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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

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Abstract

The association of genetic lesions detected by FISH with survival was analyzed in 1069 patients with newly presenting myeloma treated in the Medical Research Council (MRC) Myeloma IX trial, with the aim of identifying patients associated with the worst prognosis. A comprehensive FISH panel was performed, and the lesions associated with short PFS and OS in multivariate analysis were +1q21, del(17p13) and an adverse *IGH* translocation group incorporating t(4;14), t(14;16) and t(14;20). These lesions frequently co-segregated, and there was an association between the accumulation of these adverse FISH lesions and a progressive impairment of survival. This observation was used to define a series of risk groups based on number of adverse lesions. Taking this approach we defined a favorable risk group by the absence of adverse genetic lesions, an intermediate group with 1 adverse lesion and a high risk group defined by the co-segregation of >1 adverse lesion. This genetic grouping was independent of the ISS and so was integrated with the ISS to identify an ultra-high risk group defined by ISS II or III and >1 adverse lesion. This group constituted 13.8 % of patients and was associated with a median OS of 19.4 months.

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Authorship Contributions

GJM, JAC and GHJ were chief investigators of the MRC Myeloma IX trial.

KDB, BAW, CPW and GJM designed research, analyzed data and wrote the paper.

JAC, GHJ and FED designed research and wrote the paper.

FMR designed research, performed research, analyzed data and wrote the paper.

LC, GP and ZJC performed research.

WMG, AJS, SEB and WJT analyzed data.

Disclosure of Conflict of Interest

The authors have no relevant conflicts of interest to report.

Introduction

The natural history of myeloma is highly variable. Following induction chemotherapy some patients will remain in a durable remission for many years, and with increasingly effective salvage therapies long term survival is a realistic prospect for these patients. At the other end of the clinical spectrum there is a subset of patients with a disease course characterized by rapid relapse and early progression to a refractory disease state. These patients have ultra-high risk myeloma, and patient groups with a median overall survival (OS) of less than 24 months can be considered to have a sufficiently poor prognosis to fall into this group. Identifying these patients at diagnosis is important as they are likely to have a poor outcome with standard therapeutic approaches, and it would be appropriate to target this group with alternative treatment strategies within the context of clinical trials. However, in order to carry out this approach, robust prognostic factors are needed.

It is recognised that prognosis in myeloma is related to both patient factors and tumor variables. The most widely applied prognostic system in myeloma is the International Staging System (ISS), which stratifies patients into three groups based on serum albumin and β_2 -microglobulin levels (1). These two variables reflect both patient and tumor factors, with β_2 -microglobulin being a measure of tumor bulk and renal function whilst albumin is associated with the general state of the patient. This system is well validated and easily applied, but it fails to identify individual patient risk in a robust enough fashion to justify a change in therapeutic approach. Attempts have therefore been made to improve on this system by identifying tumor genetic variables associated with short survival. Different technologies have been utilized to identify genetic variables associated with outcome, with one approach being gene expression profiling. The UAMS (University of Arkansas for Medical Sciences) group was the first to link gene expression to adverse survival in myeloma, with a gene expression signature that was enriched with over-expressed genes on 1q and under-expressed genes on 1p, and since then other groups have identified alternative gene expression signatures linked to short survival (2-4).

An alternative strategy is the use of fluorescence in situ hybridisation (FISH) to interrogate specific genetic lesions, and several lesions have been linked to impaired survival using this method. t(4;14) leads to the deregulation of *MMSET* and *FGFR3* and has been associated with adverse prognosis in a variety of clinical settings including high dose therapy with stem cell support (5-11). There is emerging data which suggests that the outcome of this group may be improved by treatment with bortezomib (12-14). t(14;16) is a rare translocation, resulting in up-regulation of *MAF* that has also been linked with adverse clinical outcome, although its prognostic significance has recently been questioned (6, 15-16). Del(17p13) is considered to be an important prognostic marker which has been associated with a negative effect on survival in a number of different treatment contexts (6, 10, 17-19). Del(13q14) is found in ~50% of myeloma tumors and until recently was thought to be associated with short survival (20-22). However, the relationship of del(13q14) and adverse prognosis is now thought to be secondary to the close association of del(13q14) with other high risk genetic lesions such as t(4;14) (23-24). Over-expression of genes on 1q is central to the high risk gene expression signature generated by the UAMS group, and gain of 1q21 detected by FISH has been linked to adverse prognosis in a patient series treated with tandem

transplantation (2, 25). However, its value as an independent FISH biomarker of adverse prognosis has not been validated by other groups (26). The International Myeloma Working Group (IMWG) recently evaluated these data and concluded that there was currently only evidence for testing for t(4;14), t(14;16) and del(17p13) when stratifying patients into genetic risk groups(27). In order to fully understand the impact of these lesions on clinical outcome we have performed a comprehensive FISH panel on patients entering the phase III clinical trial MRC Myeloma IX, and have studied the association of these FISH lesions with survival and existing prognostic factors with the aim of defining a group of patients with ultra-high risk disease.

Materials and Methods

Patients and Treatment

1960 patients with newly diagnosed myeloma requiring treatment were enrolled in the MRC Myeloma IX trial (ISRCTN68454111), the design and results of which have been reported elsewhere (28). In summary, the trial comprised an intensive and non-intensive pathway based on patient eligibility for autologous transplantation, which was decided after informed discussion between patient and treating physician, taking into account patient performance status, co-morbidities and age. Patients in the intensive pathway underwent an initial randomisation to CVAD (cyclophosphamide, vincristine, doxorubicin and dexamethasone) or CTD (cyclophosphamide, thalidomide and dexamethasone), followed by high dose melphalan with autologous stem cell rescue. In the non-intensive pathway patients were randomised to either MP (melphalan and prednisolone) or CTDA (attenuated cyclophosphamide, thalidomide and dexamethasone) to maximum response. Both pathways incorporated a maintenance randomisation to thalidomide versus no maintenance. Primary end-points included progression free survival (PFS) and overall survival (OS). Survival data were available from 1960 patients, 1111 from the intensive pathway and 849 from the non-intensive. 556 patients received CVAD, 555 CTD, 423 MP and 426 CTDA. Median follow up is 3.7 years.

FISH

1180 patients provided a bone marrow sample for FISH analysis, of which 1140 were suitable for testing. Diagnostic bone marrow samples were initially purified for plasma cells using CD138 magnetic microbeads (Miltenyi Biotec, Bisley, UK). FISH was performed on the purified plasma cells to detect the presence of an *IGH* translocation, the common *IGH* translocation partners (*FGFR3* at 4p16, *CCND3* at 6p21, *CCND1* at 11q13, *MAF* at 16q23 and *MAFB* at 20q12), hyperdiploid status using the iFISH ploidy classification, deletion of 1p32, 13q14, 16q23, 17p13, 22q11 and gain of 1q21 as previously described(23). The FISH failure rate was 6% of analyzable bone marrow specimens, providing results for 1069 patients. A subset of 869 cases had a complete data set of adverse immunoglobulin heavy chain gene (*IGH*) translocations, +1q21 and del(17p13).

Statistical Analysis

Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, USA) and R. Survival curves were plotted using the Kaplan-Meier method. Differences between

curves were tested for statistical significance using the log-rank test, with $p < 0.05$ taken as the level of significance. Variables associated with survival in univariate analysis at the $p < 0.05$ level were entered into the model for multivariate testing. Multivariate analysis of variables associated with survival used a backwards elimination Cox proportional hazards model, with factors retained if they were significant at $p < 0.05$.

Results

In univariate analysis the FISH lesions associated with adverse PFS were $t(4;14)$, $t(14;16)$, $+1q21$, $del(13q14)$ and $del(17p13)$ (table 1). Lesions associated with adverse OS were $t(4;14)$, $t(14;16)$, $t(14;20)$, $+1q21$, $del(13q14)$ and $del(17p13)$ (table 1). $t(14;16)$ and $t(14;20)$ are rare translocations, and behaved in a similar fashion to $t(4;14)$, so consequently were grouped together to form an adverse *IGH* translocation group. These FISH groups (the adverse *IGH* group, $del(13q14)$, $del(17p13)$ and $+1q21$) were entered into a multivariate analysis together with the ISS. The genetic groups associated with impaired PFS and OS in this multivariate testing were the adverse *IGH* translocation group, $del(17p13)$ and $+1q21$ (table 2). These groups had a clear association with short survival with the median OS for each group being 25.9 months, 26.7 months and 31.0 months respectively, and they were further examined in order to refine their association with adverse prognosis.

Inter-relationships of Adverse FISH Groups

The number of patients in each of the adverse FISH groups, and their inter-relationships, was assessed (figure 1). Of the cases analyzed by FISH, 11.4% had $t(4;14)$, 3.2% had $t(14;16)$ and 1.6% had $t(14;20)$, creating a combined 'adverse *IGH*' group ($n=145$) comprising 16.2% of the total. $+1q21$ was present in 38.9% of patients and $del(17p13)$ was detected in 8.4% of patients. There was a strong positive association between adverse *IGH* translocations and $+1q21$, with 72% of the adverse *IGH* group also carrying $+1q21$ compared to 32.4% of cases outside this group ($p < 0.001$). $del(17p13)$ was found in 18/145 (12.4%) of adverse *IGH* cases, which is similar to its background rate in the overall dataset. However, in 16/18 (89%) of these cases $del(17p13)$ co-segregated with both the adverse *IGH* translocation and $+1q21$.

Prognostic Impact of Adverse Lesions in Isolation

The association of each of these lesions with survival was assessed in patients where the lesion occurred in isolation. Adverse *IGH* translocations, $+1q21$ and $del(17p13)$ were all found to have a similar negative impact on PFS and OS as individual lesions, compared to samples lacking any of these lesions (figure 2). Interestingly, the prognostic impact of these lesions when they occurred in isolation was moderate, with a median OS of 44.2 months (adverse *IGH*), 41.9 months ($+1q21$) and 38.0 months ($del(17p13)$) compared to 60.6 months for patients without any adverse lesions.

Prognostic Impact of Adverse Lesions in Combination

The association of number of adverse lesions with survival was assessed, and a clear progressive association between the accumulation of adverse FISH lesions and impairment of survival was demonstrated (figure 3). In this model, patients with two adverse lesions

were associated with a median OS of 23.4 months, whilst the triple combination of an adverse *IGH* translocation, +1q21 and del(17p13) was associated with the worst survival (median OS 9.1 months).

This progressive association of number of adverse lesions and impairment of survival can be used to group myeloma patients into a series of risk groups. Taking this approach we defined a low risk group defined by the absence of adverse genetic lesions (median OS 60.6 months, median PFS 23.5 months), an intermediate group with 1 adverse lesion (median OS 41.9 months, median PFS 17.8 months) and a high risk group defined by the co-segregation of >1 adverse lesion (median OS 21.7 months, median PFS 11.7 months)(figure 4). In subgroup analyses, this prognostic grouping remained significantly associated with PFS and OS in patients treated intensively or non-intensively, in patients treated with thalidomide-based or conventional induction therapy, and in patients receiving thalidomide maintenance or no maintenance (supplementary data).

The Integration of Biological Groupings and the ISS

The ISS stage was significantly associated with survival in the same data set, with a median OS that was not reached for ISS I, 47.7 months for ISS II and 35.7 months for ISS III. The genetic groups defined by number of adverse lesions and the ISS were independently associated with PFS and OS in multivariate analysis (table 3). The genetic risk groups significantly stratified OS within the ISS II and III groups ($p < 0.001$), and conversely the ISS stratified outcome within the groups characterised by 0 and >1 adverse lesions ($p < 0.001$ and $p = 0.006$ respectively). Integrating the genetic risk groups with the ISS, therefore, has the potential to further refine the definition of risk for individual patients. When the two systems were combined, three distinct risk groups were evident based on the median OS associated with each group (table 4). In this way we were able to identify a favourable risk group, defined as patients with ISS I or II and no adverse genetic lesions or ISS I and 1 adverse lesion (median OS 67.8 months). At the opposite end of the clinical spectrum, ultra-high risk disease was defined by ISS II or III in the presence of >1 adverse lesion. This group constituted 13.8% of patients, and was associated with a median PFS of only 9.9 months and a median OS of 19.4 months. An intermediate risk group consisted of patients with ISS I and >1 adverse lesion, ISS II and 1 adverse lesion and ISS III with 0-1 adverse lesions, and this group was associated with a median OS of 41.3 months(figure 5). These groups were significantly associated with survival in subgroup analyses of patients treated with or without autologous transplantation, and in patients treated with or without thalidomide maintenance (supplementary data).

Discussion

We show that in the context of a large clinical trial of presenting myeloma patients incorporating both conventional and thalidomide-based therapies, the genetic lesions associated with adverse survival after multivariate analysis were del(17p13), +1q21 and an 'adverse *IGH* translocation group' comprising t(4;14), t(14;16) and t(14;20). The poor prognosis associated with the presence of t(4;14), t(14;16) and del(17p13) in our data set is consistent with the findings of the IMWG review, which recommended testing for these

lesions by FISH. However, the significance of t(14;16) has recently been questioned. The original data linking t(14;16) with short survival was based on the analysis of 15 patients with this abnormality by the Mayo clinic(6), and these data were strengthened by the association of over-expression of MAF with impaired prognosis by the UAMS group(29). However, this association of t(14;16) with adverse survival was not corroborated in an analysis of 30 patients with this abnormality by the IFM group(16). We analyzed the outcome of 33 patients with t(14;16), and found it to be positively associated with +1q21, which co-segregated with t(14;16) in 66.7% of cases. Cases with co-segregation of t(14;16) and +1q21 were associated with a median OS of 12.6 months, compared to 47.7 months when t(14;16) occurred in isolation. This provides evidence that co-segregating adverse FISH lesions, especially +1q21, can modulate the prognostic impact of t(14;16). As the numbers of patients with this rare translocation analyzed by us and the IFM group are low, it is possible that the rate of +1q21 may differ in the two groups, which could be crucial to the survival analysis of these small patient series. Alternatively, the differing therapeutic approaches employed in the two data sets may explain the result, with the IFM patients receiving a tandem-autologous transplantation approach. Further studies analyzing t(14;16) in conjunction with +1q21 and del(17p) are warranted to confirm or refute these findings. However, t(14;16) was present in 12.6% of the ultra-high risk group defined by the combination of genetic lesions and FISH, so although it is a rare lesion in the overall myeloma population it is an integral part of the definition of high risk disease in our data set.

In addition to the FISH lesions previously identified as important by the IMWG review, we clearly show that it is important to test for +1q21 and t(14;20). +1q21 is common in presenting myeloma, especially in the presence of an *IGH* translocation, and its association with adverse prognosis reflects the importance of over-expression of genes on 1q in some high risk gene expression signatures(2). In comparison, t(14;20) is a rare lesion, being found in 1.6% of our cases, but it had a clear association with short survival and constituted 8% of the ultra-high risk group, so it is important that it is tested for. Based on the similar median survival times associated with adverse *IGH* translocations, +1q21 and del(17p) when they occurred in isolation, and the identical hazard ratios associated with each lesion in multivariate analysis, we conclude that none of these lesions has a greater negative impact on survival than the others.

We show that these three groups are not mutually exclusive but frequently co-segregate, with +1q21 and the adverse translocation group showing a positive association. Importantly, we show that these relationships are clinically significant, with the accumulation of adverse genetic lesions being strongly associated with a progressive impairment of PFS and OS. It is therefore important to know the presence or absence of each of these lesions in order to properly interpret their clinical significance, with combinations of lesions being associated with the worst prognosis. When considering prediction of outcome for the individual, therefore, it is as important to know how many of these lesions are present as which lesion is present. This finding is consistent with the multistep model of myeloma pathogenesis and progression (27). In this model, *IGH* translocations and hyperdiploidy are primary events, whilst +1q21 and del(17p13) are associated with disease progression. As primary events, adverse *IGH* translocations such as t(14;20) have been described in indolent disease states

such as MGUS and asymptomatic myeloma, so the fact that they are not intrinsically associated with high risk disease in the absence of co-operating genetic events fits this pathological model (30). Similarly, the prognostic impact of progression events such as +1q21 and del(17p13) will be modulated by the etiological background of the myeloma clone, so that +1q21 in association with t(4;14) is likely to have a different clinical phenotype to +1q21 in association with hyperdiploidy.

We have used these observations to develop a prognostic classification system based on the accumulation of +1q21, del(17p13) and adverse *IGH* translocations. In this classification low risk disease is defined by the absence of any of these lesions, intermediate risk by the presence of 1 adverse lesion, and high risk by the presence of >1 adverse lesion. This grouping is different from other FISH-based prognostic systems, which treat these FISH lesions as mutually exclusive groups, as it recognises the relationships of these genetic lesions and takes into account some of the complexity of the myeloma clone. This genetic grouping does not replace existing classification systems such as the ISS and there was, in fact, little overlap between the genetic risk groups and the ISS. Consequently, integrating the ISS and these genetic risk groups provides a more accurate definition of risk for an individual patient. Using this approach, an ultra-high risk group characterised by >1 adverse genetic lesions and ISS II or III was defined. This group was small, comprising 13.8% of patients, and was associated with a median PFS of 9.9 months and a median OS of 19.4 months. These patients require alternative therapeutic strategies and should be the focus of clinical trials incorporating dose intensification, allogeneic transplantation or novel agents.

Gene expression signatures associated with high risk myeloma, such as those produced by the UAMS and IFM groups, generally had hazard ratios of >5 in their respective data sets, falling to >2 when applied to other data sets(2-3). In comparison, the ultra-high risk group formed by the combination of FISH and the ISS was associated with a hazard ratio of 4.16. The difficulty with gene expression signatures in myeloma is that there is currently no firm consensus; the UAMS 17-gene signature, IFM 15-gene signature and our own 6-gene signature share no common genes, and more work is required to define common genes, regions or gene functionality before these signatures can be more widely applied. Until then, whilst high risk gene expression signatures remain valid and useful tools within the context of specialist centers, and provide valuable insight into myeloma biology, their application in a multi-centered setting is uncertain. In comparison there is an emerging consensus regarding FISH lesions associated with short survival, and this study demonstrated that defining cases with co-segregation of these lesions, and incorporating the ISS, can refine these associations to identify a group of patients associated with a very poor clinical outcome. This system was strongly associated with survival in the overall data set, and in subgroup analyses of all treatment contexts: patients treated with conventional induction therapy, patients treated with thalidomide-based induction therapy, patients treated with high dose melphalan, patients treated non-intensively, patients treated with thalidomide maintenance and patients who did not received maintenance. The system would require validation in other treatment settings such as bortezomib therapy. Some reports have suggested that bortezomib therapy may overcome the adverse prognosis associated with some adverse genetic lesions, although the largest study addressing this question found that

t(4;14) and del(17p13) remained prognostic parameters in patients receiving bortezomib induction(14). This suggests that this model may remain prognostic in bortezomib treated patients, and even if the individual lesions included in the model require reassessment in alternative therapeutic contexts, the concept of a summative impact on survival of co-segregating adverse lesions is likely to remain relevant.

In conclusion, this study demonstrates that t(4;14), t(14;16), t(14;20), +1q21 and del(17p13) can be used to define adverse prognosis in myeloma in the context of thalidomide and conventional induction therapy, with or without autologous transplantation, and patients with the worst clinical outcome are identified by the co-segregation of more than one of these lesions. We also show the importance of combining clinical and genetic factors for predicting patient outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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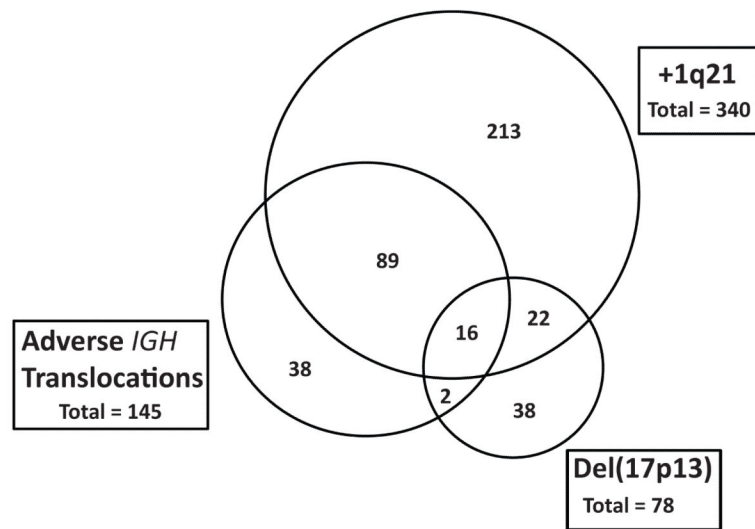


Figure 1. Venn diagram of the relationship of each of the adverse FISH groups (adverse *IGH* translocations, +1q21 and del(17p13)).

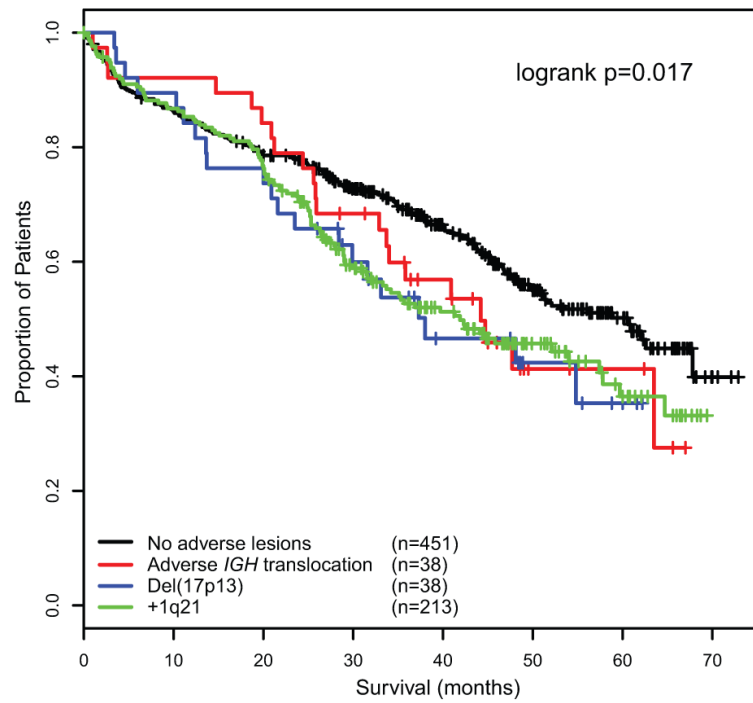


Figure 2. OS of each of the adverse FISH lesions when they occur in isolation compared to samples lacking any adverse lesions.

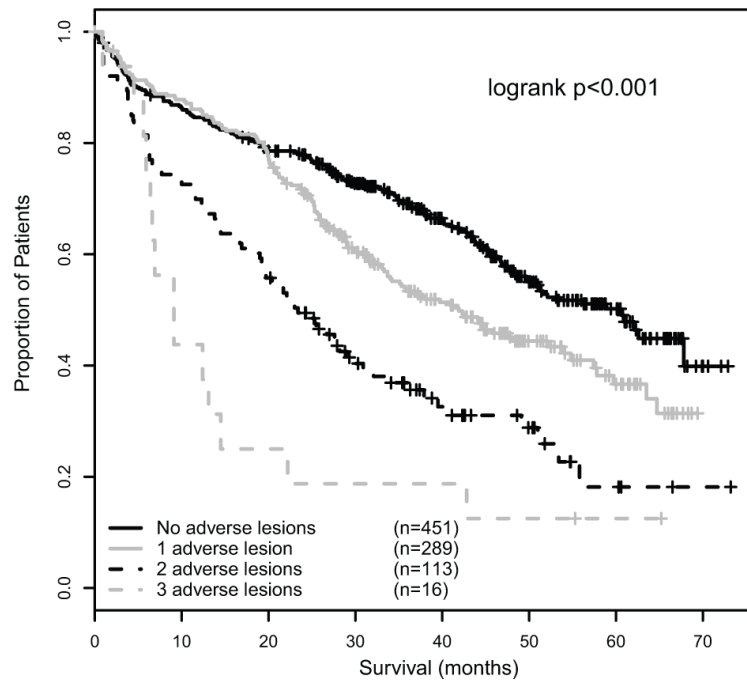


Figure 3. OS graded by number of adverse lesions, showing the progressive impact of accumulation of adverse lesions.

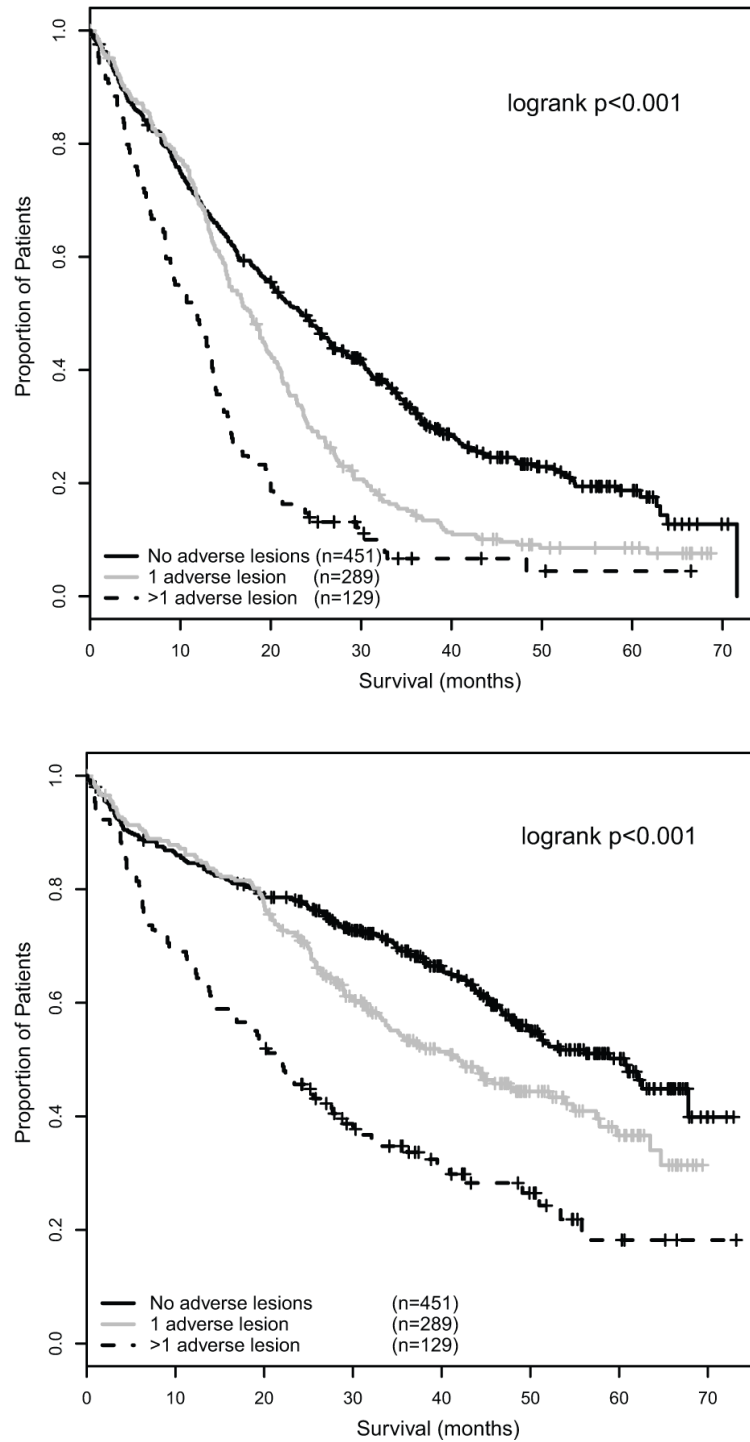


Figure 4.

A – PFS of genetic risk groups defined by the presence of 0, 1 and >1 adverse FISH lesions respectively.

B – OS of genetic risk groups defined by the presence of 0, 1 and >1 adverse FISH lesions respectively.

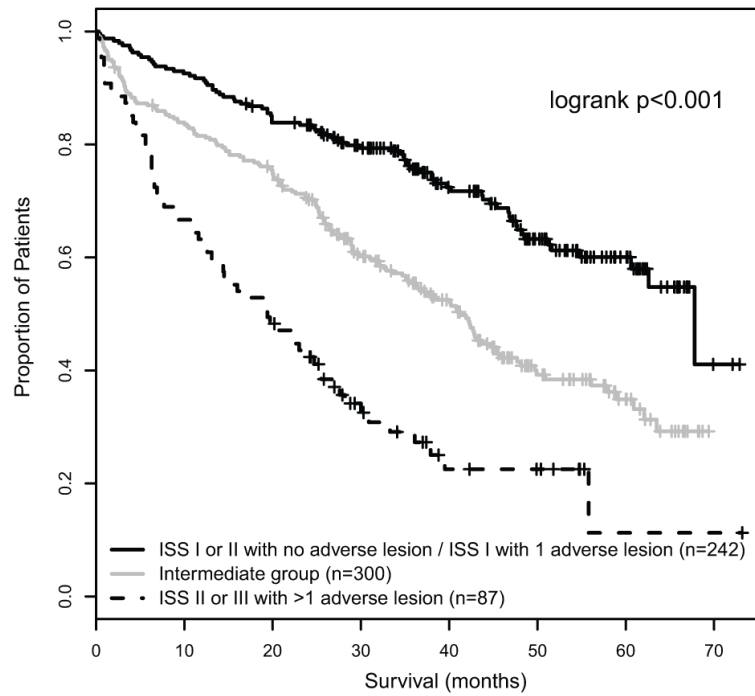


Figure 5. Integration of the ISS and FISH groups to identify an ultra-high risk group, defined by ISS II or III with >1 adverse lesion.

Table 1

Median PFS and OS associated with each FISH lesion, compared to samples without the lesion.

FISH Lesion	PFS			OS		
	Lesion Present	Lesion Absent	p=	Lesion Present	Lesion Absent	p=
	Median PFS (months)	Median PFS (months)		Median OS (months)	Median OS (months)	
Hyperdiploidy	18.9	17.8	0.110	49.7	43.7	0.150
t(4;14)	13.1	19.3	<0.001	27.7	50.9	<0.001
t(6;14)	27.2	18.2	0.361	not reached	47.7	0.426
t(11;14)	21.3	17.5	0.292	51.6	46.9	0.209
t(14;16)	13.6	18.6	0.028	32.9	48.3	0.025
t(14;20)	10.2	18.5	0.152	16.9	48.3	<0.001
del(1p)	19.0	18.7	0.701	36.4	47.7	0.216
+1q	13.8	22.1	<0.001	31.0	54.8	<0.001
del(13q)	16.3	20.1	0.002	40.9	52.1	0.005
del(16q)	19.9	18.2	0.200	43.7	48.3	0.462
del(17p)	14.7	18.3	0.002	26.7	48.5	<0.001
del(22q)	18.7	18.0	0.265	53.2	45.8	0.653

Table 2

Variables significantly associated with survival in multivariate analysis.

Variable	PFS			OS		
	Hazard Ratio	95% CI	p=	Hazard Ratio	95% CI	p=
Adverse <i>IGH</i> Translocation	1.65	1.31 - 2.07	<0.001	1.54	1.16 - 2.03	0.003
+1q21	1.46	1.21 - 1.76	<0.001	1.53	1.20 - 1.94	0.001
del(17p13)	1.41	1.05 - 1.90	0.022	1.53	1.06 - 2.19	0.02
ISS (I vs II)	1.36	1.07 - 1.74	0.012	1.79	1.24 - 2.58	0.002
ISS (I vs III)	1.55	1.21 - 1.97	<0.001	2.69	1.89 - 3.84	<0.001

Table 3

Multivariate analysis of the ISS and the genetic groups defined by number of adverse lesions

Variable	PFS			OS		
	HR	95% CI	p	HR	95% CI	p
Number of adverse lesions (0 vs 1)	1.48	1.21 - 1.80	<0.001	1.30	1.00 - 1.69	0.048
Number of adverse lesions (0 vs >1)	2.42	1.89 - 3.09	<0.001	2.59	1.93 - 3.47	<0.001
ISS (I vs II)	1.34	1.05 - 1.70	0.019	1.79	1.24 - 2.57	0.002
ISS (I vs III)	1.53	1.20 - 1.96	0.001	2.72	1.91 - 3.89	<0.001

Table 4

The median OS associated with patient groups defined by the combination of ISS stage and number of adverse lesions. These values were used to define favourable, intermediate and ultra-high risk patients groups.

Group	Median OS	Combined FISH_ISS Risk Group	Median OS	HR for OS (95% CI)
ISS I and no adverse lesions	Not reached	Favorable Risk	67.8	-
ISS I and 1 adverse lesion	Not reached			
ISS II and no adverse lesion	62.6			
ISS I and >1 adverse lesion	42.8	Intermediate Risk	41.3	2.03 (1.55 - 2.66)
ISS II and 1 adverse lesion	42.3			
ISS III and no adverse lesion	42.9			
ISS III and 1 adverse lesion	35.3			
ISS II and >1 adverse lesion	25.5	Ultra-High Risk	19.4	4.16 (2.98 - 5.83)
ISS III and >1 adverse lesion	14.4			