ALDH2 Polymorphism rs671 is A Predictor of Immune Checkpoint Inhibitor Efficacy Against Chest Malignancies

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Abstract

Background

Aldehyde dehydrogenase 2 (ALDH2) plays an important role in the endogenous aldehyde detoxification of various types of cells. *ALDH2*2*, a variant allele of the *ALDH2* polymorphism rs671, leads to decreased enzymatic activity. *ALDH2*2* may enhance tumor antigen presentation due to aldehyde-induced DNA damage while suppressing peripheral blood T cell counts and T cell activation.

Methods

On the basis of our hypothesis that rs671 affects the sensitivity of immune checkpoint inhibitors (ICIs), we evaluated the effects of rs671 on patients with chest malignancies who started ICI therapy in 2016–2019. The cohort consisted of 105 cases, including 64 adenocarcinoma and 30 squamous cell carcinoma patients, 49 of whom were *ALDH2*2* carriers. Nivolumab, Pembrolizumab, or Atezolizumab was used for the first ICI treatment.

Results

The best response to ICI therapy (partial response/stable disease/progressive disease) was 36%/50%/14% in the rs671(−) cases; however, the response was relatively poor in the rs671(+) cases (27%/29%/45%, respectively) ($p = 0.002$). The hazard ratio (95% confidence interval) of disease progression within the observation period of 6 months for the rs671(+) cases was estimated to be 5.0 (2.5–10) after the adjustment for covariates, including sex, Brinkman index, treatment line, tumor tissue programmed death-ligand 1 positivity rate, and tumor tissue *EGFR* mutation. This association was also maintained in a stratified analysis, suggesting that *ALDH2*2* is an independent negative predictive factor for the short-term prognosis of ICI therapy. Thus, the progression-free survival (PFS) ratio of the rs671(+) cases decreased rapidly after ICI initiation but was eventually higher than that of the rs671(−) cases (restricted mean survival time in 12 months from 2 to 3 years afterward was 1.3 times that of the rs671(−) cases). Moreover, the highest PFS ratio after 2 years among sub-groups was found in the first-line treatment group of rs671(+) patients (40%).

Conclusions

Our study suggests that rs671 may be an accurate and cost-effective predictor of ICI treatment, in which optimal case selection is an important issue.

1 Background

Aldehyde dehydrogenase 2 (ALDH2) is expressed in many tissues, including blood cells[1, 2], and metabolizes endogenous aldehydes, such as formaldehyde, acetaldehyde, and 4 hydroxynonenal (4HNE) [3, 4]. Approximately half of the Japanese population and at least 2% of the global population shows the low-activity phenotype derived from the *ALDH2* genetic polymorphism rs671 (the variant allele is named *ALDH2*2*), which is associated with differences in lifestyle habits, disease risks, and drug sensitivities[5, 6]. The association is complicated, bidirectional, and rather strong[7]. For example, esophageal cancer is less common among *ALDH2*2* carriers due to reduced drinking habits, but *ALDH2*2* carriers with drinking habits show the highest risk because of accumulated aldehydes[8]. Additionally, *ALDH2*2* is reported to increase the risk for leprosy[9], whereas viral hepatitis is mild in *ALDH2*2* carriers[10], likely due to the alleviation of inflammation by the presence of aldehydes[11, 12]. Because hepatitis is a primary carcinogenesis promoter, it is reasonable that *ALDH2*2* is reported as a protective factor against liver cancer[13, 14].

Immune checkpoint inhibitors (ICIs) are an innovative cancer treatment that provides benefits for some but not the majority of patients; therefore, understanding the ICI-sensitive population is an important challenge. To date, rs671 has not been studied as a potential predictor of ICI treatment, but it may have a complicated, bidirectional, and strong effect on ICI therapy for the following reasons: 1) Cancer cells of *ALDH2*2* carriers may show more DNA damage induced by aldehyde exposure during smoking and drinking[15, 16], resulting in an increased presentation of antigens to immune cells, which is advantageous in ICI treatment. 2) Because endogenous 4HNE, a typical endogenous aldehyde that accumulates in *ALDH2*2* carriers, delays cell proliferation[3, 17–20], ICI resistance due to genetic mutations in cancer cells[21, 22] is less likely to occur. 3) However, high aldehyde concentrations can suppress immune cell activation[12], making the short-term effect of ICIs difficult to detect. 4) Nevertheless, T cell exhaustion is unlikely to occur[23, 24], and this may be advantageous in long-term ICI therapy. 5) Lastly, the low T cell count in the peripheral blood of *ALDH2*2* carriers reported previously may have a negative effect on ICI treatment[25]. Thus, to verify the hypothesis that *ALDH2*2* carriers show a different ICI sensitivity compared with non-carriers, we investigated patients with ICI-treated chest malignancies.

2 Methods

2.1 Patients

The subjects were 106 patients with chest malignancies who received ICI treatment at the Division of Hematology, Respiratory Medicine and Oncology, Saga University School of Medicine from February 2016 to May 2019 and provided written consent for genetic analyses. There was no restriction on the number of ICI doses, type of ICI, and chemotherapy after the first ICI dose. We obtained relevant information from the electronic medical records. The *ALDH2* genotype (rs671) was determined in peripheral blood mononuclear DNA stored at − 20°C using a TaqMan® SNP genotyping assay system in accordance with the instructions (ThermoFisher Scientific, Waltham, MA, USA). One patient was excluded from the study

after less than 3 months of observation without disease progression. The study was approved by the clinical study ethics review committee of Saga University (project ID R1-16) and conducted accordingly.

2.2 Statistics

2.2.1 Main outcomes: best response to ICI therapy

One of the main outcomes was the best response to ICI treatment. Best responses were classified as Complete Response (CR), Partial Response (PR), Stable Disease (SD), and Progressive Disease (PD) according to RECIST Ver1.1.[26]. CR is defined as the disappearance of all target lesions with any pathological lymph nodes reduced in the short axis to < 10 mm and PR as at least a 30% decrease in the sum of target lesion diameters, taking as reference the baseline sum diameters. PD is defined as at least a 20% increase in the sum of target lesion diameters, taking as reference the smallest sum, and the sum must also demonstrate an absolute increase of at least 5 mm. Lastly, SD is defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters.

2.2.2 Main outcomes: restricted mean survival time (RMST)

The RMST introduced by Royston and Parmar[27] of the progression-free survival (PFS) was used because a proportional hazard assumption has not been established between the rs671 groups for PFS (Figs. 1, S1). RSMT was estimated as the area under the survival curve between the time points (LIFETEST procedure in SAS 9.4, SAS Institute Inc., Cary, NC, USA).

2.2.3 Secondary outcomes: PFS ratio during a 6-month observation period

From the biological background described in the Background section, the effect of ICIs on short-term prognosis and long-term prognosis may differ for each rs671 group. From the report on the adhesion time of programmed cell death-1 (PD-1) antibodies and memory T cells[21], the half-life of ICIs is estimated to be several months. The longer the observation period, the more effects of time-dependent covariates that cannot be adjusted; for example, the number of ICI administrations after the second dose (up to 68 times in this study) (Table S1), drug used, presence or absence of adverse reactions, and onset time of adverse reactions. Thus, we aimed to directly compare the short-term effects of the initial ICI by limiting the observation period. Because proportional hazard assumption during this period is established (Fig. S1), a Cox proportional hazard model was used to estimate the hazard ratio (HR) after multivariate adjustment (PHREG procedure in SAS 9.4). Covariates were identified as attributes suspected to be associated with disease progression or rs671. For example, *EGFR* mutations in tumor tissue, female sex, non-smoker, and adenocarcinoma tissues are predictors of poor prognosis[28–30], and *EGFR* mutations and programmed death-ligand 1 (PD-L1) expression are associated with smoking habits[30, 31]. Smoking habit is also associated with rs671[32, 33]. Thus, sex, age (continuous), Brinkman index (< 100, < 1000, ≥1000) (ordinal), type of first ICI, tumor histotype, TNM classification (categorical), number of lines (first, second, third, and later) (categorical), chemistry with ICIs, PD-L1 positivity ratio (< 1%, < 50%, -100%, unassessed)

(categorical), and *EGFR* mutation ((+), (-), unassessed) (categorical) were set as the covariates. Additionally, as a time-dependent covariate, the presence or absence of immune-related adverse events (irAEs) (defined as ICI withdrawal or prednisolone administration due to immune-related side effects) that occurred prior to disease progression was used. The number of days before the appearance of irAEs was entered as a continuous variable (Supporting information, SAS code). Chemotherapy, which started before disease progression, was also considered. However, because it was applied to only one case, it was not used as a variable. In addition, stratified analyses were performed in case the multivariate adjustment was inadequate.

2.2.4 PFS ratio per rs671 group

The PFS ratio was determined by rs671 groups because the effects of covariates on ICI treatment are likely to differ between rs671 groups based on biological assumptions. For example, chemotherapy before ICI for rs671(+) patients may cause more T cell immunity loss because their basic T cell count is lower than that of rs671(−) patients[25]. If grouped by rs671, the proportional hazard assumption for the total observation period is maintained between sub-groups (Fig. 2). Therefore, the HR was estimated using a Cox proportional hazard model (PHREG procedure in SAS 9.4).

3 Results

3.1 Basic characteristics

The characteristics of the patients are shown in Table 1. The distribution of tumor tissue PD-L1 positivity rates was different between the *ALDH2* genotypes. In the time-dependent variables shown in Table S1, the number of ICI administrations was low in the rs671(+) group.

Table 1
Characteristics of patients by ALDH2 genotype.

	Total		Rs671(−)		Rs671(+)		p
All	105		56		49		
Before ICI							
Sex							
Male	89	(85%)	44	(79%)	45	(92%)	0.059
Female	16	(15%)	12	(21%)	4	(8%)	
Age (years)							
Median (IQR)	69		69		69		0.835
40–49	6	(6%)	4	(7%)	2	(4%)	0.970
50–59	12	(11%)	6	(11%)	6	(12%)	
60–69	37	(35%)	20	(36%)	17	(35%)	
70–79	46	(44%)	24	(43%)	22	(45%)	
80–89	4	(4%)	2	(4%)	2	(4%)	
Brinkman Index							
< 100	14	(13%)	10	(18%)	4	(8%)	0.330
< 1000	39	(37%)	19	(34%)	20	(41%)	
≥ 1000	52	(50%)	27	(48%)	25	(51%)	
Type of ICI (first dose)							
Nivolumab	40	(38%)	23	(41%)	17	(35%)	0.658
Pembrolizumab	45	(43%)	24	(43%)	21	(43%)	
Atezolizumab	20	(19%)	9	(16%)	11	(22%)	
Tumor histotype							
Adenocarcinoma	64	(61%)	31	(55%)	33	(67%)	0.176
Squamous cell carcinoma	30	(29%)	19	(34%)	11	(22%)	
Pleomorphic carcinoma	5	(5%)	1	(2%)	4	(8%)	
Mesothelioma	2	(2%)	2	(4%)	0	(0%)	

Rs671(−); *ALDH2*1/*1*, rs671(+); *ALDH2*1/*2* or *ALDH2*2/*2*, ICI; immune checkpoint inhibitor, IQR; interquartile range, PD-L1; programmed death-ligand 1, *EGFR*; epidermal growth factor receptor.

	Total		Rs671(−)		Rs671(+)		p
Other	4	(4%)	3	(5%)	1	(2%)	
TNM classification							
Stage III	25	(24%)	15	(27%)	10	(20%)	0.148
Stage IV	51	(49%)	30	(54%)	21	(43%)	
Unknown	29	(28%)	11	(20%)	18	(37%)	
Treatment line							
First-line	41	(39%)	21	(38%)	20	(41%)	0.840
Second-line	31	(30%)	16	(29%)	15	(31%)	
Third-line and later	33	(31%)	19	(34%)	14	(29%)	
Chemotherapy with first ICI							
Yes	6	(6%)	4	(7%)	2	(4%)	0.683
No	99	(94%)	52	(93%)	47	(96%)	
PD-L1 (+) ratio in cancer tissue							
< 1%	20	(19%)	5	(9%)	15	(31%)	0.012
< 50%	22	(21%)	13	(23%)	9	(18%)	
≥ 50%	48	(46%)	26	(46%)	22	(45%)	
Unassessed	15	(14%)	12	(21%)	3	(6%)	
EGFR mutation in cancer tissue							
(+)	8	(8%)	5	(9%)	3	(6%)	0.400
(−)	81	(77%)	38	(68%)	43	(88%)	
Unassessed	16	(15%)	13	(23%)	3	(6%)	

Rs671(−); *ALDH2*1/*1*, rs671(+); *ALDH2*1/*2* or *ALDH2*2/*2*, ICI; immune checkpoint inhibitor, IQR; interquartile range, PD-L1; programmed death-ligand 1, *EGFR*; epidermal growth factor receptor.

3.2 ICI best response and RMST

The best response to ICI therapy is shown in Table 2. In all cases, the best effect was observed at the initial ICI evaluation, and the response was better in the rs671(−) group than in the rs671(+) group. The PFS curve (Fig. 1) also suggested that *ALDH2*2* has a negative effect early after ICI initiation, but after 2 years, the PFS ratio was higher in the rs671(+) group than in the rs671(−) group, and after 2–3 years (12-month period), the RMST was 0.26 in the rs671(+) group and 0.20 in the rs671(−) group (Table 3).

Table 2
Overall best response per RECIST Ver1.1. by ALDH2 genotype.

	Total		Rs671(−)		Rs671(+)		p
Best response to immune checkpoint inhibitor							
Complete response	0	(0%)	0	(0%)	0	(0%)	0.0022
Partial response	33	(31%)	20	(36%)	13	(27%)	
Stable disease	42	(40%)	28	(50%)	14	(29%)	
Progressive disease	30	(29%)	8	(14%)	22	(45%)	
Disease control rate	71%		86%		55%		0.0005

p; probability value for Chi-squared test, Fisher's exact test, or Wilcoxon rank-sum test.

Rs671(−); *ALDH2*1/*1*, rs671(+); *ALDH2*1/*2* or *ALDH2*2/*2*, disease control rate; (all − progressive disease)/all, p; probability value for Chi-squared test.

Table 3
Progression-free survival rate after the initiation of immune checkpoint inhibitors.

Observation period	Restricted mean survival time	
	Rs671(−)	Rs671(+)
0–6 months	0.82	0.58
6–12 months	0.46	0.31
12–24 months	0.25	0.26
24–36 months	0.20	0.26

Rs671(−); *ALDH2*1/*1*, rs671(+); *ALDH2*1/*2* or *ALDH2*2/*2*.

3.3 Multivariate-adjusted HR during a 6-month observation period

The multivariate-adjusted HR during a 6-month observation period is shown in Table 4. In model 3 with all variables, the HR (95% confidence interval (CI), p-value) of the rs671(+) group was estimated to be 5.0 (2.5–10, $p < 0.0001$). It was 4.9 (2.4–10, $p < 0.0001$) (Akaike's Information Criterion = 462) and almost unchanged after the adjustment of the time-dependent variables (the presence or absence of irAEs and timing of onset). In the stratified analysis, the HR was estimated to be high in the rs671(+) group almost consistently (Table 5).

Table 4
Hazard ratio of cancer progression for *ALDH2*2* carriers estimated from a 6-month observation.

	Rs671	HR	95% CI	p	AIC
Model 1	(−)	1.00	(reference)		464
	(+)	3.33	(1.80–6.15)	0.0001	
Model 2	(−)	1.00	(reference)		465
	(+)	4.89	(2.37–10.1)	< 0.0001	
Model 3	(−)	1.00	(reference)		460
	(+)	5.04	(2.48–10.2)	< 0.0001	

Rs671(−); *ALDH2*1/*1*, rs671(+); *ALDH2*1/*2* or *ALDH2*2/*2*, HR; hazard ratio by Cox proportional hazard model, CI; confidence interval, AIC; Akaike's Information Criterion.

Model 1: adjusted for sex, age (continuous), Brinkman Index (< 100, < 1000, ≧1000) (ordinal), type of first immune checkpoint inhibitor (ICI), tumor histotype, TNM classification (categorical), number of lines (first, second, third, and later) (categorical), and chemotherapy with ICI.

Model 2: adjusted for the covariates in model 1 and the PD-L1 positivity ratio (< 1%, < 50%, -100%, unassessed).

Model 3: adjusted for the covariates in model 2 and *EGFR* mutation ((+), (-), unassessed).

Table 5
Stratified hazard ratio of cancer progression for ALDH2*2 carriers estimated from a 6-month observation.

	HR	95% CI	p
Sex			
Male (N = 89)	8.72	3.36–22.6	< 0.0001
Female (N = 16)	Non-estimable		
Age			
< 70 (N = 55)	2.81	0.85–9.27	0.0892
≥ 70 (N = 50)	7.55	1.74–32.6	0.0068
Brinkman Index			
< 1000 (N = 53)	5.82	1.60–21.1	0.0074
≥ 1000 (N = 52)	6.07	1.56–23.5	0.0091
Type of immune checkpoint inhibitor			
Pembrolizumab (N = 45)	6.02	1.46–24.8	0.0131
Nivolumab and Atezolizumab (N = 60)	4.34	1.36–13.8	0.0131
Tumor histotype			
Adenocarcinoma (N = 64)	6.77	2.43–18.9	0.0003
Others (N = 41)	3.07	0.47–19.9	0.2394
TNM classification			
Stage III (N = 25)	Non-estimable		
Stage IV (N = 51)	21.3	3.62–126	0.0007
Treatment line			
First-line (N = 41)	3.70	0.86–15.9	0.0783
Second-line and later (N = 64)	8.39	2.94–24.0	< 0.0001
Chemotherapy with first ICI			
Yes (N = 6)	Non-estimable		
No (N = 99)	4.91	2.41–9.99	< 0.0001
PD-L1 (+) ratio in cancer tissue			
< 50% (N = 42)	10.9	2.38–49.9	0.0021

	HR	95% CI	p
≥ 50% (N = 48)	4.01	1.31–12.3	0.0152
EGFR mutation in cancer tissue			
(+) (N = 8)	Non-estimable		
(−) (N = 81)	14.1	4.30–46.5	< 0.0001

Reference = *ALDH2*1/*1* carriers. HR; hazard ratio by Cox proportional hazard model adjusted for covariates used in Model 3 in Table 2, CI; confidence interval, PD-L1; programmed death-ligand 1, EGFR; epidermal growth factor receptor. Cases with unknown TNM classification (N = 29), unknown PD-L1 (+) ratio in cancer tissue (N = 15), and unknown *EGFR* mutation in cancer tissue (N = 16) were excluded.

3.4 Association between PFS and the other variables by rs671 groups

The PFS curve is shown in Fig. 2, and the multivariate-adjusted HR is shown in Table 6. The multivariate-adjusted HR of PFS showed significant differences by sex, type of ICI, treatment line, PD-L1 antibody positivity rate, and *EGFR* mutation for either type of rs671; an association with rs671 was suggested for treatment line, PD-L1 antibody positivity rate, and *EGFR* mutation based on the interaction analysis. The treatment line was associated with PFS only in the rs671(+) group, and the first-line group showed the best treatment outcome. The PD-L1 positivity rate was also associated with PFS only in the rs671(+) group; however, there was no dose-response relationship (a middle level was associated with the highest HR). Only the rs671(−) group showed short PFS among the groups with *EGFR* mutations.

Table 6
Hazard ratio of cancer progression by *ALDH2* genotype estimated from the entire observation period.

	Rs671(−) (N = 56)				Rs671(+) (N = 49)				p for interaction
Explanatory variables	**HR**	**95% CI**		**p**	**HR**	**95% CI**		**p**	
Sex (reference = male)									
Female	5.07	1.22	21.1	0.0255	0.87	0.16	4.59	0.8662	0.0785
Age									
Per one year	0.99	0.93	1.06	0.7345	1.04	0.98	1.11	0.2365	
Brinkman Index (< 100, < 1000, ≥1000) (ordinal)									
Per category	1.64	0.70	3.81	0.2539	1.45	0.67	3.17	0.346	
Type of ICI (reference = pembrolizumab)									
Atezolizumab	0.93	0.10	8.93	0.9476	0.84	0.15	4.71	0.8403	0.8472
Nivolumab	1.02	0.15	6.84	0.9877	0.07	0.01	0.50	0.0078	0.0521
Tumor histotype (reference = Squamous cell carcinoma)									
Adenocarcinoma	0.78	0.10	6.33	0.8167	1.93	0.56	6.60	0.2955	
Pleomorphic carcinoma	0.00	0.00	NE	0.9932	0.92	0.10	8.86	0.9417	
Mesothelioma	2.35	0.11	48.8	0.5817	—				
Other	1.05	0.07	17.0	0.9742	1.45	0.06	37.3	0.824	
TNM classification (reference = stage III)									
Stage IV	1.57	0.22	11.4	0.6546	0.50	0.13	1.98	0.3233	
Unknown	1.68	0.30	9.30	0.5527	1.33	0.35	5.04	0.6753	
Treatment line (reference = third-line and later)									
First-line	4.03	0.45	36.4	0.2147	0.07	0.01	0.53	0.0098	0.0049

	Rs671(−) (N = 56)				Rs671(+) (N = 49)				p for interaction
Explanatory variables	**HR**	**95% CI**		**p**	**HR**	**95% CI**		**p**	
Second-line	2.69	0.32	22.9	0.3640	0.56	0.13	2.43	0.4336	0.1810
Chemotherapy with first ICI (reference = no)									
Yes	3.94	0.30	51.8	0.2964	> 999	0.00	NE	0.9877	
PD-L1 (+) ratio in cancer tissue (reference = < 1%)									
≥50%	0.46	0.06	3.83	0.4753	27.0	4.64	158	0.0002	0.0116
1-100%	0.49	0.06	3.71	0.4879	1.99	0.27	14.4	0.4969	0.2838
Unassessed	1.21	0.15	9.44	0.8589	176	12.9	> 999	0.0001	0.0079
EGFR mutation in cancer tissue (reference = (-))									
(+)	28.4	2.36	342	0.0084	0.32	0.03	2.95	0.3127	0.0041
Unassessed	1.65	0.20	13.4	0.6401	0.23	0.03	2.20	0.2033	0.0602

Rs671(−); *ALDH2*1/*1*, rs671(+); *ALDH2*1/*2* or *ALDH2*2/*2*, HR; hazard ratio by the Cox proportional hazard model includes all explanatory covariates shown above, CI; confidence interval, ICI; immune checkpoint inhibitor, NE; non-estimable, PD-L1; programmed death-ligand 1, *EGFR*; epidermal growth factor receptor. The p-value for interactions was estimated by the Cox proportional hazard model including all explanatory covariates shown above, rs671, and interaction in terms of sex*rs671, type of ICI*rs671, treatment line*rs671, PD-L1 ratio*rs671, and *EGFR* mutation*rs671.

4 Discussion

As expected, the *ALDH2* polymorphism rs671 influenced the effects of ICI therapy on chest malignancies. *ALDH2*2* had a negative effect on short-term prognosis, although it was unlikely to affect long-term prognosis. According to multivariable and stratified analyses, the negative effect was independent of sex, smoking habit, PD-L1 expression rate, and *EGFR* mutation. Compared with *EGFR* mutation, which has been shown to be associated with poor prognosis independent of ICI or initial ICI efficacy[30, 34], rs671 may be more strongly associated with initial ICI efficacy. However, *ALDH2*2* showed no negative effect on long-term survivors, especially the first treatment line group; thus, we found that *ALDH2*2* is not consistently associated with negative effects.

Several findings that support the negative impact of *ALDH2*2* on the short-term prognosis of ICI therapy have been reported. Gao et al. (2018) showed that drug-induced T-cell hepatitis is suppressed by exogenous acetaldehyde. Mechanistically, aldehyde suppresses the secretion of cytokines by inhibiting the phosphatidylinositol 3-kinase (PI3K)-Akt pathway in T cells or promotes the secretion of glucocorticoids that suppresses the activation of T cells[12]. This suppression of the PI3K-Akt pathway has also been confirmed in the cardiomyocytes of *Aldh2−/−* mice, in which endogenous aldehydes accumulate in the absence of exogenous aldehyde[35]. These findings suggest that endogenous aldehyde also suppresses the PI3K-Akt pathway in T cells. The PI3K-Akt pathway is important for T cell differentiation[36] and has been shown to decrease the number of T cells in the thymus gland when activity is impaired[37, 38]. In fact, we found that the number of T cells in the peripheral blood of *Aldh2−/−* mice and *ALDH2*2* carriers is low[25]. On the basis of these findings, we hypothesize that *ALDH2*2* negatively affects the initial ICI efficacy via suppression of the PI3K-Akt pathway in T cells due to endogenous aldehyde accumulation.

However, as explained above, endogenous aldehyde can also be advantageous. In the present study, the PFS ratio in the rs671(+) group decreased rapidly but was eventually higher than that of the rs671(−) group (0.21 vs. 0.27). For the first-line group, the PFS ratio after 2 years was 0.37 in the rs671(−) group and 0.40 in the rs671(+) group and was substantially higher compared with that in the other groups (the PFS ratio after 2 years was 0.18–0.23 in the rs671(−) and rs671(+) groups after the second-line treatment). This finding suggests that preventing a decrease in the number of T cells caused by pre-ICI treatment may increase the chances of obtaining a good ICI effect, especially in rs671(+) patients because *ALDH2*2* carriers have lower T cell counts[25].

The response rate of patients to ICIs is currently insufficient. For example, only 10%–20% of non-small-cell lung cancer (NSCLC)[39, 40] patients respond to this therapy. Therefore, optimal case selection is important. The effects of ICI are affected by the immunity of the host, the intestinal bacterial environment of the host, and tumor tissue factors[41, 42]. The PD-L1 expression level and *EGFR* mutation rate, which are tumor tissue factors, are currently used as predictors in clinical settings. In the present study, there was no association between the PFS and PD-L1 ratio. However, *EGFR* mutation was shown to be a negative predictor for rs671(−), as previously reported. It also has been shown that tissue infiltrating lymphocytes and tumor mutation burden can be predictors of treatment effects, although they have not been applied clinically[42-44]. The most significant limitation of these factors is that highly invasive biopsies are required. Because the microenvironment and gene mutations of tumor cells are known to fluctuate dynamically, collecting tumor tissues immediately before treatment is ideal. However, this may often be difficult due to the condition of patients and the site of lesions. Meanwhile, Hatae et al. (2020) recently showed that blood metabolites reflecting the state of intestinal bacteria and tumor-specific T cell rates are good predictors of ICI effects on NSCLC, although there are still difficulties owing to the number of tested parameters after the start of treatment[45]. Ohue et al. (2019) demonstrated that the effects of ICIs on NSCLC could be predicted by tumor antigens in blood samples collected prior to ICI initiation [HR (95% CI) of PFS in antigen-positive patients is 0.4 (0.2 to 0.9)] [46], and its clinical application is expected.

Compared with these predictors, the analysis of *ALDH2* polymorphisms has some advantages: non-invasive, inexpensive, 100% determinable, and polymorphisms do not change throughout life.

The limitations of the present study are as follows: 1) The sample size was insufficient to establish prognostic factors specific to rs671(+) patients. 2) Because the present study was limited to Japanese patients with chest malignancies who were mostly men, it cannot be generalized to other types of cancers and populations. 3) Because several time-dependent covariates can affect the outcome, such as adverse reactions and types and doses of second and subsequent ICIs, controlling covariates is insufficient for long-term observation. 4) The biological mechanism is not well supported.

Conclusion

The variant allele of the *ALDH2* polymorphism rs671 was found to be a negative predictor in the early stage of ICI treatment. However, the long-term survivor rate was the highest in the sub-group of patients with the variant allele who received an ICI as first-line treatment. The rs671 polymorphism test is expected to be a cost-effective predictor of ICI efficacy for clinical application. We need to present better personalized strategies by accumulating evidence with a larger sample size and examining the mechanism underlying the findings.

List Of Abbreviations

Aldehyde dehydrogenase 2 (ALDH2)

Immune checkpoint inhibitors (ICIs)

Programmed cell death-1 (PD-1)

Programmed death-ligand 1 (PD-L1)

Progression-free survival (PFS)

4 hydroxynonenal (4HNE)

Complete Response (CR)

Partial Response (PR)

Stable Disease (SD)

Progressive Disease (PD)

Restricted mean survival time (RMST)

Hazard ratio (HR)

Immune-related adverse events (irAEs)

Phosphatidylinositol 3-kinase (PI3K)

Non-small-cell lung cancer (NSCLC)

Declarations

Ethics approval and consent to participate: Informed written consent was obtained from all participants for the study, including genetic analyses and publication. The study was approved by the clinical study ethics review committee of Saga University (project ID R1-16) and conducted accordingly. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated and/or analyzed during the current study are not publicly available due to a potential infringement of privacy but are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: CN and NA built the database. AM and CN were the major contributors to the analysis and interpretation of the database. AM wrote the manuscript while organizing discussion from CN, SK, ES and NA. CN, SK, ES and NA read and approved the final manuscript.

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Figures

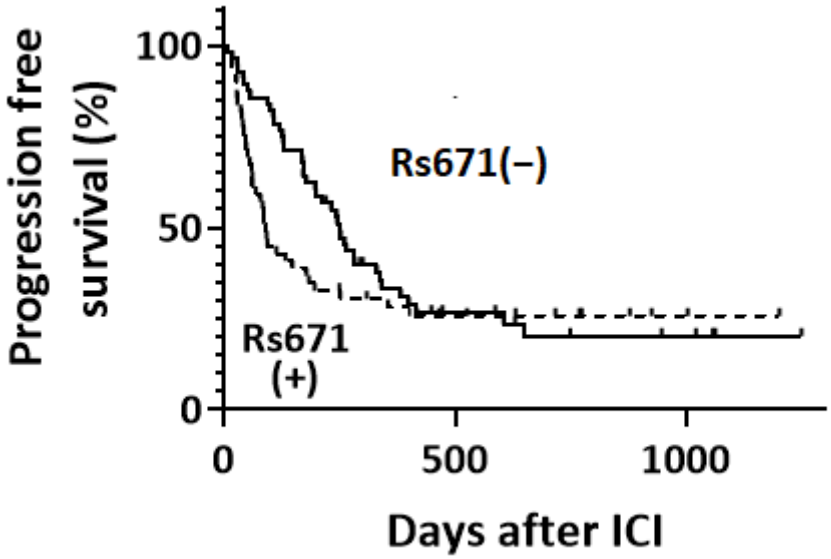

Figure 1

Progression-free survival after the initiation of immune checkpoint inhibitor therapy. Kaplan–Meier plots were shown for patients with a chest malignancy. ICI, immune checkpoint inhibitor; Rs671(−), ALDH2*1/*1 (n = 56), rs671(+); ALDH2*1/*2 or ALDH2*2/*2 (n = 49). Gahan–Breslow–Wilcoxon test indicates a significant difference (p = 0.0084).

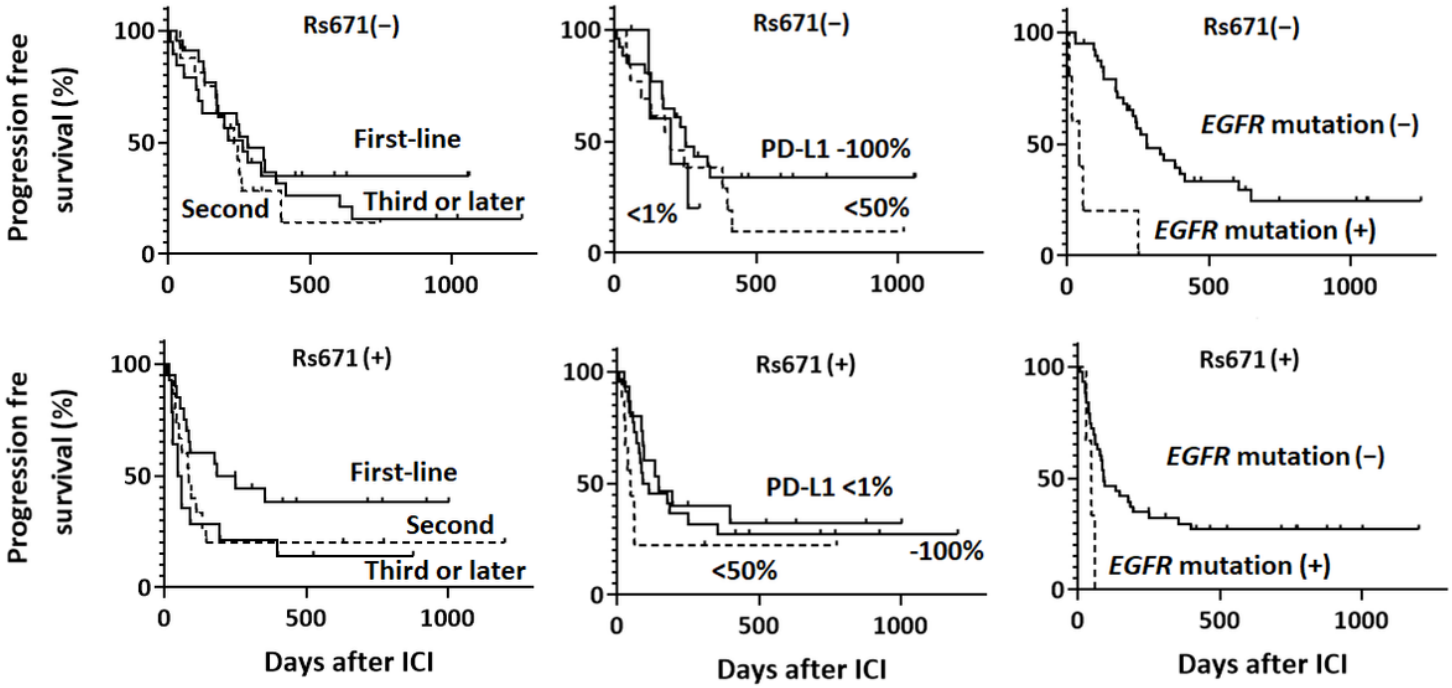

Figure 2

Progression-free survival after the initiation of immune checkpoint inhibitor therapy by ALDH2 rs671 polymorphism. Kaplan–Meier plots were shown for patients with chest malignancies. ICI; immune checkpoint inhibitor, Rs671(−); ALDH2*1/*1, rs671(+); ALDH2*1/*2 or ALDH2*2/*2.

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