BRIEF REPORT

Long-Term Recurrence of Completely Resected NSCLC

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ABSTRACT

Introduction: The aim of this study is to evaluate the clinical backgrounds, including driver mutations, of those patients with early stage NSCLC who experienced recurrence beyond 5 years after complete resection.

Methods: We used a cohort of 512 consecutive cases of surgically resected NSCLC without other malignances from 2006 to 2011 in Aichi Cancer Center Hospital. The inclusion criteria for this cohort were patients with primary NSCLC who underwent a surgically curable operation.

Results: A total of 172 patients (32.8%) had recurrence after the surgery. Among the recurrent cases, 17 patients (3.3%) had a relapse more than 5 years after the surgery, and all except one (16 of 17, 94.1%) had driver mutations, including gene rearrangements.

Conclusions: Even in early stage NSCLC after complete resection, it was found that some cases had a relapse more than 5 years after the surgery. Most of these cases had some kind of driver mutations; so more than 5 years of postoperative surveillance may be beneficial, especially in those with driver gene mutants.

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Keywords: Driver mutation; Long-term recurrence; Non–small-cell lung cancer; Postoperative surveillance

Introduction

Surgery is one of the standard treatments for patients with clinical early stage NSCLC, and there is no evidence that the mediastinum is involved before the surgical resection. The favorable results reported in the surgery series and the long-term survival data of these patients have established surgery as the treatment of choice.[1]

The rationale for surveillance after initial treatment of NSCLC is to detect recurrence early so that survival and quality of life can be improved. However, there are no randomized trials comparing different postoperative surveillance strategies, including the appropriate length of the observation period.[2]

Guidelines from the International Association for the Study of Lung Cancer and the College of American Pathologists encourage testing for *EGFR* mutations and *ALK* rearrangement in patients with newly diagnosed localized NSCLC.[1] However, there is no evidence to support molecular marker testing at the time of the original diagnosis as a predictive marker of recurrence and a prognostic marker.

The aim of this study is to confirm the usefulness of the initial driver mutation status as a guide for postoperative surveillance period in patients with early stage NSCLC and to reveal the clinical backgrounds of those who had recurrence more than 5 years after the surgery.

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Materials and Methods

Patients

We used a cohort of consecutive series of surgically resected patients with NSCLC from 2006 to 2011 in Aichi Cancer Center Hospital, Nagoya, Japan. The inclusion criterion of this cohort was patients with primary NSCLC who underwent a surgically curable operation. This retrospective study was approved by the Institutional Review Board of Aichi Cancer Center (no: 2019 1 449), and the need for informed consent was waived given the retrospective nature of the study design. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Genomic Test

Exons 18 to 21 of the *EGFR* and *KRAS* genes were analyzed as previously described.[3] Immunohistochemical analysis of ALK, with a mouse monoclonal antibody for ALK (5A4, Dako),[4,5] was done as previously described. Next-generation sequencing with tumor RNA was performed with Ion 540 chips on Ion Torrent S5 Sequencer using barcoded libraries prepared with AmpliSeq Library Preparation Kits (Thermo Fisher Scientific, Wilmington, DE) according to the manufacturer's protocols in patients with recurrence more than 5 years after surgery. In the 10 cases, in which the tumor tissue could be collected at the time of recurrence, the presence or absence of the gene mutations was determined in pairs (Table 1).

Statistical Analysis

The univariate relationship between each independent variable was evaluated using the chi-square test. All statistical analyses were performed using JMP 12 software (SAS Institute, Cary, NC).

Results

The median time of follow-up was 61.6 months. The patients' backgrounds are listed in Table 2. Briefly, a total of 172 patients (32.8%) had recurrence after the surgery. Among the recurrent cases, 17 patients (3.3%) had a relapse more than 5 years after the surgery and all except one of them(16 of 17, 94.1%) had driver mutations, including gene rearrangements (Tables 1 and 3), which were statistically significant when compared with the cases recurrent within 5 years.

The distribution of *EGFR* and *KRAS* mutations according to histologic patterns and pathologic stages is reported in Table 3. Papillary predominant adenocarcinoma (59.2%) was the most common histologic subtype for *EGFR* mutants. Most cases harboring *EGFR* mutations were of pathologic stage IA and IB (74.2%).

Table 1. The Driver Mutation Statuses of the 17 Patients of Primary and Recurrent Lesions

Driver Gene Alteration of Primary Lung Lesion	Recurrent Lesion	Driver Gene Alteration of Reccurnt Lesion
CD74-ROS1	Bone	Rebiopsy was not done
L858R	Brain	Rebiopsy was not done
19 deletion	Lung	Rebiopsy was not done
CD74-ROS1	Lung	CD74-ROS1
G12C	Pleural effusion	G12C
Wild type	Pleural effusion	Rebiopsy was not done
19deletion	Brain	Rebiopsy was not done
L858R	Lung	L858R
L858R	Lung	L858R
19 deletion	Lung	19 deletion
19 deletion	Lung	19 deletion
19 deletion	Lung	19 deletion
KIF5-RET	Lung	KIF5-RET
G12C	Lung	Rebiopsy was not done
L858R	Mediastinal lymph node	L858R
19 deletion	Lung	Rebiopsy was not done
S768I	Lung	S768I

The precise data for somatic gene alterations are reported in Table 4. A total of 11 patients (64.7%) had *EGFR*-activating mutations (four with L858R, six with exon 19 deletions, and one with S768I), followed by two *KRAS* G12C, two CD74-ROS1, and one KIF5-RET. Only one patient had no driver mutations – none of *EGFR*, *KRAS*, *BRAF*, and *HER2*, and *ALK* or *ROS1* gene rearrangement.

The cumulative hazard ratio (HR) of recurrence is illustrated in Figure 1 and that of *EGFR* mutants and cases without driver gene alterations in Figures 2 and 3, respectively. The increase in HR after 5 years is caused by the *EGFR* mutant, with peaks appearing at 6 and 9 years (Fig. 2). It is apparent that the risk of recurrence is highest 9 months after surgery and decreases over time in cases without driver mutations (Fig. 3).

Discussion

In this study, we analyzed the genomic background of patients who had experienced recurrence more than 5 years after complete resection for early stage NSCLC and revealed that most of them harbored driver mutations, including oncogenic fusion genes. In particular, in the *EGFR* mutation

Table 2. Patient Background Characteristics

Characteristic	All, n (%)	Recurrence Within 5 Years	Recurrence Beyond 5 Years	*p* Value
Sex				
Male/female	313 (61.1)/199(38.9)	104/51	10/7	0.678
Age median [range]	66 [22-86]	66 [39-85]	65 [38-80]	
Histologic type				
Adeno/nonadeno	370 (72.0)/142 (38.0)	98/57	15/2	0.073
With/without STAS	149 (29.0)/363 (71.0)	64/91	5/12	0.491
Pathologic stage				
IA/IB	235 (45.9)/63 (12.3)	24/22	9/1	0.030[a]
IIA/IIB	25 (4.9)/82 (16.0)	9/35	0/5	
IIIA	107 (20.9)	65	2	
Operative procedure				
Lobectomy/others	474 (92.5)/38 (7.5)	139/16	17/0	0.341
Adjuvant chemotherapy				
UFT	37 (7.2)	11	1	
Platinum doublets	88 (17.1)	58	1	
Cytotoxic monotherapy	7 (1.4)	1	0	
Driver mutation				
EGFR mutant	201 (39.2)	56	11	0.0004[b]
KRAS mutant	42 (8.2)	11	2	
Other mutants[c]	21 (4.1)	4	3	
Wild type	248 (48.5)	84	1	
Total	512	155	17	—

[a]Chi-square test was performed according to stage I, II, and III between the two groups.
[b]Chi-square test was performed with and without driver mutations between the two groups.
[c]*ALK*, *ROS1*, and *RET* rearrangement.
Adeno, adenocarcinoma; STAS, spread through air spaces; UFT, an oral 5-fluorouracil derivative agent.

cases, the HR of recurrence did not reach zero even after 5 years of follow-up and recurrence cases were also recognized after 9 years.

It is well known that the treatment for breast cancer is longer than for other solid cancers. In 2012, a randomized controlled trial, Adjuvant Tamoxifen: Longer Against, revealed that extending adjuvant tamoxifen treatment from 5 years to 10 years could markedly reduce the risk of death from breast cancer.[6] A hypothesis has been proposed for the mechanism by which the risk of recurrence in breast cancer continues over a long period of time, in which breast cancer cells enter the bone marrow and maintain a long-term dormancy.

Table 3. The Driver Mutation Statuses of Patients With NSCLC Who Had a Relapse More Than 5 Years After Surgery

	Long Interval Recurrence (N = 17)
Driver mutation	
EGFR: p.(Leu858Arg)	4
EGFR: g.(exon19del)	6
EGFR: p.(Ser768Ile)	1
KRAS: p.(Gly12Cys)	2
c. CD74:ROS1 (C6;R34)	2
c. KIF5:RET (K:R12)	1
Wild type	1

However, few reports have examined the risk of recurrence after more than 10 years in solid tumors other than breast cancer. For prostate cancer, recurrence more than 5 years after surgery was reported to be found in several percent of completely resected cases[7]; therefore, monitoring of prostate-specific antigen for recurrence for more than 5 years after surgery is recommended.[8]

In NSCLC, follow-up for 5 years after surgery is recommended[1] and postoperative follow-up ends in 5 years in effect. In our cohort, a lot of censored cases were found in term between 60 months and 63 months after surgery, which may well reflect this guideline recommendation (Fig. 1). In a report comparing institutional surveillance patterns after NSCLC treatment with stereotactic body radiation therapy and lobectomy, a certain risk of recurrence beyond 5 years was found in the lobectomy group.[9] In a study evaluating the patterns and risks of postoperative recurrence in completely resected *EGFR*-mutant NSCLC, thoracic recurrence, in particular, also seems to have certain risks beyond 5 years.[10] Tamiya et al.[11] reported the follow-up data of a prospective, multicenter, genomic examination of 876 surgically resected NSCLC cases and revealed that *EGFR* mutations were significantly associated with improved overall survival, given the potential value of the results

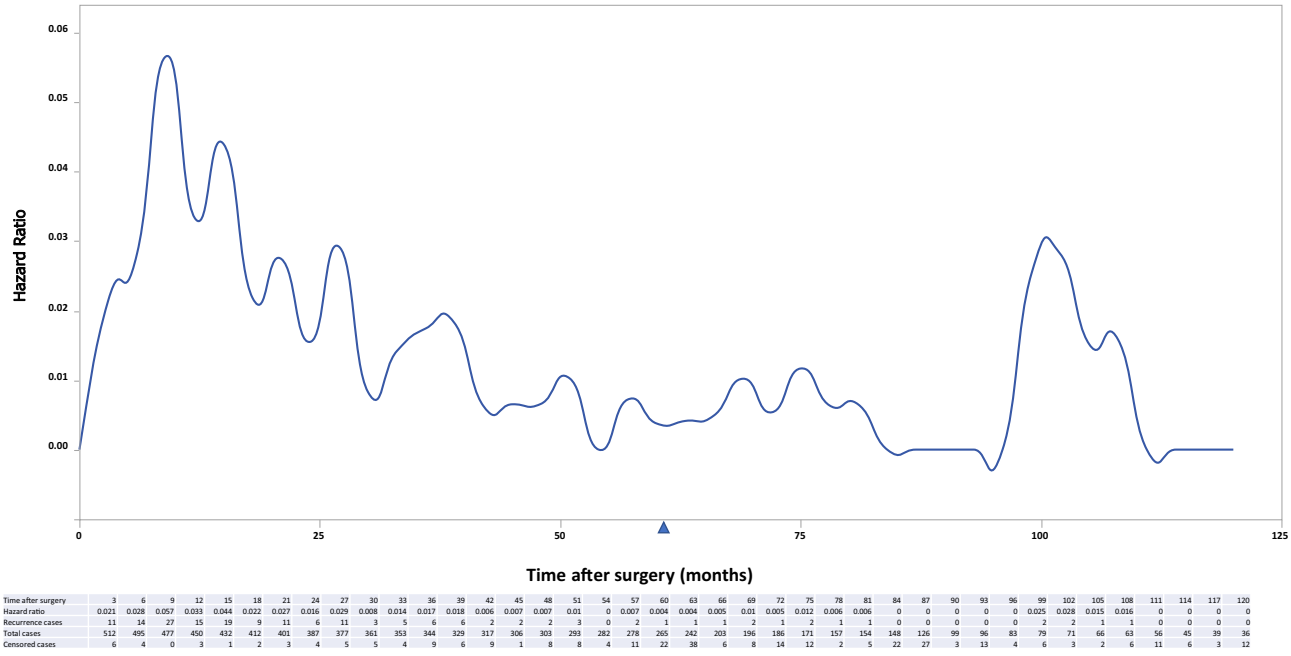
Table 4. Distribution of *EGFR* and *KRAS* Mutations According to Histologic Subtypes and Pathologic Stage

EGFR and *KRAS* Mutations	Total Number = 512, n (%)
EGFR mutations, n	201
Histologic subtypes	
Adenocarcinoma	196 (97.5)
Papillary predominant	119 (59.2)
Acinar predominant	35 (17.5)
Lepidic predominant	24 (11.9)
Solid predominant	18 (8.9)
Pleomorphic	1 (0.5)
Adenosquamous	2 (1)
Squamous	1 (0.5)
Clear cell	1 (0.5)
Pathologic stage	
IA	132 (65.7)
IB	17 (8.5)
IIA	4 (1.9)
IIB	21 (10.4)
IIIA	27 (13.5)
KRAS mutations, n	42
Histologic subtypes	
Adenocarcinoma	35 (83.4)
Papillary predominant	12 (28.5)
Invasive mucinous	11 (26.1)
Lepidic predominant	3 (7.1)
Solid predominant	3 (7.1)
Acinar predominant	1 (2.3)
Large	7 (16.6)
Pathologic stage	
IA	17 (40.4)
IB	7 (16.7)
IIA	3 (7.2)
IIB	8 (19.0)
IIIA	7 (16.7)

in predicting outcome in patients with NSCLC and the possibility of intervention after surgery.

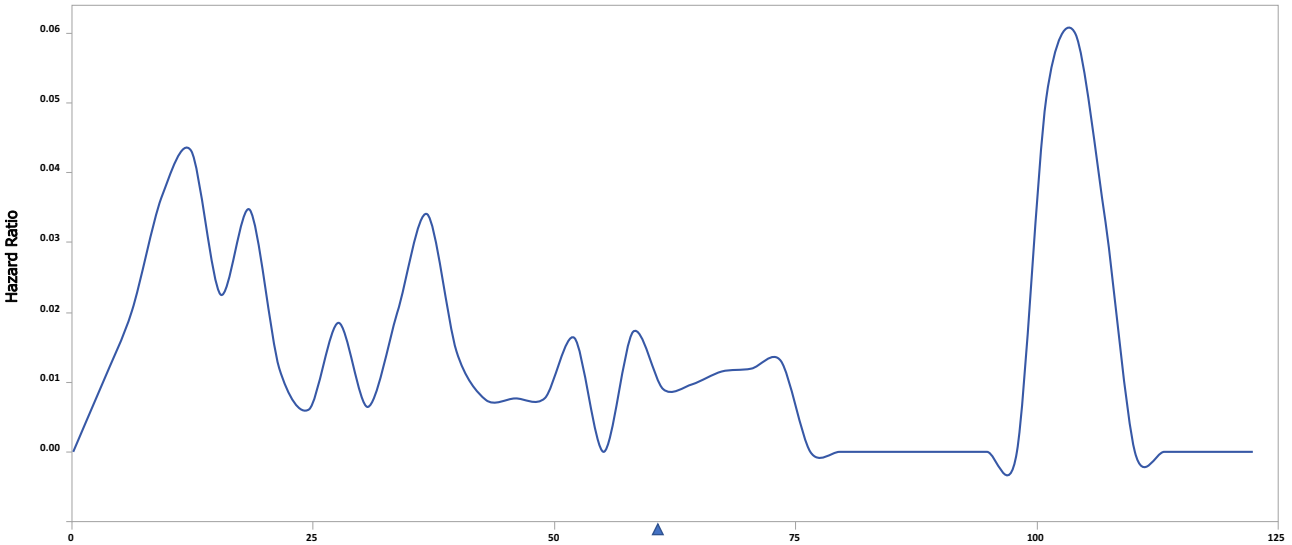
Several mechanisms that explain these findings may be speculated upon. First, taking *EGFR* L858R as an example, the transiently transfected *EGFR*-L858R mutant in CL1-0 lung cancer cells can promote lung cancer invasion.[12] In other words, *EGFR*-mutant cells easily form micrometastases from the early stage of the disease and a niche. However, driver mutation–related tumor cells have less tumor mutational burden[13] and are more likely to escape the host immune surveillance. In addition, it is speculated that the driver mutant tumor cells rely on the activated cascade for the survival of the tumor cells, and thus are unlikely to cause spread from the niche. Recently, Chalela et al.[14] explored the prognosis impact of *EGFR* and *KRAS* mutations in the nontumoral lung of patients with adenocarcinoma and found that 21.3% of the patients had *EGFR* or *KRAS* mutations in their adenocarcinoma and also in their histologically normal lung tissue. In addition to the possible relationship of this finding with both the carcinogenesis process and the extension of a primary tumor to other organs, mutations in the normal lung tissue seem to be associated with a worse short-term prognosis.[14] The long-term recurrence cases found in our study may also be derived from normal cells harboring *EGFR* mutations.

In a post hoc analysis of a randomized phase III trial (ADJUVANT and CTONG1104) of adjuvant gefitinib therapy in the treatment of Chinese patients who had undergone complete resection for *EGFR*-mutant stage II to IIIA NSCLC, temporal distribution analysis revealed that the rate of recurrence was lower in the gefitinib group in the early period after surgery, which is also



Time after surgery	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108	111	114	117	120
Hazard ratio	0.021	0.028	0.057	0.033	0.044	0.022	0.027	0.016	0.029	0.008	0.014	0.017	0.018	0.006	0.007	0.007	0.01	0	0.007	0.004	0.004	0.005	0.01	0.005	0.012	0.006	0.006	0	0	0	0	0	0.025	0.028	0.015	0.016	0	0	0	0
Recurrence cases	11	14	27	15	19	9	11	6	11	3	5	6	6	2	2	2	3	0	2	1	1	1	2	1	2	1	1	0	0	0	0	0	2	2	1	1	0	0	0	0
Total cases	512	495	477	450	432	412	401	387	377	361	353	344	329	317	306	303	293	282	278	265	242	203	196	186	171	157	154	148	126	99	96	83	79	71	66	63	56	45	39	36
Censored cases	6	4	0	3	1	2	3	4	5	5	4	9	6	9	1	8	8	4	11	22	38	6	8	14	12	2	5	22	27	3	13	4	6	3	2	6	11	6	3	12

Figure 1. Cumulative HR of recurrence after surgery of all patients. HR, hazard ratio.



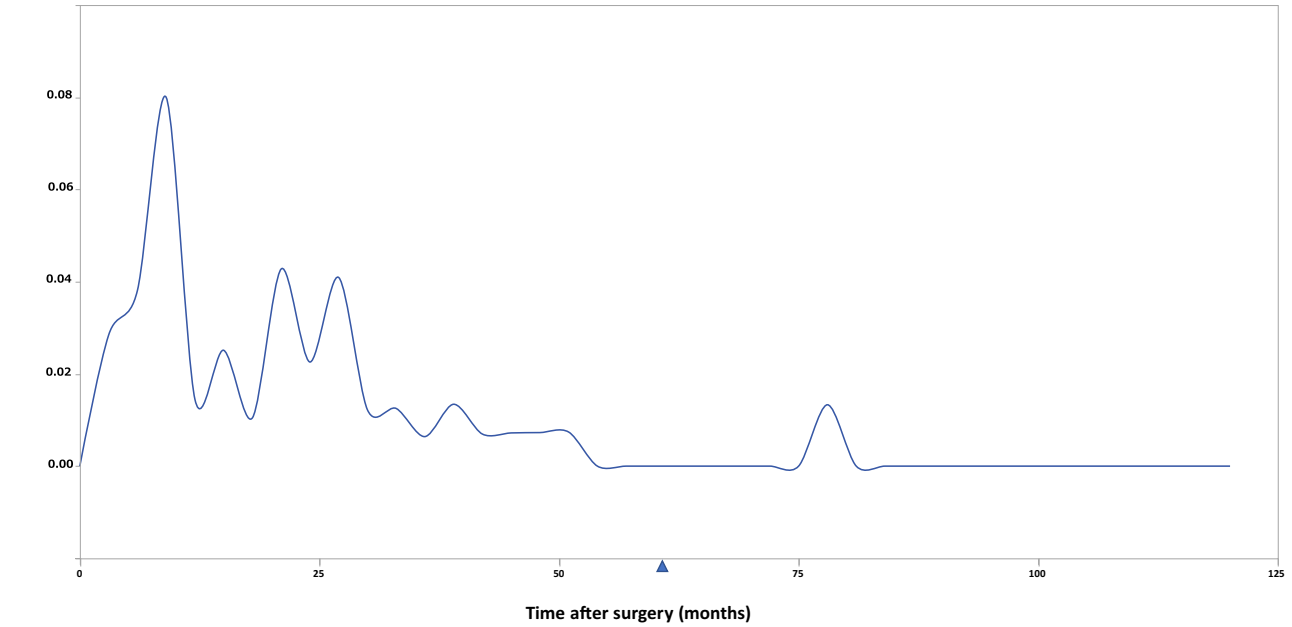
Time after surgery	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108	111	114	117	120
Hazard Ratio	0.01	0.02	0.037	0.044	0.023	0.035	0.012	0.006	0.019	0.006	0.02	0.034	0.015	0.008	0.008	0.008	0.017	0	0.017	0.009	0.01	0.012	0.012	0.013	0	0	0	0	0	0	0	0	0.051	0.061	0.033	0	0	0	0	0
Recurrence cases	2	4	7	8	4	6	2	1	3	1	3	5	2	1	1	1	2	0	2	1	1	1	1	1	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0	0
Total cases	201	196	190	183	175	171	165	162	160	154	149	145	137	133	129	128	121	116	115	110	102	86	83	76	67	66	65	64	55	46	44	40	39	33	30	28	25	20	18	16
Censored cases	3	2	0	0	0	0	1	1	3	4	1	3	2	3	0	6	3	1	3	7	15	2	6	8	1	1	1	9	9	2	4	1	4	1	1	3	5	2	2	5

Figure 2. Cumulative HR of recurrence after surgery of patients harboring *EGFR*-activating mutations. HR, hazard ratio.

consistent with our results.[15] Considering these results, it may be useful to evaluate the molecular profile as much as possible during surgery in terms of postoperative surveillance.

The limitations of this study include its small sample size and retrospective nature. In the future, a prospective study, preferably multicenter, may be needed to confirm our results.

In conclusion, even in early stage NSCLC after complete resection, it was found that some cases had a relapse more than 5 years after surgery, as in breast cancer. Most of these cases had some kind of driver mutations; so it is proposed that more than 5 years of postoperative surveillance is needed, especially for these cases, and also suggesting that postoperative therapy with molecular targeted drugs might be necessary.



Time after surgery	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108	111	114	117	120
Hazard Ratio	0.028	0.038	0.08	0.014	0.025	0.01	0.043	0.023	0.041	0.012	0.013	0.006	0.013	0.007	0.007	0.007	0.007	0	0	0	0	0	0	0	0	0.013	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Recurrence cases	7	9	18	3	5	2	8	4	7	2	2	1	2	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total cases	246	236	225	207	199	191	187	177	171	163	160	156	149	144	139	137	135	132	129	123	109	94	91	89	86	75	73	71	63	49	47	43	40	38	36	35	32	26	22	21
Censored cases	3	2	0	5	3	2	2	2	1	1	2	6	3	4	1	1	2	3	6	14	15	3	2	3	11	1	2	8	14	2	4	3	2	2	1	3	6	4	1	5

Figure 3. Cumulative HR of recurrence after surgery of patients without driver gene alterations. HR, hazard ratio.

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