



A Study on the Detection of ALK Fusion Genes in Genetic Testing Using Surgical Specimens from Cases of Postoperative Recurrence

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Introduction

The number of molecularly targeted therapies available for lung cancer is increasing year by year, and genetic testing has become essential for drug treatment. For cases that recur after surgery alone, genetic testing is often performed using surgical specimens at the time of recurrence. ALK fusion genes are detected in 3–5% of patients regardless of ethnicity, and many molecularly targeted drugs with high response rates have been developed. In cases of postoperative recurrence, if an ALK fusion gene is confirmed, a significant prolongation of survival can be expected with molecularly targeted therapy; therefore, appropriate genetic testing is crucial. In cases of stage p-Stage I lung cancer, we do not carry out multi-gene testing immediately after the pathological diagnosis has been confirmed. If recurrence is detected during post-operative follow-up, genetic testing is carried out at that point using the surgical specimen. Consequently, in cases of recurrence occurring several years after surgery, a considerable amount of time may have elapsed between the specimen being embedded in paraffin and its submission for testing. We investigated whether appropriate ALK fusion gene testing was performed in cases of postoperative recurrence at our institution and whether this led to treatment.

Methods

- We included cases at our hospital where lung cancer recurred after surgical treatment between January 2020 and December 2025, and for which multigene testing was performed using surgical specimens. Cases in which a histological diagnosis had been obtained prior to surgery and in which multi-gene testing had been performed were excluded.
- We examined the presence or absence of genetic abnormalities, the appropriateness of the results, and the number of days from surgery to specimen collection.
- For the group in which all genetic mutations tested negative (All Negative), a t-test was used to compare the time (in months) from sample collection to testing between the subgroups with and without RNA quality issues.
- We compared the detection rate of ALK fusion genes with general detection rates.

Results

- During the study period, multiplex companion diagnostic tests was performed in 48 cases (Oncomine DX Target Test Multi-CDx system: 40 cases; AmoyDx PLC Panel: 8 cases). The identified actionable mutations were as follows: EGFR 15 (Exon 21: 7, Exon19: 8); ALK 2; ROS1 3; HER2 3; MET: 4; KRAS(G12C): 1. (figure1)
- Seventeen cases were all negative; however, in 5 of these cases, fusion gene testing for ALK, ROS1, and others could not be performed due to poor RNA quality (PRQ) in the specimens.
- The 5 cases with poor RNA quality had an average of 11 months (4–24 months) between surgery and specimen collection for genetic testing, while the 12 cases without poor RNA quality could be performed had an average of 25 months (5–62 months). (Table2)
- In the ‘All Negative’ group, the time from surgery to tissue sample collection was significantly shorter in the PRQ (+) group than in the (–) group. (Figure2)

Table1 Clinicopathological characteristics of 48 cases in which genetic mutations were investigated following recurrence

Variables		n	
Age (years)			
	median	72	(52-85)
Sex	male	25	(52%)
	female	23	(48%)
multiplex companion diagnostic tests			
	Oncomine	40	(83%)
	Amoy	8	(17%)
gene mutations			
EGFR		16	(33%)
	Exon19 deletion	8	
	Exon21 L858R	7	
	G719X + others	1	
ALK		2	(4%)
ROS1		3	(6%)
HER2	Exon20 Insertion	3	(6%)
MET	Exon14 Skipping	4	(8%)
KRAS	G12C	1	(2%)
	G12D/S	1	(2%)
PIK3CA/FGFR3		1	(2%)
All Negative		17	(35%)

Oncomain:Oncomine DX Target Test Multi-CDx system Amoy:AmoyDx PLC Panel

Conclusion

Genetic testing carried out on specimen slides submitted for analysis following confirmation of recurrence revealed that ALK fusion genes were detected in 4 per cent of cases; this figure showed no significant difference from generally reported figures. However, this detection rate tended to be lower than that for ROS1 or MET. Among the cases classified as ‘all negative’, approximately 10 per cent of the total 48 cases were those in which testing was not possible due to poor RNA quality. It is possible that ALK-positive cases were included among these. As fusion genes can also be detected by FISH testing, it may be worth considering the addition of FISH testing, where necessary, for cases with poor RNA quality in future. Furthermore, immunostaining is also considered effective for ALK and ROS1. In the PRQ (+) group, the interval between surgery and the collection of the specimen for testing was shorter than in the PRQ (–) group. However, given the small number of cases, there are limitations to the interpretation of this result. These findings suggest that the appropriate handling of specimens (e.g. temperature and time) may be more important than extending the interval until testing. There are many types of ALK inhibitors, and their efficacy is extremely high. We believe that every effort should be made to identify as many cases of ALK-positive lung cancer as possible.

Figure1 Proportion of genetic mutations

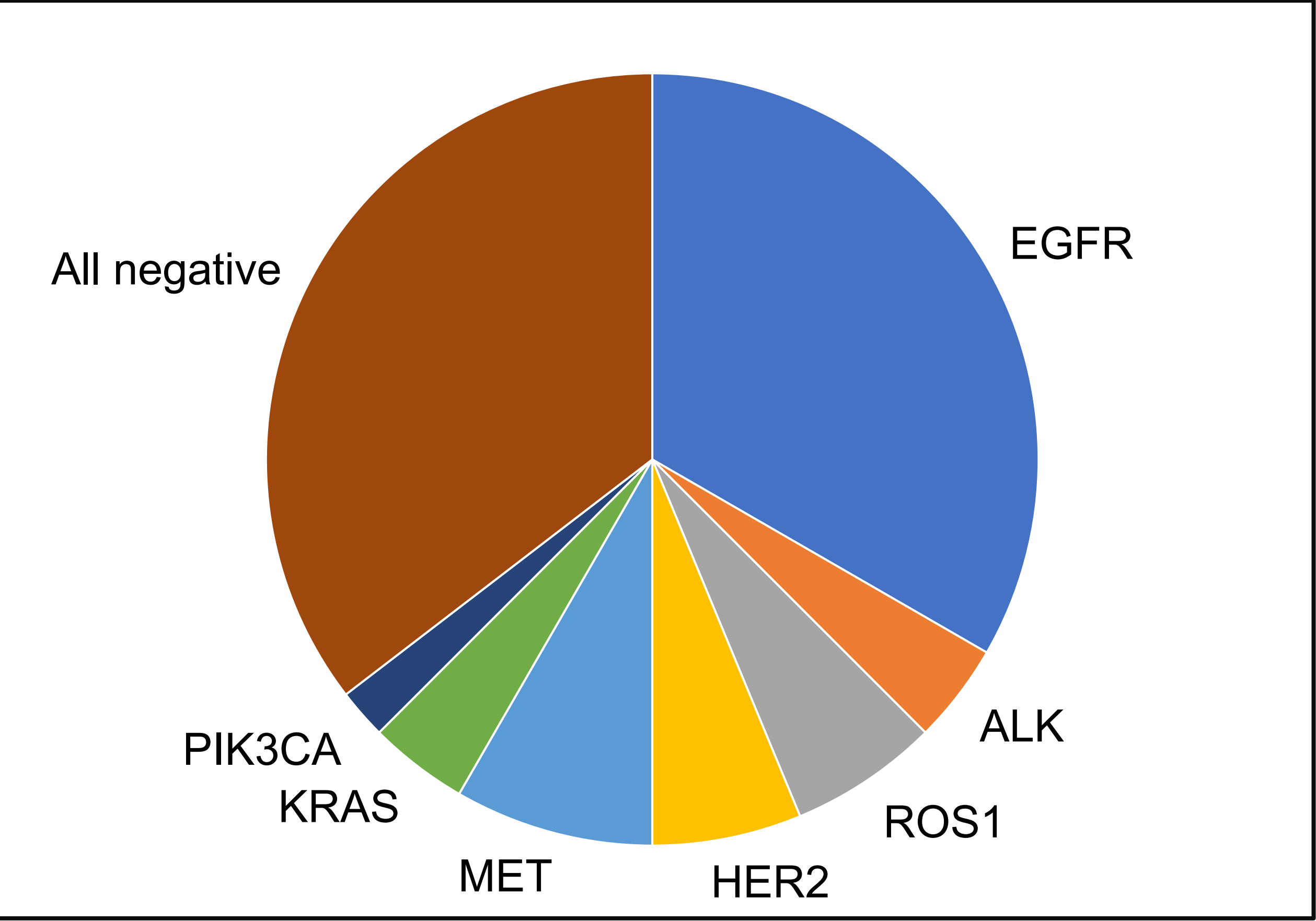
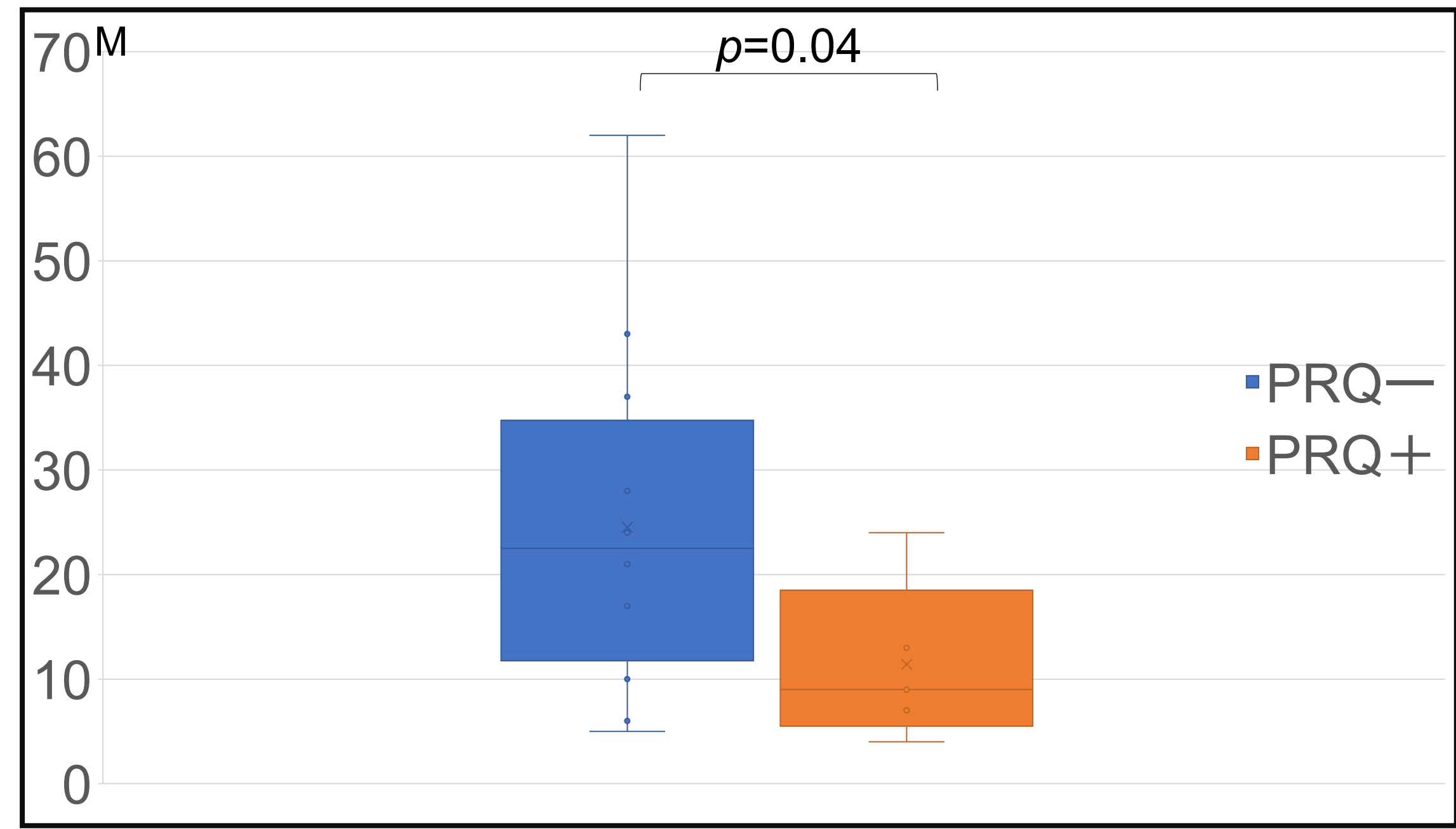


Table2 Number of months from surgery to testing, by presence or absence of genetic mutations and RNA quality

genetic mutation	n	time until the test(M)
presence	31	27 (1-114)
All Negative		
poor RNA quality -	12	25 (5-62)
poor RNA quality +	5	11 (4-24)

Figure2 t-test of the number of months until testing, by presence or absence of poor RNA quality, in cases of ‘All Negative’



PRQ : poor RNA quality