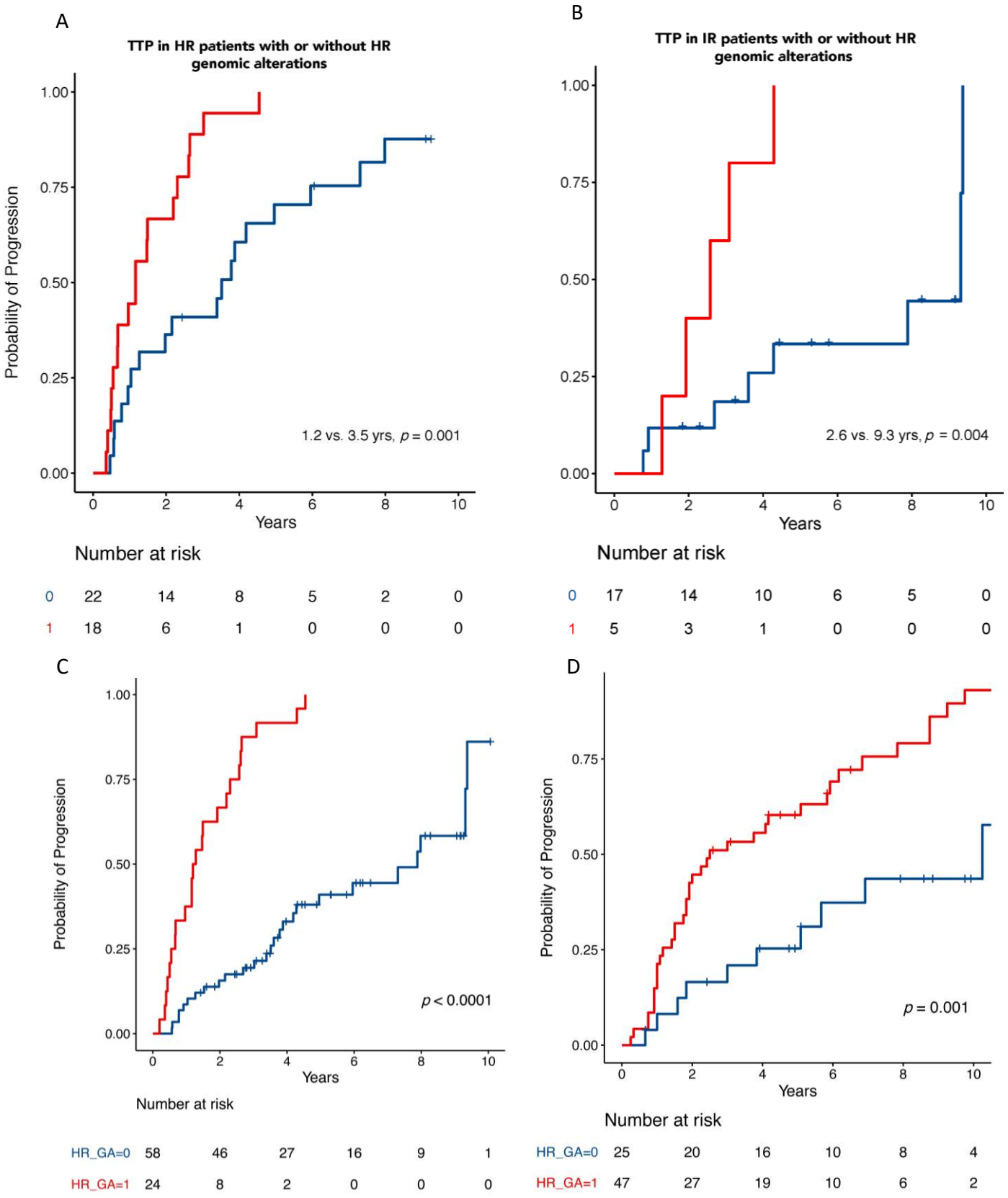

A
TTP in HR patients with or without HR genomic alterations
Probability of Progression
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
Years
1.2 vs. 3.5 yrs, p = 0.001
Number at risk
0 22 14 8 5 2 0
1 18 6 1 0 0 0
B
TTP in IR patients with or without HR genomic alterations
2.6 vs. 9.3 yrs, p = 0.004
Number at risk
0 17 14 10 6 5 0
1 5 3 1 0 0 0
C
p < 0.0001
Number at risk
HR_GA=0 58 46 27 16 9 1
HR_GA=1 24 8 2 0 0 0
D
p = 0.001
Number at risk
HR_GA=0 25 20 16 10 8 4
HR_GA=1 47 27 19 10 6 2

Table 1 :

Model	Likelihood ratio test statistic	Chi-square p-value	C-statistic (95% CI)
Primary cohort			
Mayo 2008	32.02	<0.001	0.66 (0.57 - 0.74)
Mayo 2008 + Genetic model			0.75 (0.65 - 0.86)
Mayo 2018	20.46	<0.001	0.72 (0.64 - 0.80)
Mayo 2018 + Genetic model			0.77 (0.70 - 0.85)
Validation Cohort			
Mayo 2008	12.62	<0.001	0.57 (0.47 - 0.67)
Mayo 2008 + Genetic model			0.66 (0.56 - 0.76)
Mayo 2018	10.19	0.001	0.61 (0.49 - 0.74)
Mayo 2018 + Genetic model			0.67 (0.56 - 0.77)