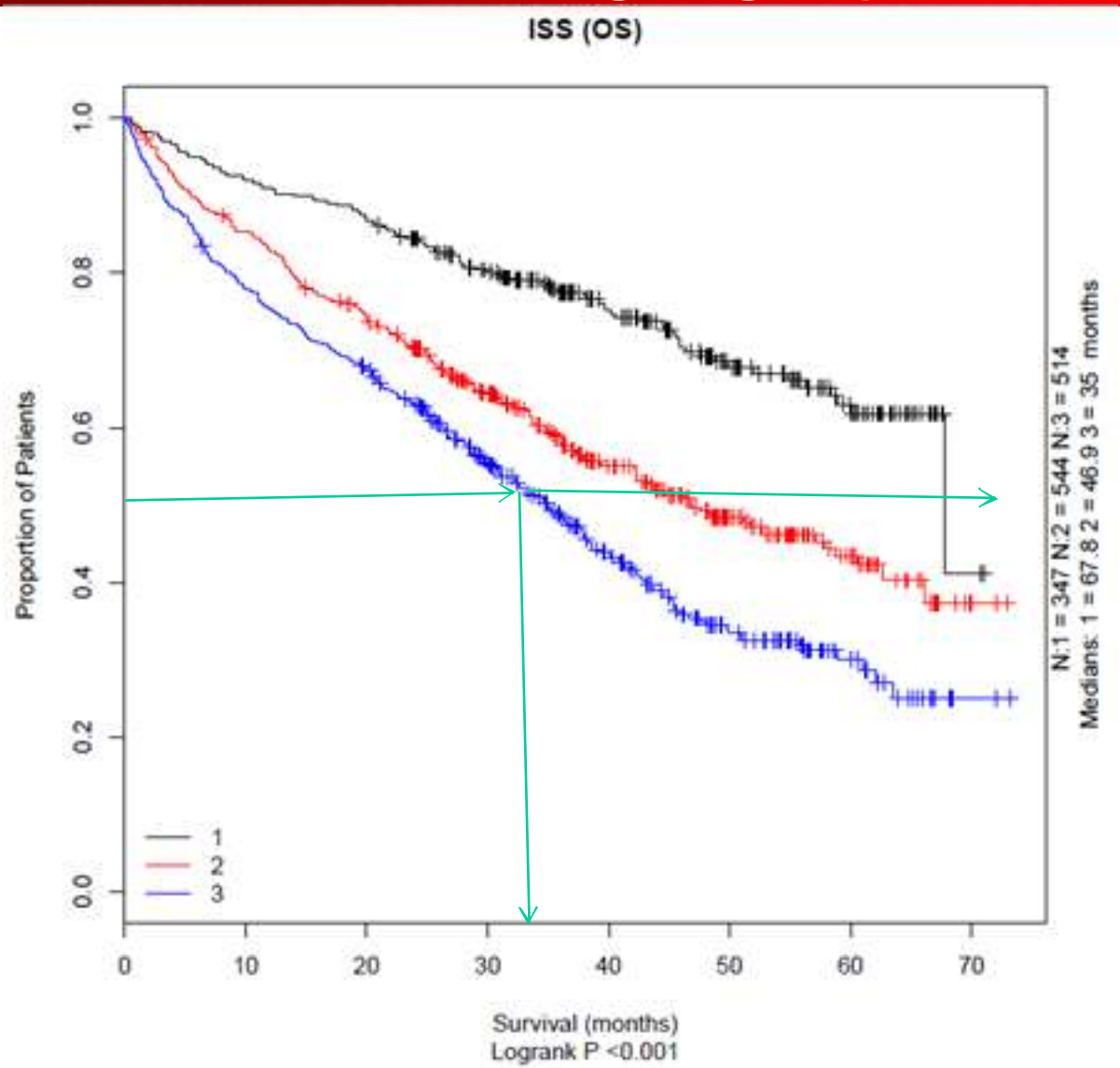
Impact of risk status on treatment

G Morgan

What is the question being addressed

- Myeloma looks homogeneous down the microscope
- There are a range of survival outcomes that can't be recognised at disease presentation
- At a molecular level myeloma is heterogeneous
- Hypothesis
  - Molecular subgroups have different clinical outcomes
  - Targeting treatment to these groups will improve outcomes

The international staging system

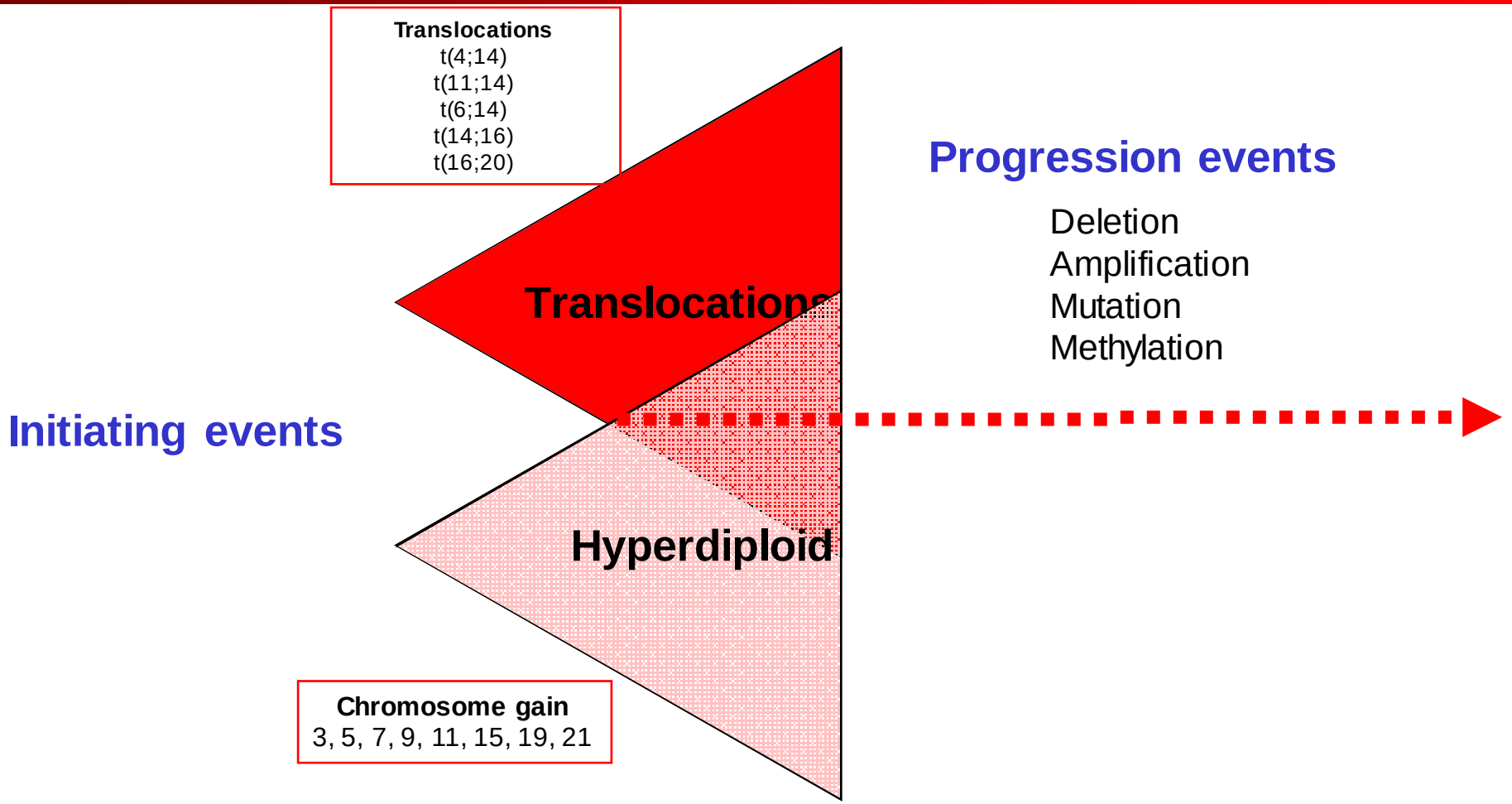


Due to the number of events early analyses with low median follow up will have power to study impacts on higher risk subgroups

Lessons from cytogenetics

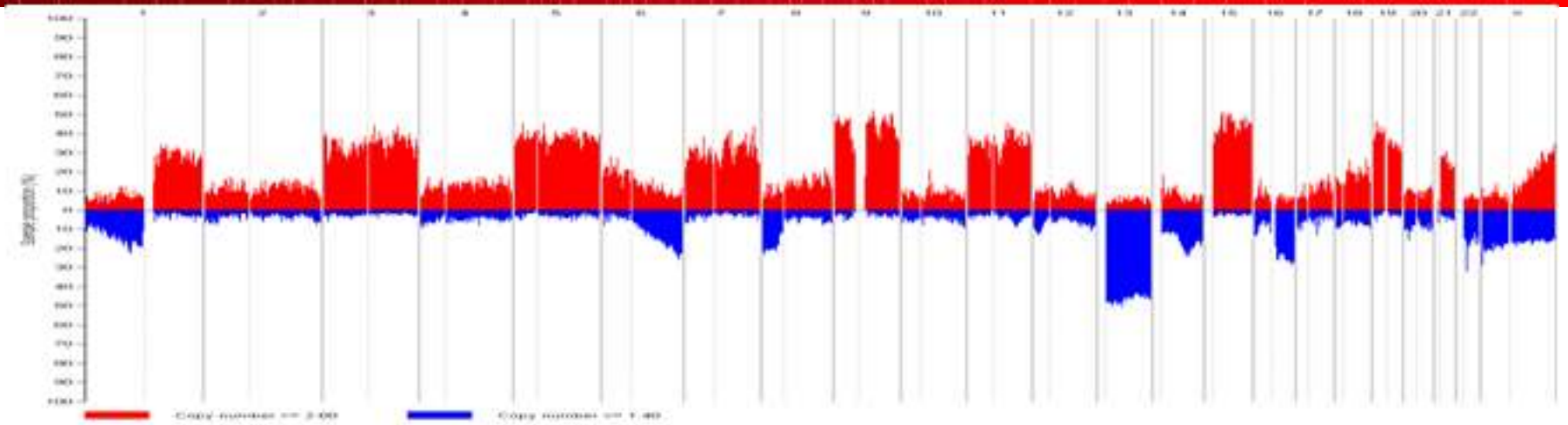
- If we are to target treatment based on biology then we need to focus on the knowledge gained from genetic analysis.
- Translocations
  - t(4;14) 10-15% MMSET/Fgfr3
  - t(11;14) 15-20% cyclin D1
  - t(6;14) <4% cyclin D3
  - t(14;16) <5% maf
  - t(14;20)
- Hyperdiploidy

Molecular classification of myeloma



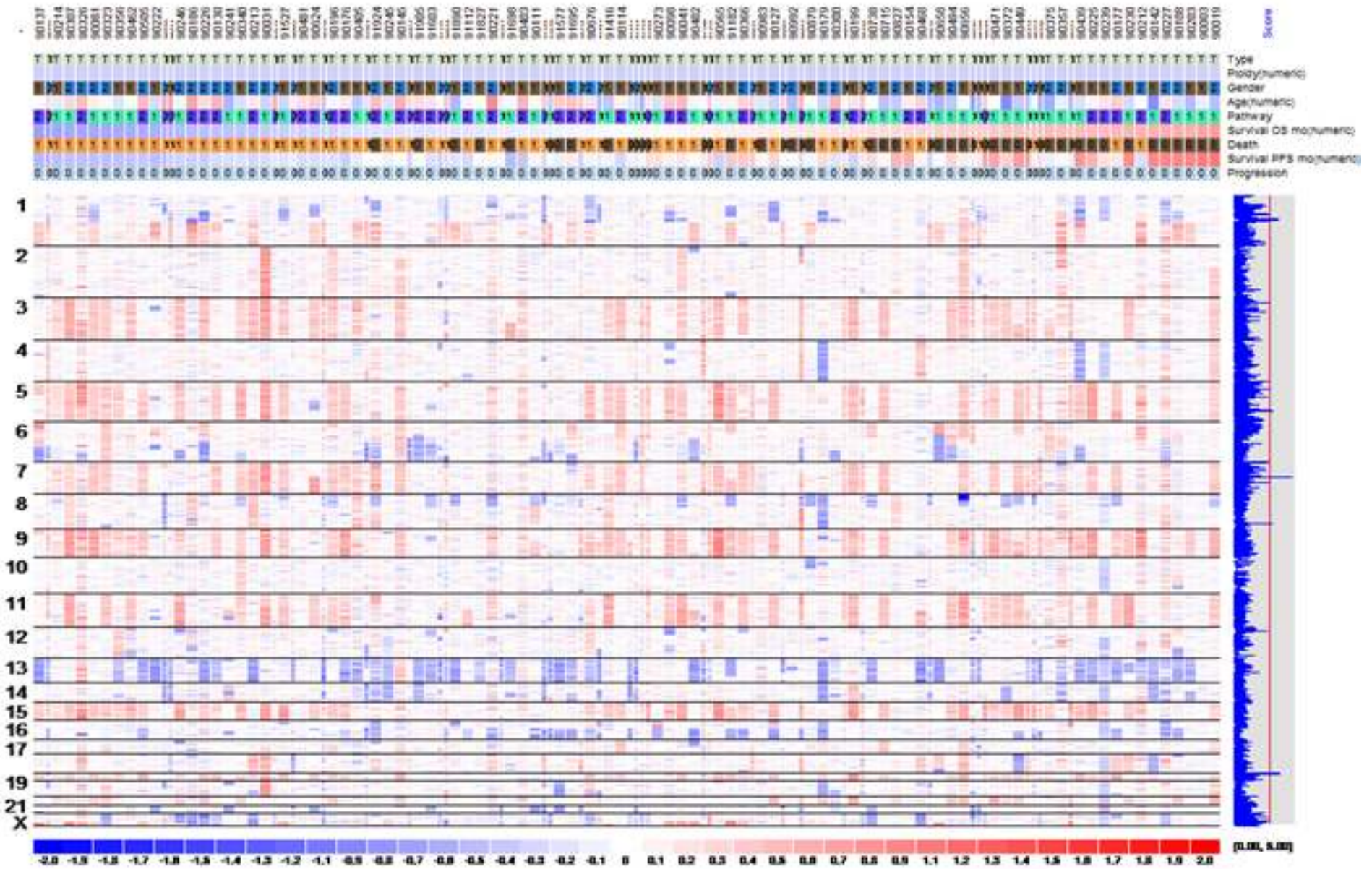
The acquisition of additional genetic events may impact on the prognosis determined by the initiating genetic lesions leading to myeloma.

Summary of myeloma gains and deletions



Chromosome	Deletions (%)	Chromosome	Gains (%)
1p	29.8 + 4.4 UPD	**1q**	36.0
6q	33.3	**3**	27.2
8p	25.4	**5**	33.3
12	21.9	**7**	21
13q	58.7	**9**	35.9
14q	38.1	**11**	24.6
16q	35.0 + 8.7 UPD	**15**	36.8
17p	7.0 + 1.75 UPD	**19**	33.3
18	15.8	**21**	12.3
20	12.3		
22	18.4		
X	28.0 + 21.0 UPD	**X**	8.7

Survival module in dCHIP



Associations of Genetic Lesions and Survival

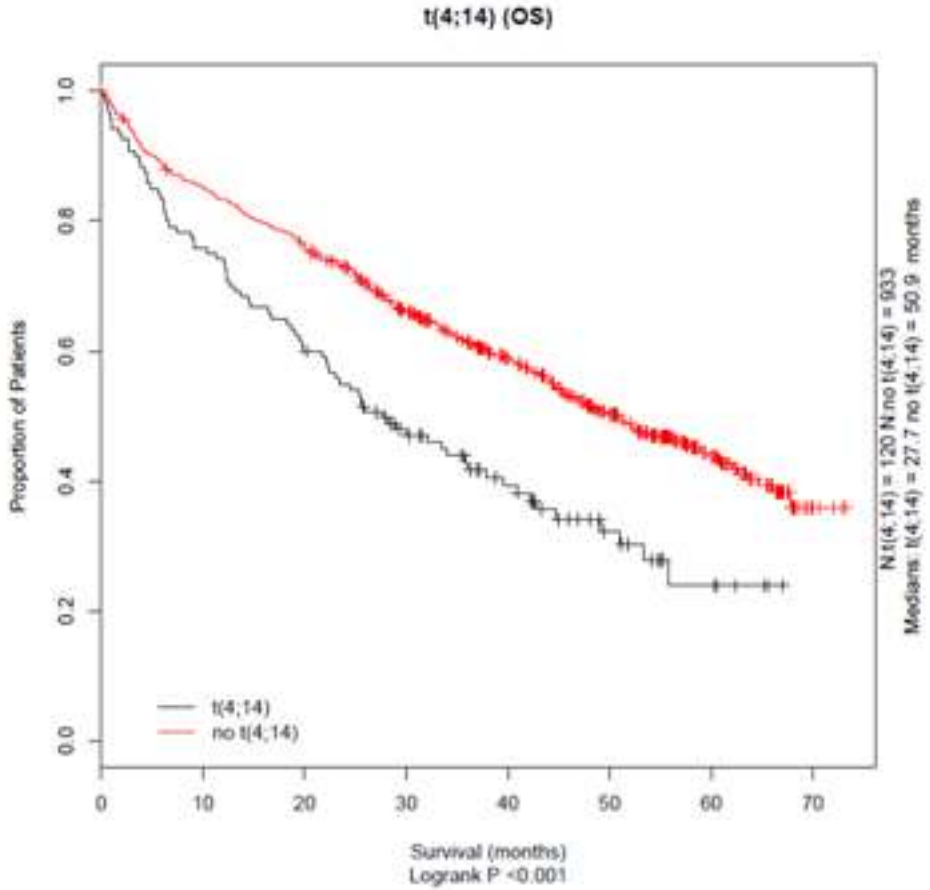
Univariate Analysis

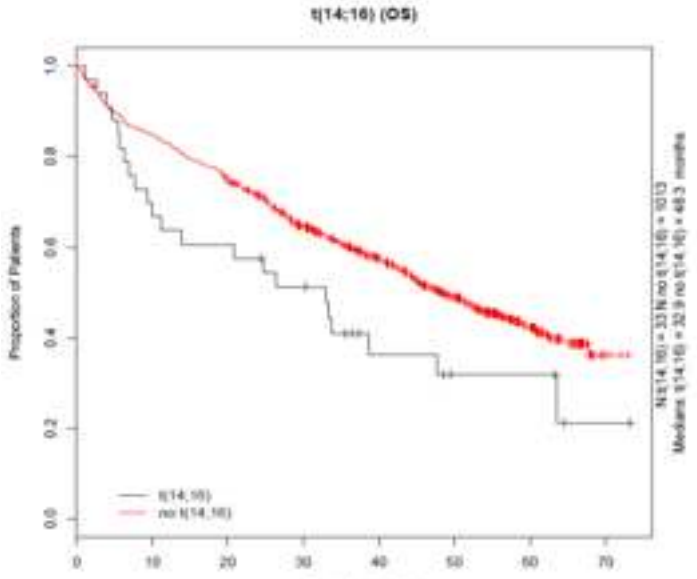
FISH Lesion	PFS			OS		
	Lesion Present Median PFS (months)	Lesion Absent Median PFS (months)	p=	Lesion Present Median OS (months)	Lesion Absent Median OS (months)	p=
Hyperdiploidy	18.9	17.8	0.110	49.7	43.7	0.150
t(4;14)	13.1	19.3	<0.001	27.7	50.9	<0.001
t(6;14)	27.2	18.2	0.361	not reached	47.7	0.426
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

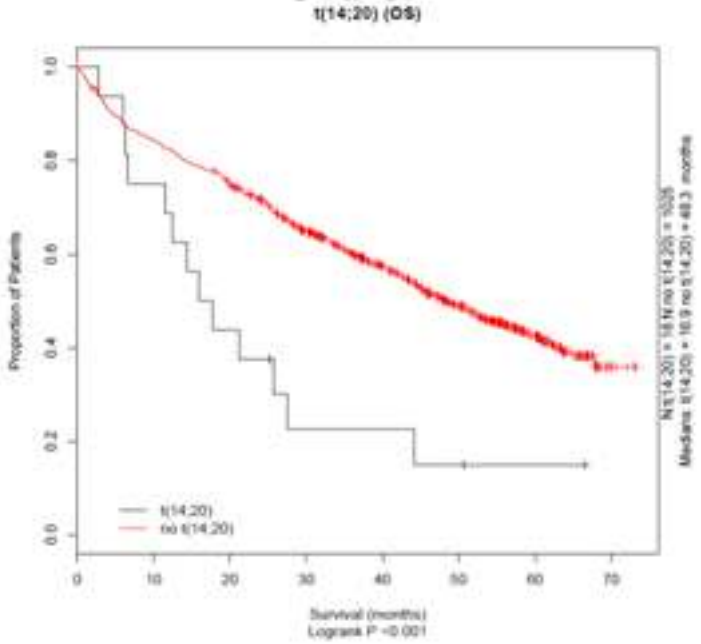
Multivariate Analysis

Variable	PFS			OS		
	Hazard Ratio	95% CI	p=	Hazard Ratio	95% CI	p=
Adverse *IGH* 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

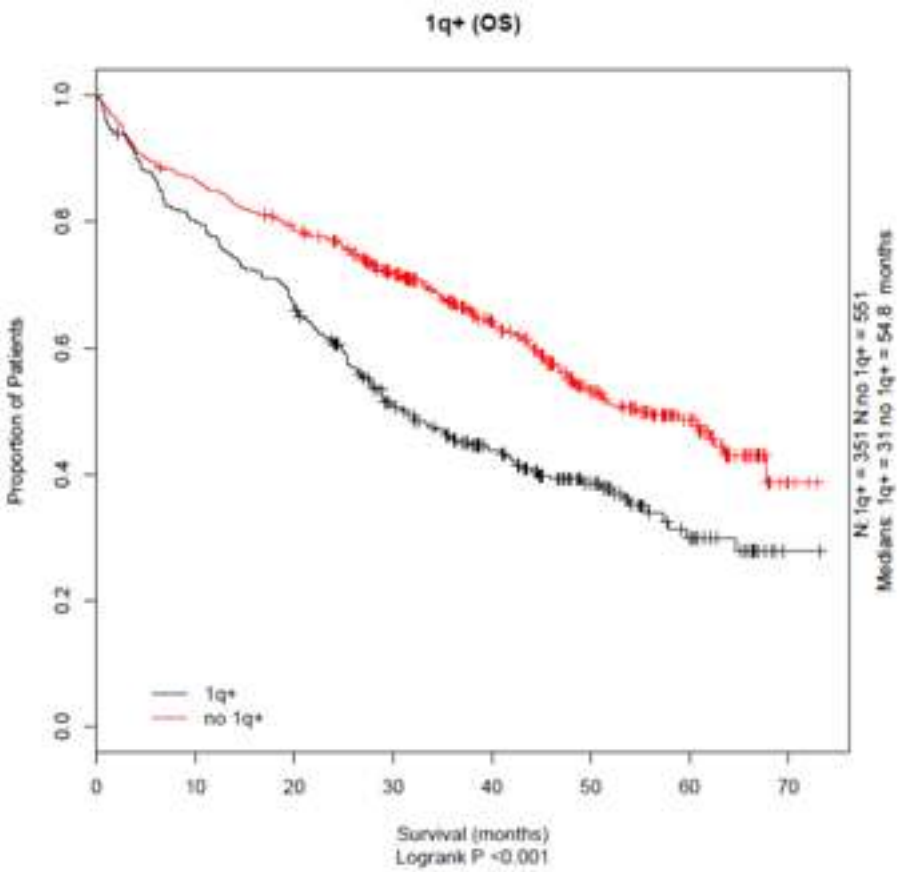
Prognostic translocations

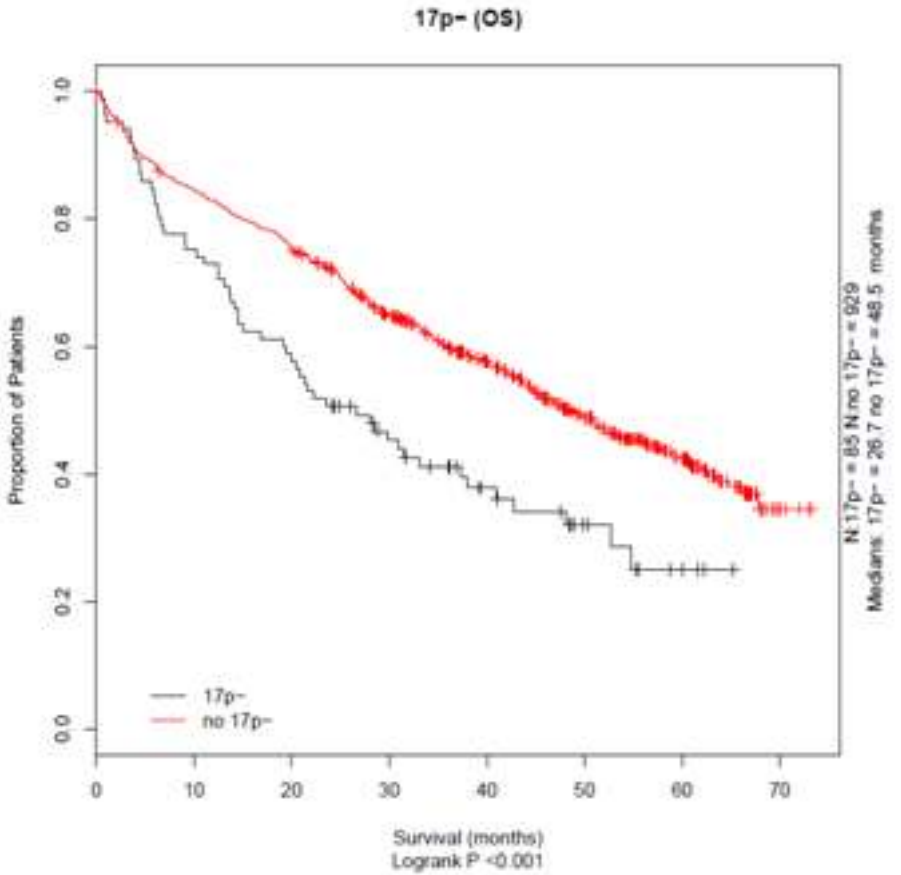




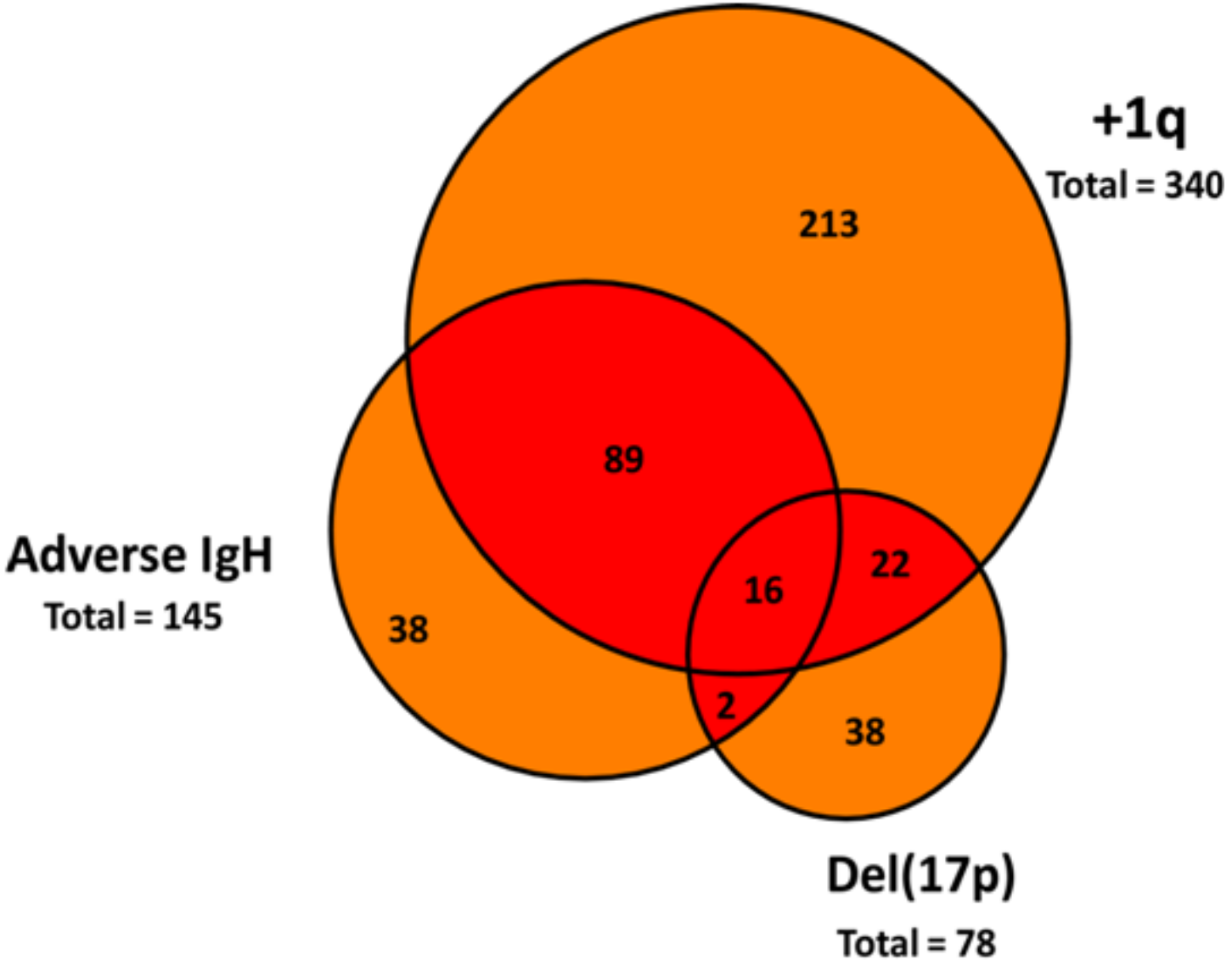


Prognostic copy number variants

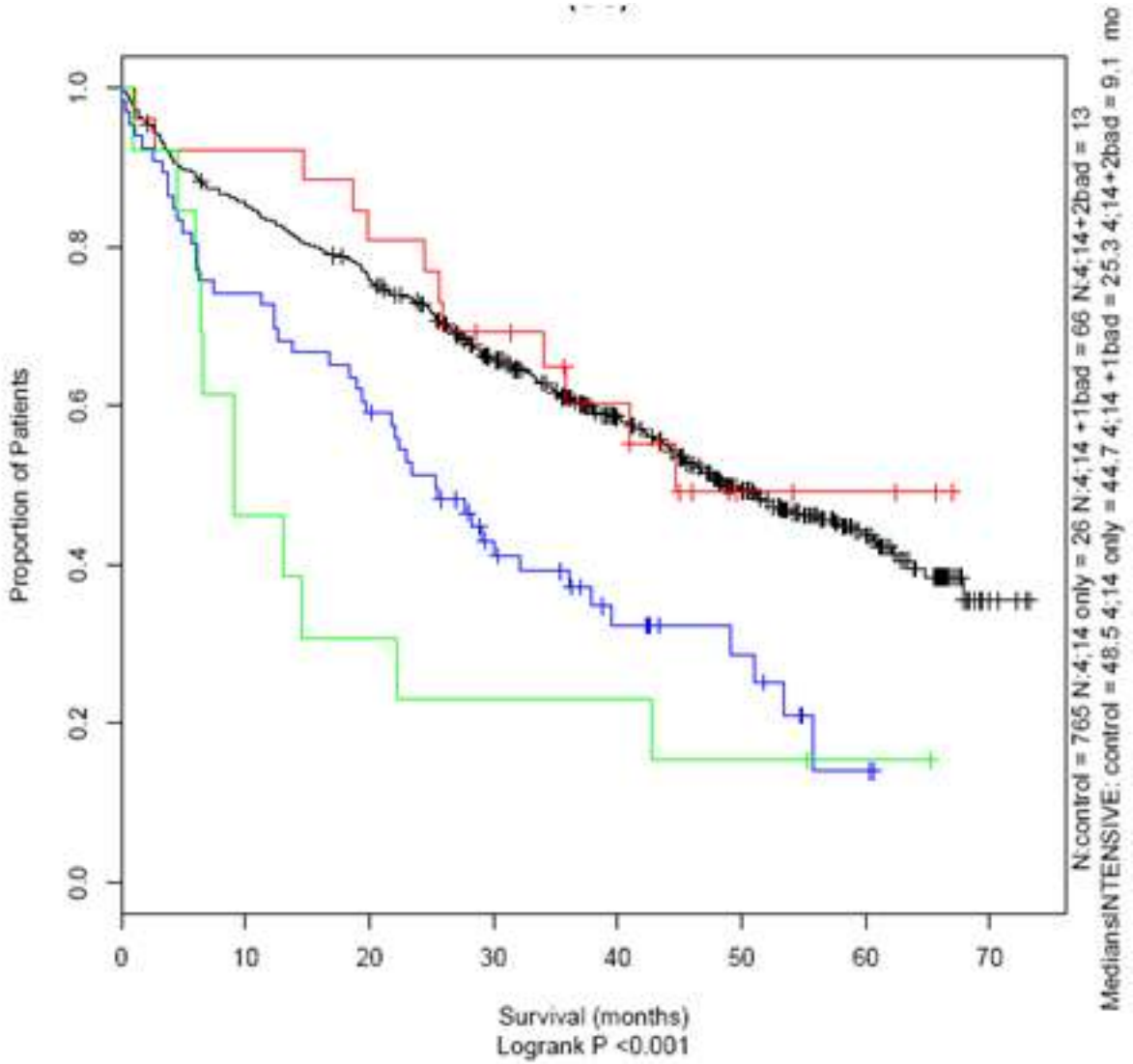




Inter-relationship of adverse genetic lesions



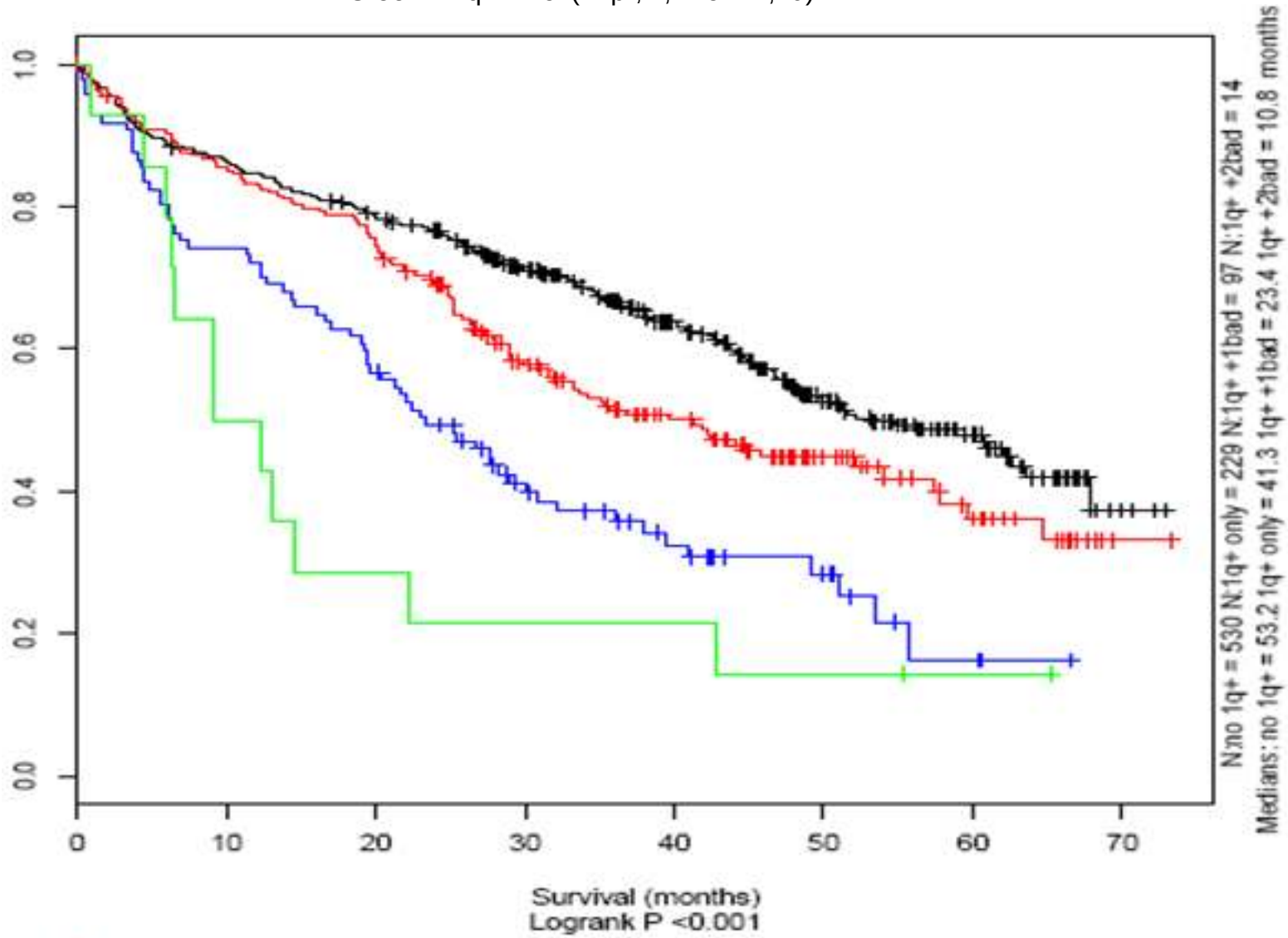
Are all t(4;14) bad



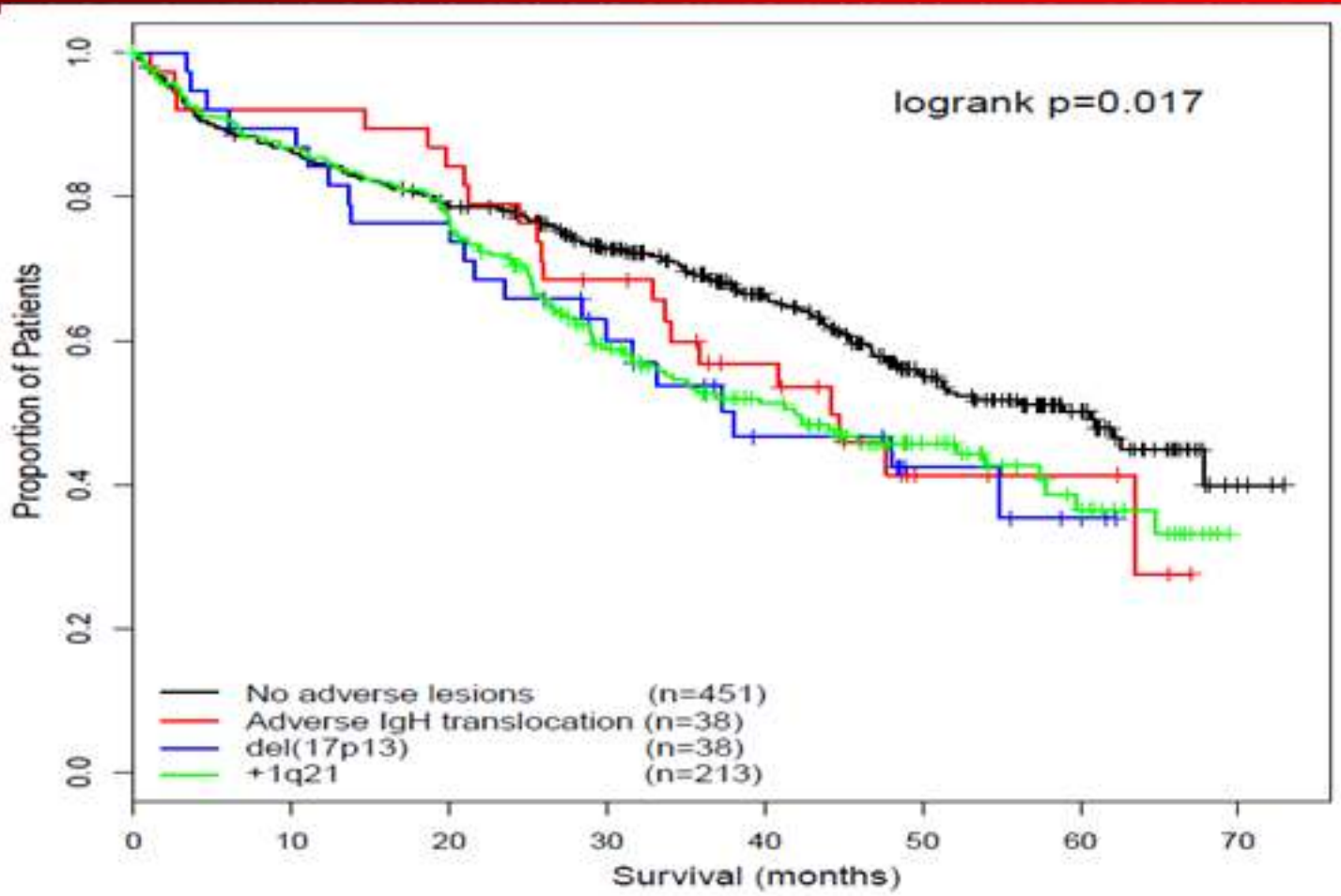
Black = no 4;14
Red = 4;14 only
Blue = 4;14 + either 17p- **or** 1q+
Green = 4;14 + 17p- **and** 1q+

1q+

Black = no 1q+
Red = 1q+ only
Blue = 1q+ + either 4;14 **or** 17p- **or** 14;20
Green = 1q+ +2 of (17p-, 4;14 or 14;20)

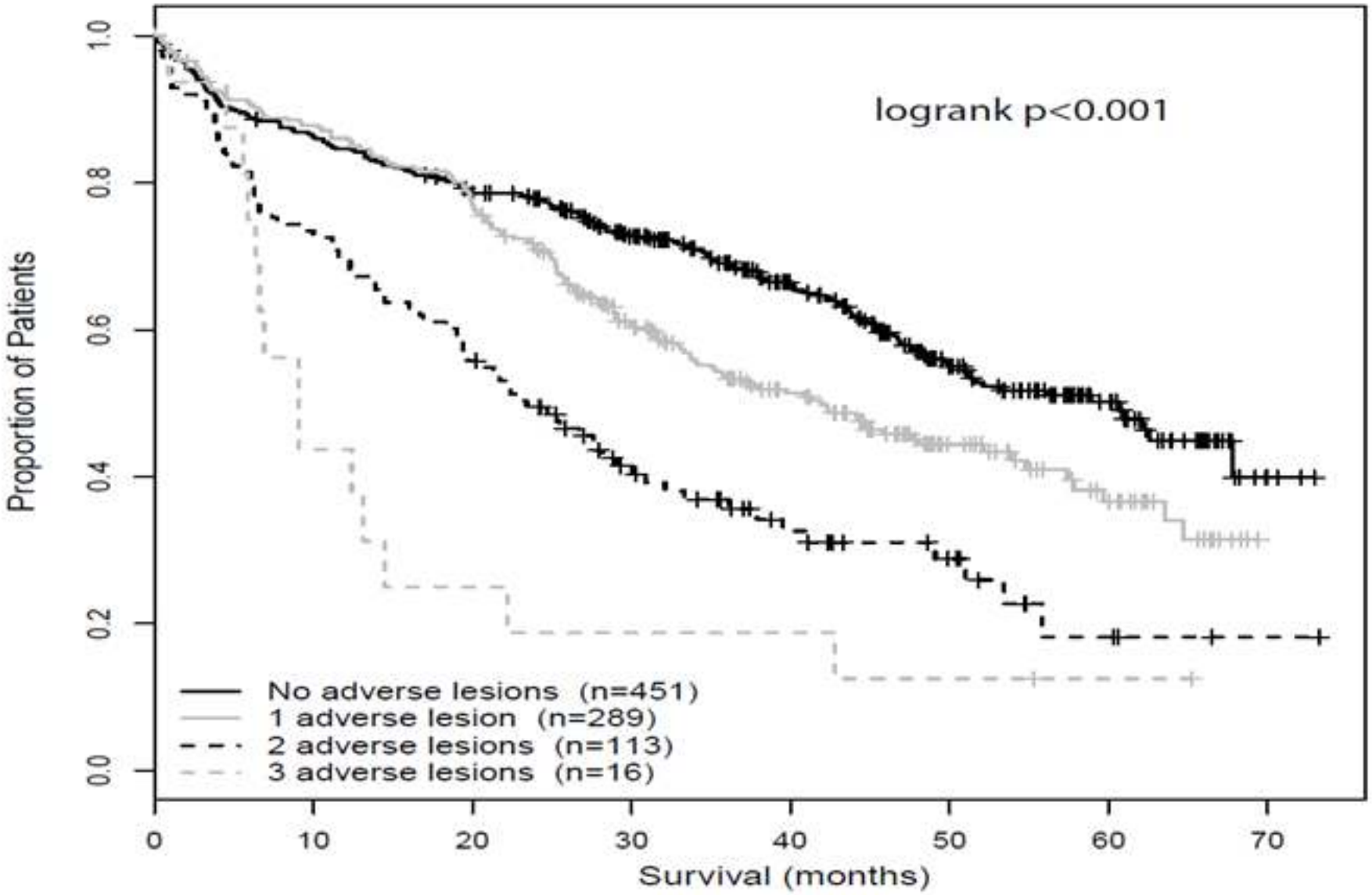


OS of single adverse lesions compared to no adverse lesions



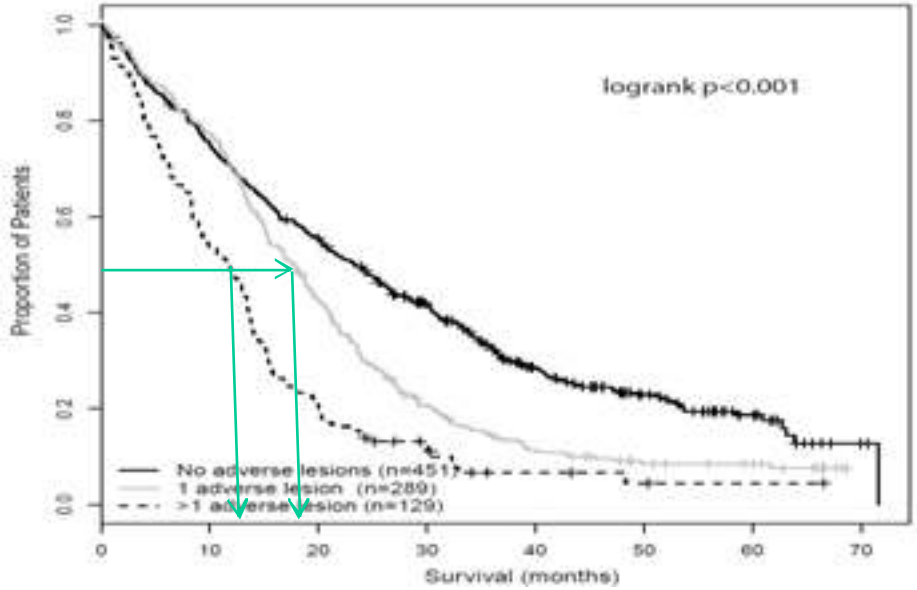
1. If you wish to determine risk status for a patient you need to determine whether one or more of the important prognostic variables is present.
2. Build model based on adverse IgH, 17p- and 1q+.

Effect of 0 vs 1 vs 2 vs 3 lesions on OS

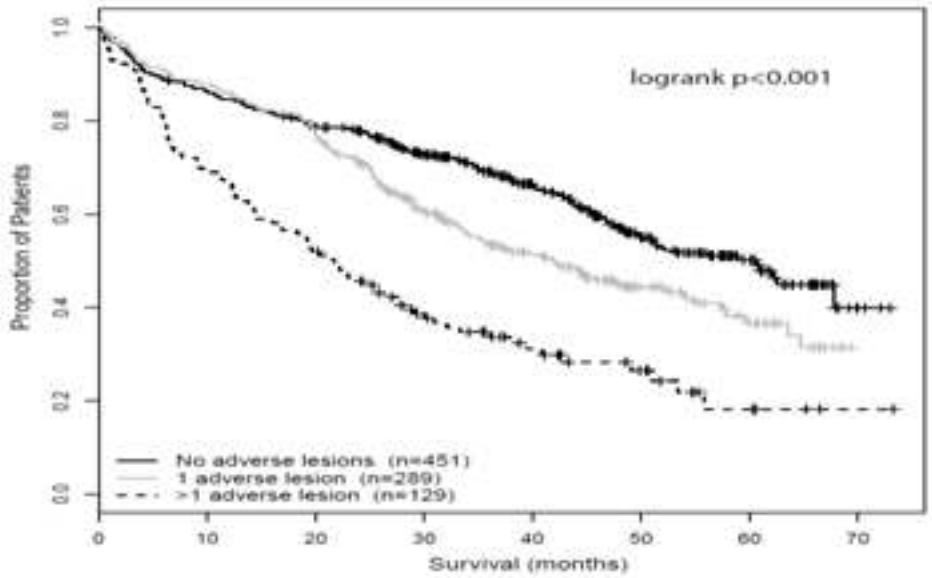


0 vs 1 vs >1 adverse lesion and OS

PFS

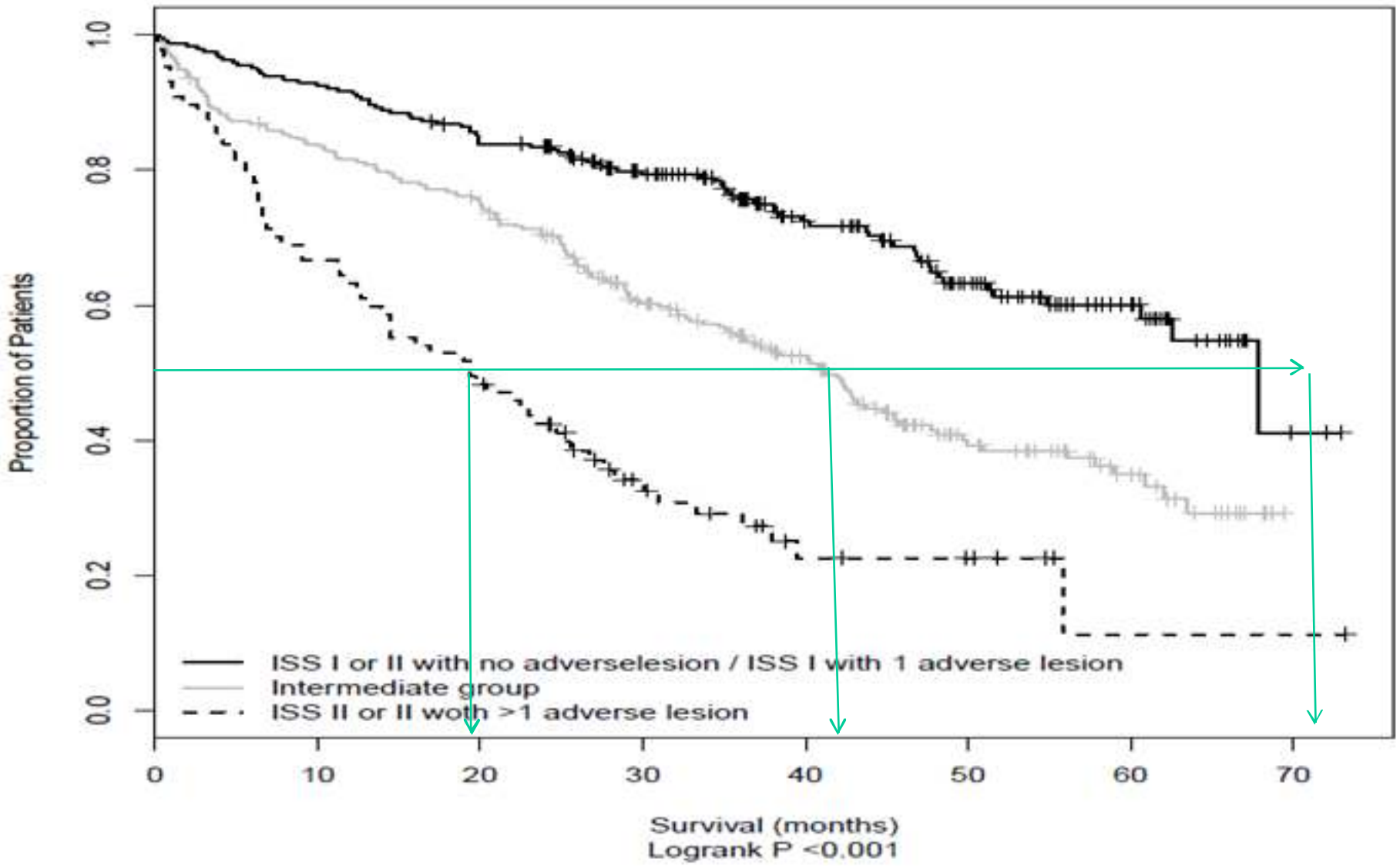


OS

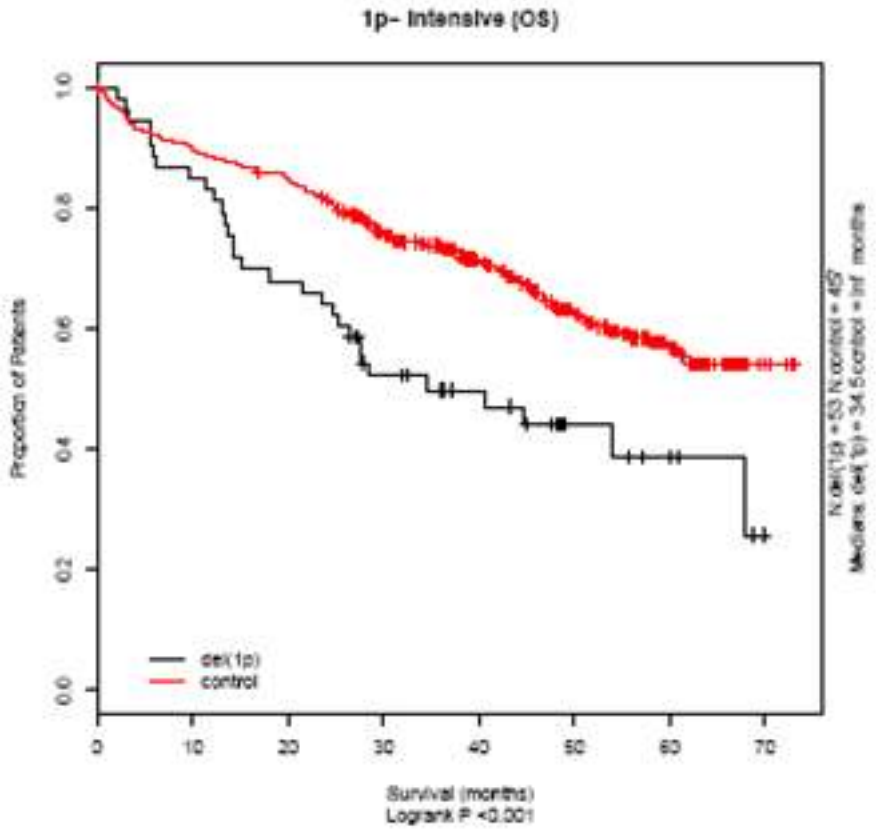


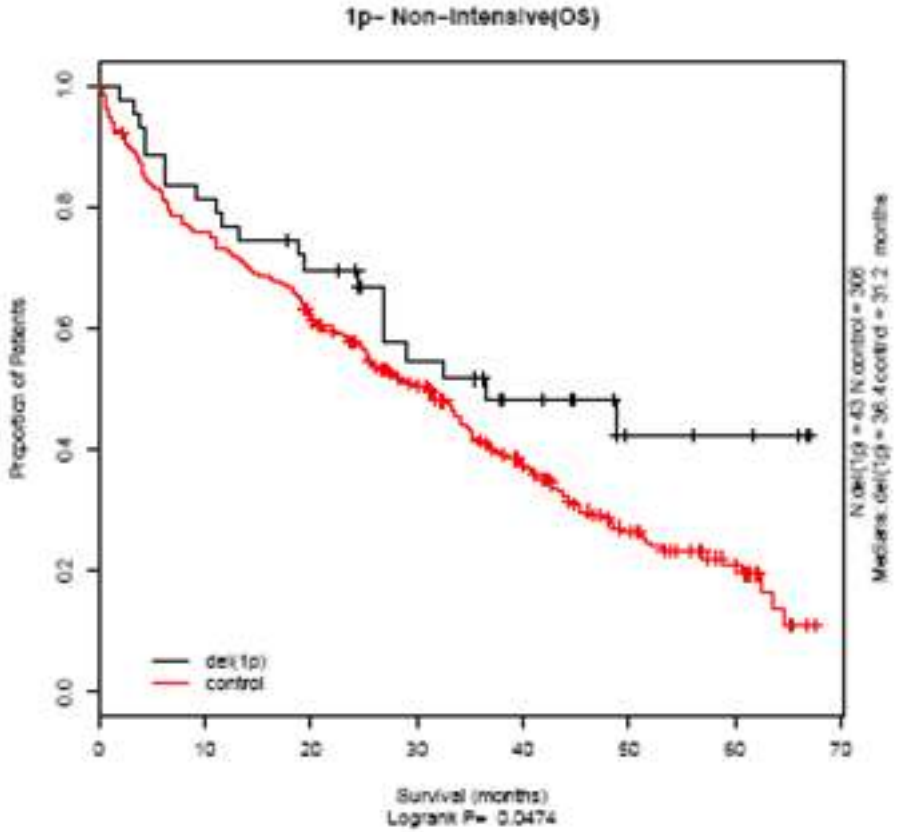
1. Most high risk cases relapse by a year and a half.
2. Because of the number of events occurring, early analyses of trials with short median follow up will be predominently looking at the impact of treatment on high risk cases.
3. The impact of treatments on low risk disease will be seen in later analyses with longer follow up, which are needed to capture the number of events in this group of patients.

OS combining genetics and the ISS

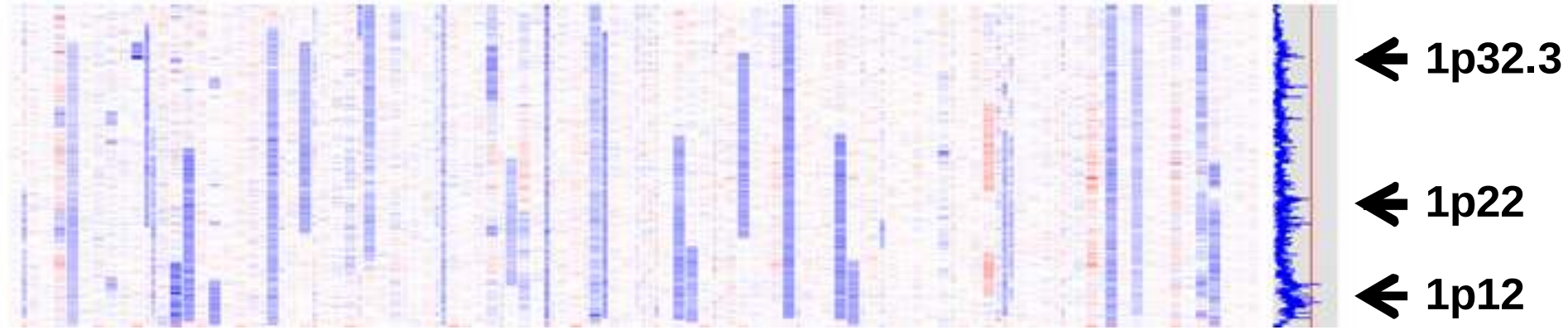


1p32- intensive and non intensive





In depth mapping of 1p vs survival



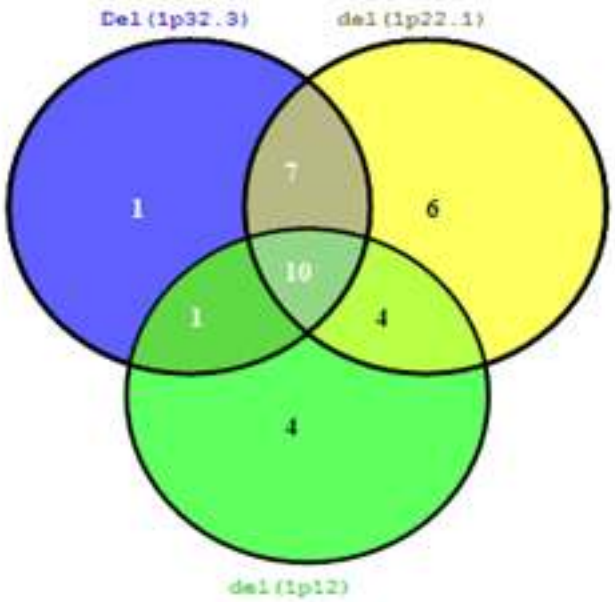
Regions on 1p

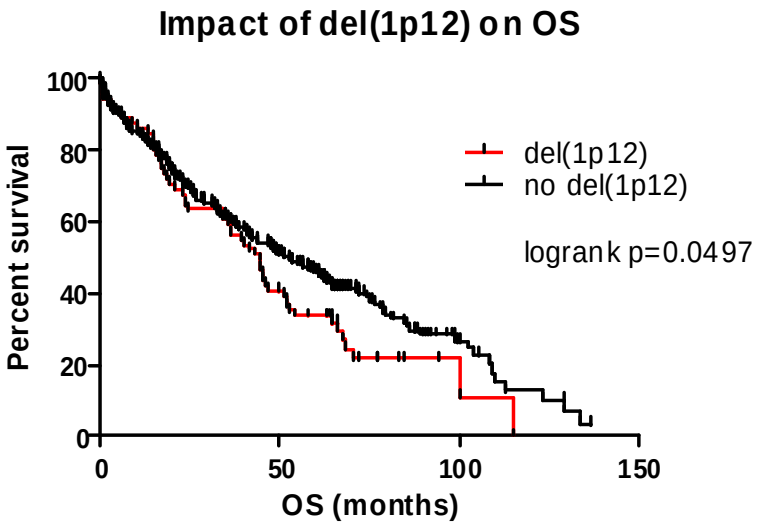
Overall there are 3 main regions of interest:

1p32 (FAF1/CDKN2C)	homozygous deletions
1p22.1 (EVI5 to TMED5)	unknown
1p12 (FAM46C)	deletion and mutation

Mapping Data Set	Total	Any 1p Deletion		1p32 Deletion		1p22.1 Deletion		1p12 Deletion	
		n=	%	n=	%	n=	%	n=	%
MRC Myeloma IX	114	34	29.8	18	15.8	25	21.9	22	19.3
IFM	192	68	35.4	23	12	47	24.5	43	22.4
MMRC	254	78	30.7	32	12.6	56	22	54	21.3
Mayo Clinic	53	17	32.1	6	11.3	10	18.9	11	20.8
Carrasco	66	20	30.3	7	10.6	15	22.7	11	16.7
Overall	679	217	32	86	12.7	153	22.5	141	20.8

Inter-relationship of deletions on 1p





Screened 160 cases ndMM
FAM46C mutations = 3.4%

Sample no.	Base Change	Amino-Acid Change	1p12 mapping
323	c357 C>G	F118L	no deletion
1527	c463 A>T	I154F	deleted
245	c537 C>A	F178C	no deletion
326	c872 A>G	Y290C	no deletion
127	c1068 C>G	Y355X	deleted

1. Need probes for 1p32 and 1p12
2. Integration of 1p- into the model didn't help overall
3. Helped in defining a group of good risk with long median survivals

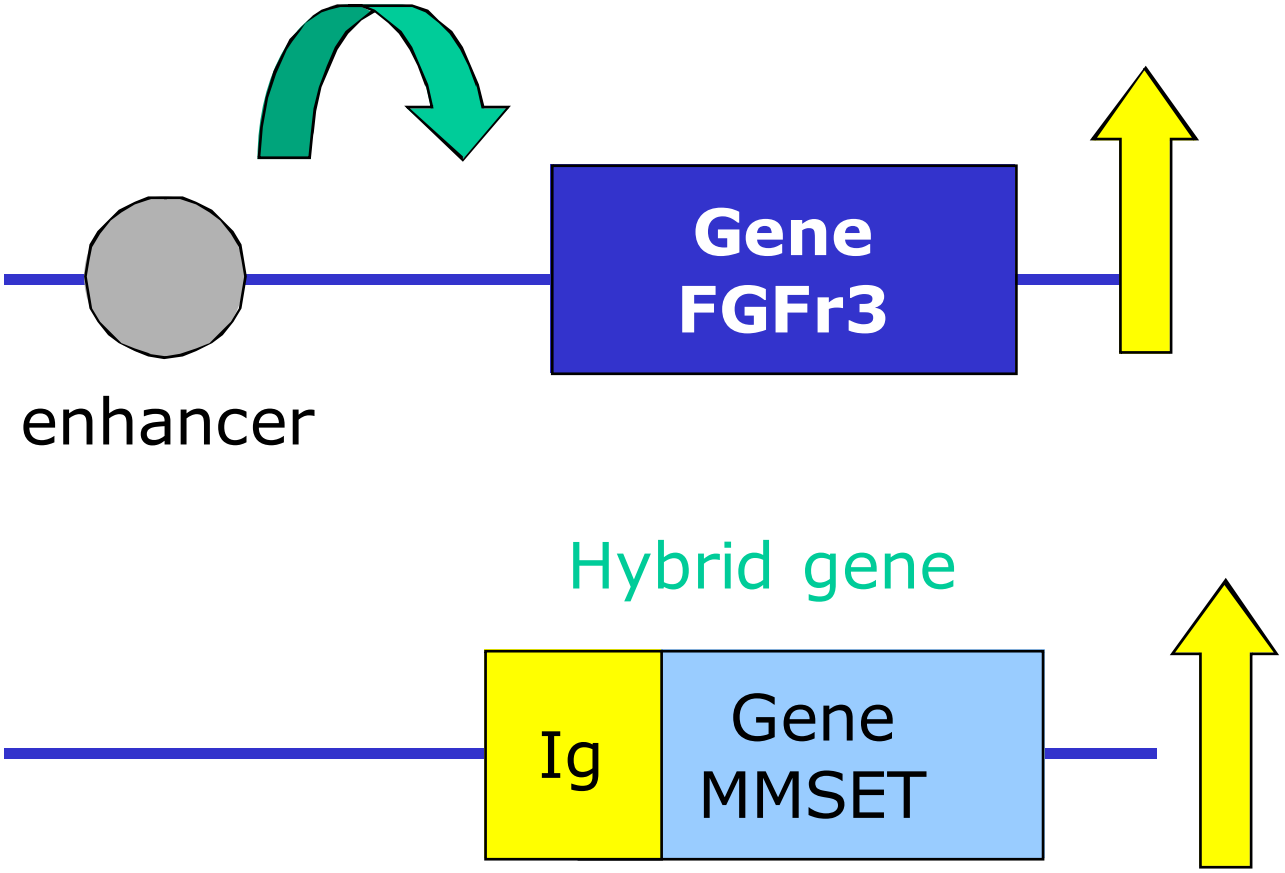
Impact on trial design

- Examining currently available datasets
- We are seeing considerable improvements in the outcome of low risk disease subsets
  - Median survivals are long in this subset
  - In responders they are even longer
    - CR in cases lacking adverse genetics with low B2M
  - Demonstrating efficacy of novel agents will require large studies and both early (median 3 yrs) and late (median 6 yrs) analyses
- Currently Minimal impact on “ultra high risk” subsets of disease
  - Defined by genetic events
  - Needs a new treatment strategy for this subset of disease
  - Design can be simple relatively small studies with short follow up
  - Needs a way of reliably identifying these groups at presentation
- Suggest we need to work on infra-structure of trial groups and to develop molecular diagnostic platforms

The development of personalised medicine strategies for myeloma

- We have discussed “prognostic factors” and their use for risk stratification.
- Predictive strategies are essential for the personalised treatment approach for myeloma.
- What is required is a “diagnostic test” that predicts the response to a specific therapy.
- Where are we in myeloma?

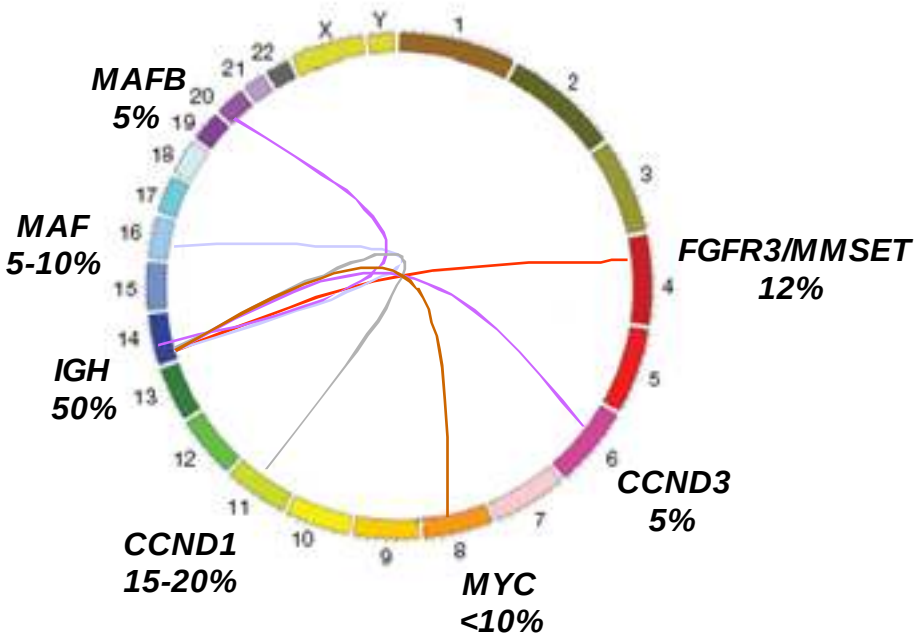
t(4;14) oncogene deregulation



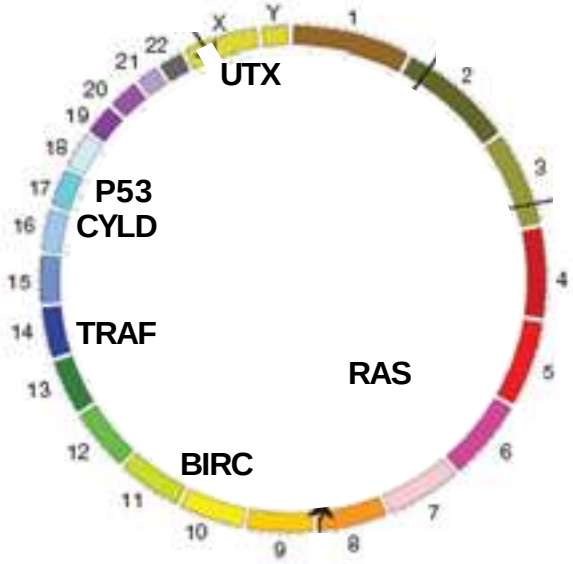
If translocation is present
Target FGFr3 tyrosine kinase
Target MMSET histone methyl transferase
Diagnostic test for the translocation

Myeloma Genome

Known IGH translocations in myeloma (50% samples)



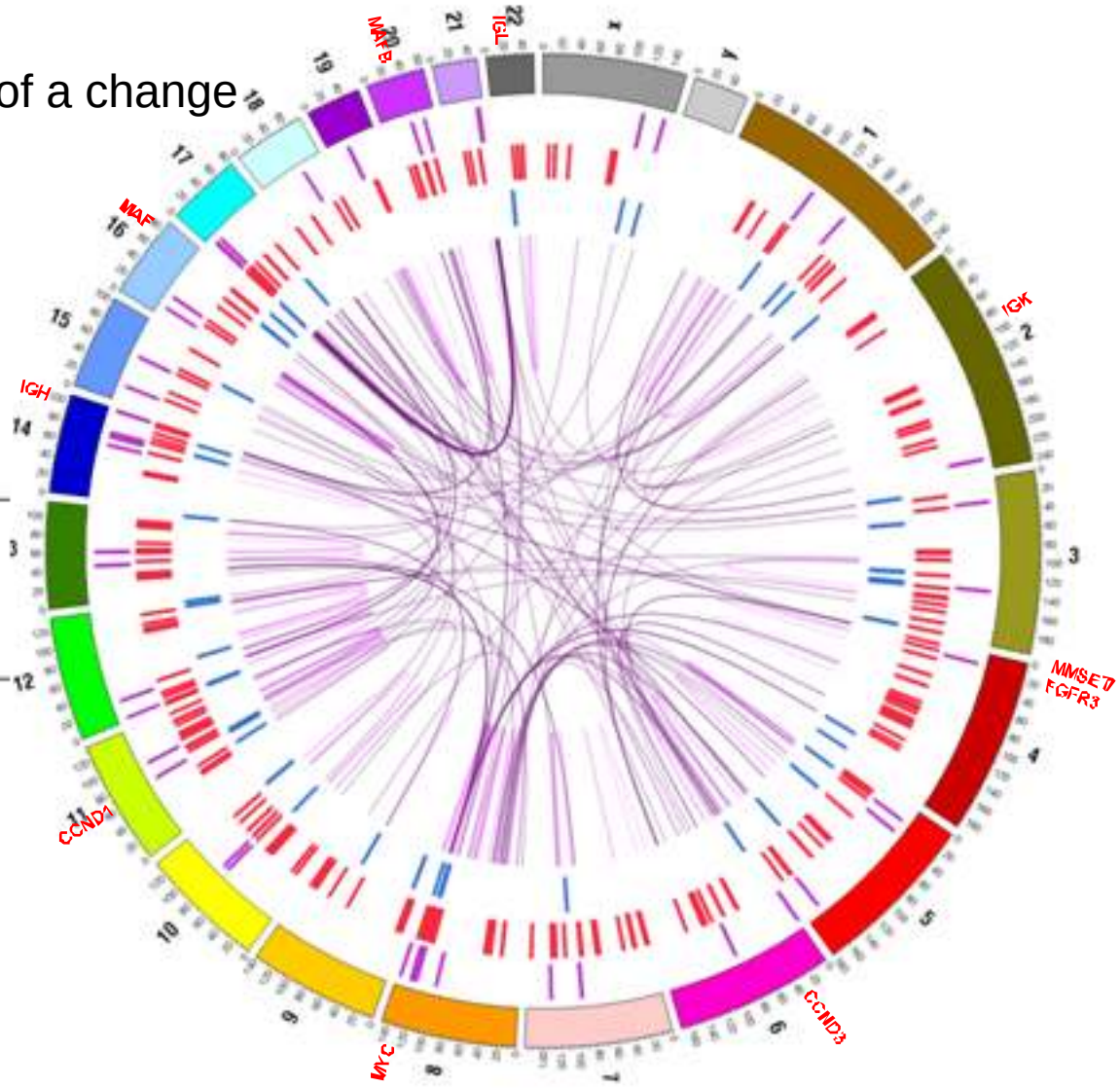
Known mutated genes



Impact of NGS on our understanding

1. Multiple genetic changes
2. Not all driver mutations
3. Important to define relevance of a change

Whole Genome (n = 23)	Total	Mean
Nonsynonymous point mutations		
Missense	721	31.3
Nonsense	49	2.1
Splice site	22	1.0
Read-through	1	0.04
	793	34.6
Small indels		
Frame-shift deletion	5	0.2
In-frame deletion	5	0.2
Frame-shift insertion	2	0.09
In-frame insertion	1	0.04
	13	0.6
Larger rearrangements		
Translocations	204	8.7
Deletions	105	4.6
Insertions	23	1.0
Tandem duplications	32	1.4
Long-range	78	3.4
Complex	33	1.4
	475	20.7



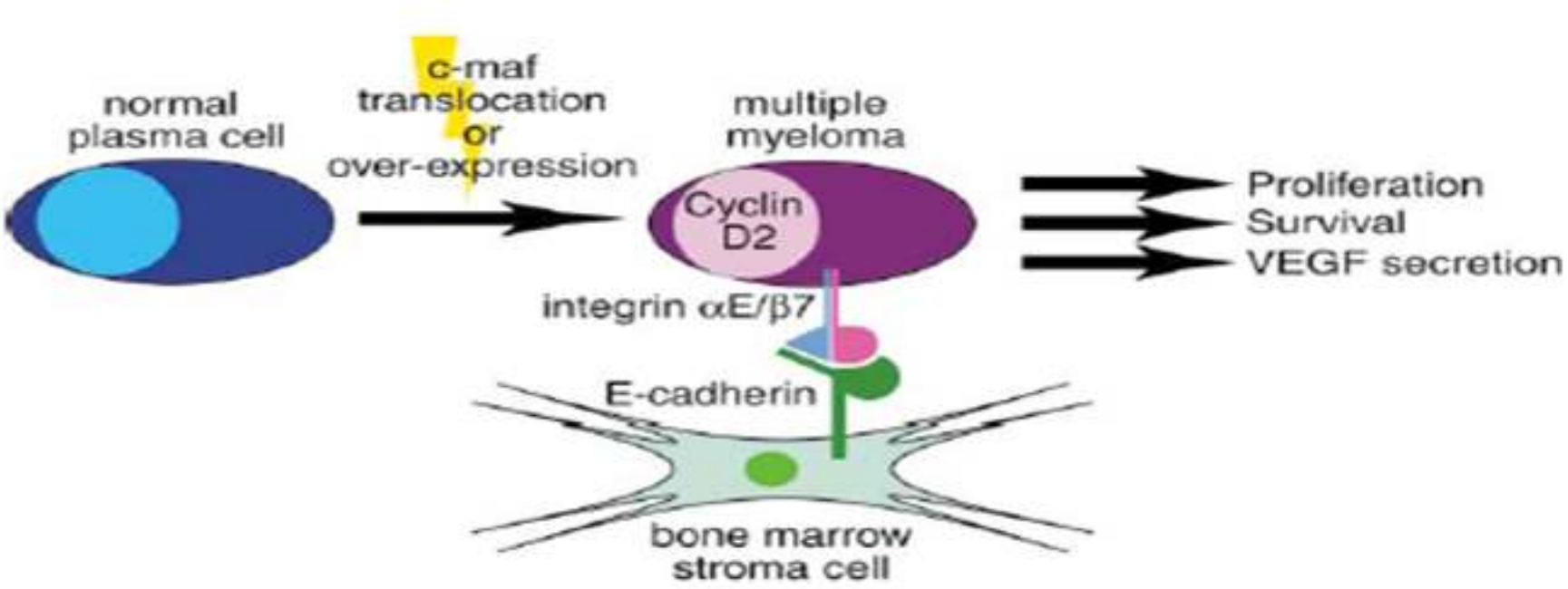
Campbell et al 2011

Potential clinical value of mutation testing

- **BRAF mutations present in 4% of samples**
  - **Confirm this rate in representative trial samples**
  - **Demonstrate the mutation is a driver not a passenger event**
- **BRAF inhibitors have been developed**
  - **Functional in melanoma**
- **Strategy for myeloma**
  - **Mutation detection strategy**
  - **Screen presenting cases**
  - **Trials of BRAF inhibitors in mutation positive cases**

The end

Targeting MAF



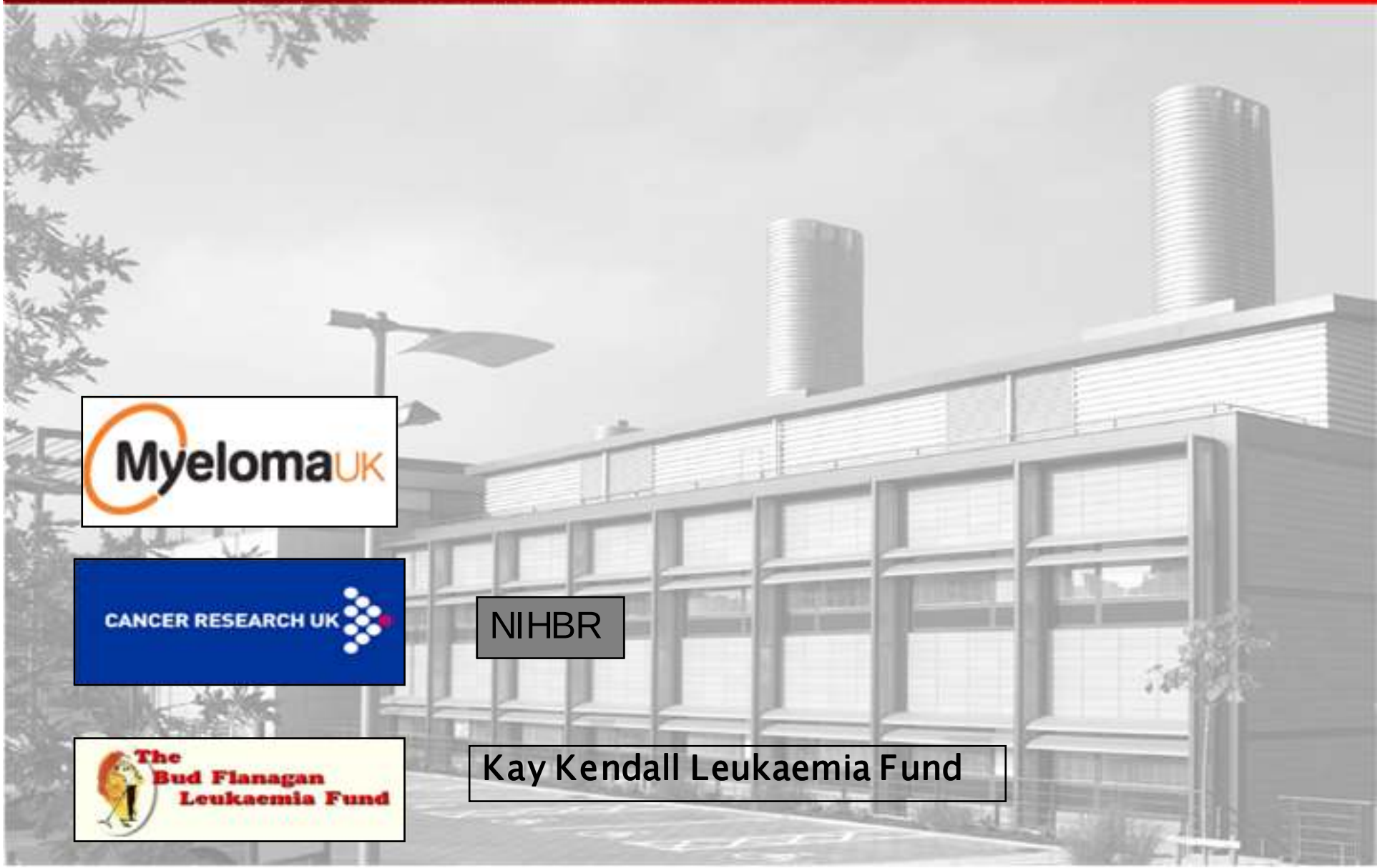
- **Frequent upregulation of Deptor**
- **Frequent upregulation of the PI3K parhway**
- **Suggests targeting this pathway may be perticularly effective**

Prognostic – RQ-PCR

- Multiplexed PCR reaction
  - FGFr3
  - MMSET
  - MAF
  - CyclinD1
- CD138 selected cells
- Extract RNA
- cDNA
- PCR

- In order to define clinical outcomes
- Not sufficient to simply report single variants
- Essential to report yes/no for each important variable
- Variables
  - t(4;14)
  - MAF
  - 1q+
  - 17p-
  - 1p- ?

The end

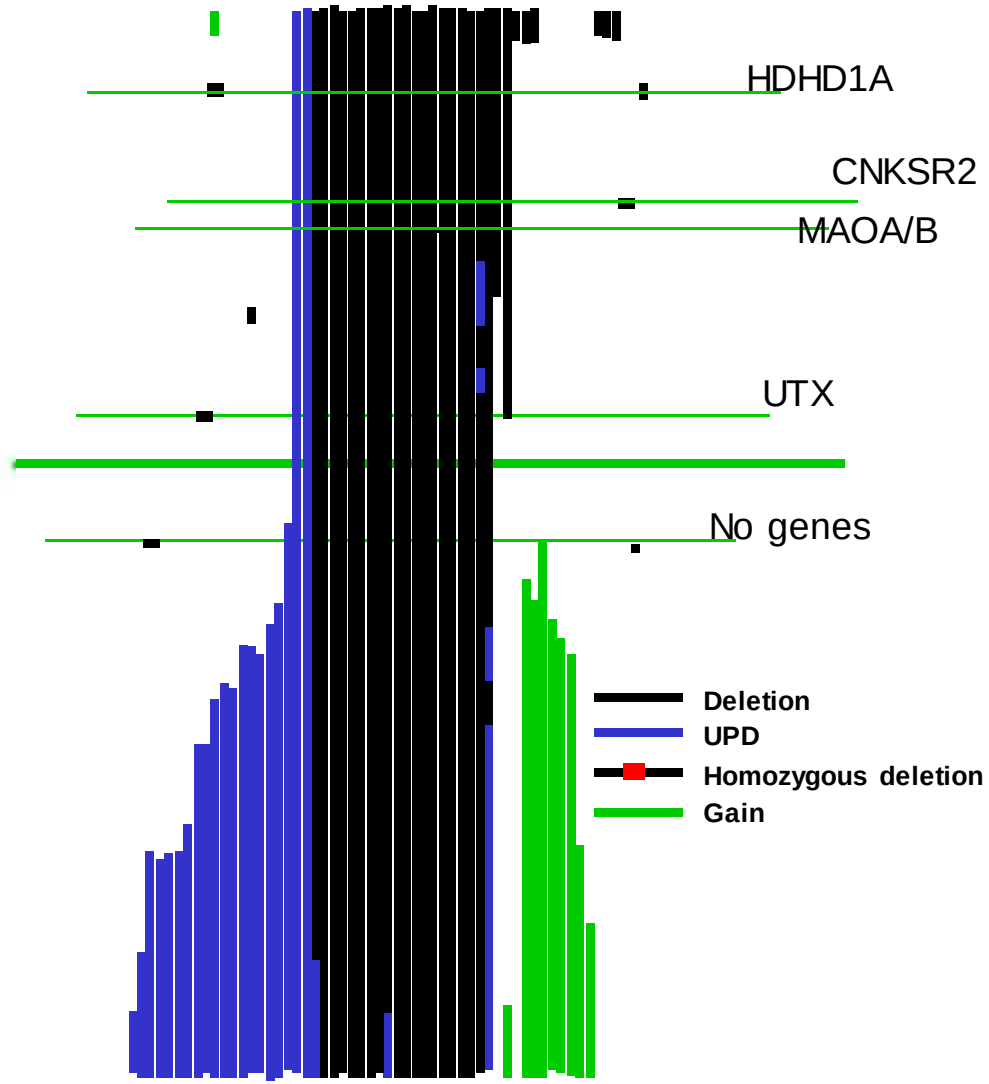

MyelomaUK
CANCER RESEARCH UK
NIHBR
The Bud Flanagan Leukaemia Fund
Kay Kendall Leukaemia Fund

Chromosome X and the UTX gene

Somatic mutations of the histone H3K27 demethylase gene *UTX* in human cancer

Gijs van Haaften[1,10,11], Gillian L Dalgliesh[1,11], Helen Davies[1,11], Lina Chen[1], Graham Bignell[1], Chris Greenman[1], Sarah Edkins[1], Claire Hardy[1], Sarah O'Meara[1], Jon Teague[1], Adam Butler[1], Jonathan Hinton[1], Calli Latimer[1], Jenny Andrews[1], Syd Barthorpe[1], Dave Beare[1], Gemma Buck[1], Peter J Campbell[1], Jennifer Cole[1], Simon Forbes[1], Mingming Jia[1], David Jones[1], Chai Yin Kok[1], Catherine Leroy[1], Meng-Lay Lin[1], David J McBride[1], Mark Maddison[1], Simon Maguire[1], Kirsten McLay[1], Andrew Menzies[1], Tatiana Mironenko[1], Lee Mulderrig[1], Laura Mudie[1], Erin Pleasance[1], Rebecca Shepherd[1], Raffaella Smith[1], Lucy Stebbings[1], Philip Stephens[1], Gurpreet Tang[1], Patrick S Tarpey[1], Rachel Turner[1], Kelly Turrell[1], Jennifer Varian[1], Sofie West[1], Sara Widaa[1], Paul Wray[1], V Peter Collins[2], Koichi Ichimura[2], Simon Law[3], John Wong[3], Siu Tsan Yuen[4], Suet Yi Leung[4], Giovanni Tonon[5,10], Ronald A DePinho[5], Yu-Tzu Tai[6], Kenneth C Anderson[6], Richard J Kahnoski[7], Aaron Massie[7], Sok Kean Khoo[8], Bin Tean Teh[8], Michael R Stratton[1,9] & P Andrew Futreal[1]

- Mutation screen identifies mutation of UTX in 10% myeloma cell lines (Futreal 2009)
- In our screen UTX deleted and expression changed



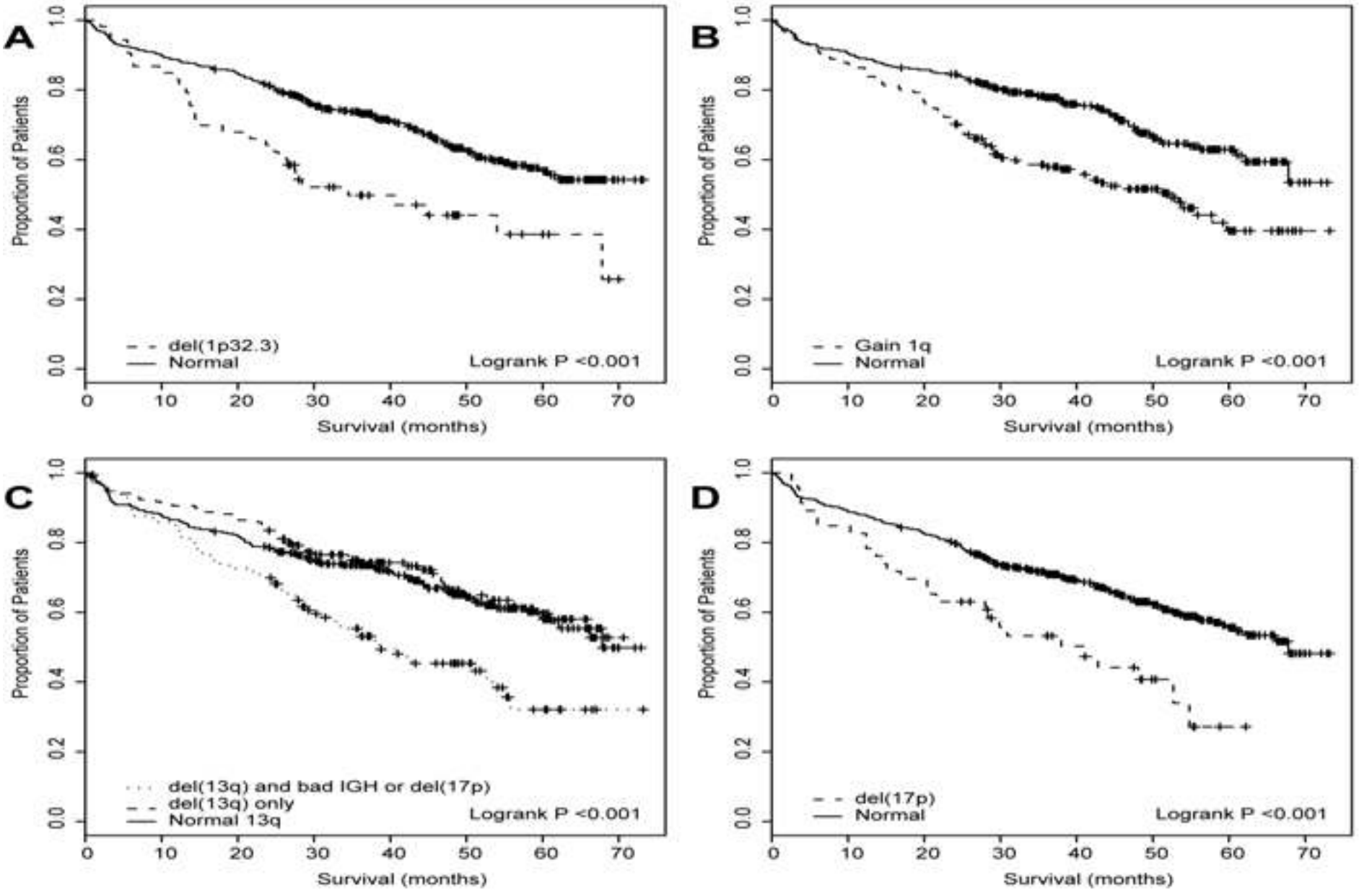
Impact of aberrant IgH rearrangements

We have identified evidence for an impact of *IGH* translocation on chromosomal abnormalities

MYC locus at 8q affected in 9% of samples
CCND1 at 11q (6%), *CCND3* at 6p (4%)
FGFR3/*MMSET* at 4p (6%)

25% of samples with common CNAs associated with translocations.

Prognostic lesion



Prognostic regions verified by FISH analysis

Copy Number Abnormality	Prognostic Significance	Genes Identified	Gene Function
del(1p)	Yes	*FAF1**	Fas associated
		*CDKN2C**	Cell cycle inhibitor
1q+	Yes	*CKS1B**	cyclin dependent kinase
		ANP32E^	histone acetyltransferase inhibitor
del(8p)	No	NA	NA
del(13q)	No#	NA	NA
del(16q)	No	NA	NA
del(17p)	Yes	*TP53**	regulator of transcription

* = significant by FISH
^ = significant by expression quartile analysis
# = not significant by FISH when del(17p), t(4;14), t(14;16) and t(14;20) samples are removed from the analysis
NA = not applicable

Combining Genetics and the ISS

Combined Risk Group	Group	Median OS
Favourable Risk	ISS I and no adverse lesions	Not reached
	ISS I and 1 adverse lesion	Not reached
	ISS II and no adverse lesion	62.6
Intermediate Risk	ISS I and >1 adverse lesion	42.8
	ISS II and 1 adverse lesion	42.3
	ISS III and no adverse lesion	42.9
	ISS III and 1 adverse lesion	35.3
Ultra-High Risk	ISS II and >1 adverse lesion	25.5
	ISS III and >1 adverse lesion	14.4