Assessment of VAV2 Expression Refines Prognostic Prediction in Adrenocortical Carcinoma

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. 1 **CLINICAL RESEARCH ARTICLES**

. 2 Assessment of VAV2 expression refines prognostic prediction

. 3 in adrenocortical carcinoma

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32 **Short title**: VAV2 in adrenocortical carcinoma 33

. 34 **Précis**: We studied VAV2 expression in a large multicentric cohort of adrenocortical

. 35 carcinoma cases and validated its role as a prognostic marker.

. 36 **Keywords:** adrenocortical carcinoma, VAV2, Ki67, prognostic markers

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. 54 **Abstract**

. 55 **Background**: Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with overall

. 56 poor prognosis. The Ki67 labeling index (LI) has a major prognostic role in localized ACC

. 57 after complete resection but its estimates may suffer from considerable intra- and

. 58 interobserver variability. VAV2 overexpression induced by increased SF-1 dosage is an

. 59 essential factor driving ACC tumor cell invasion.

. 60 **Objective:** To assess the prognostic role of VAV2 expression in ACC by investigation of a

. 61 large cohort of patients.

. 62 **Design, Setting and Participants:** 171 ACC cases (157 primary tumors, 6 local

. 63 recurrences, 8 metastases) from seven ENS@T centers were studied.

. 64 **Outcome Measurements:** H-scores were generated quantifying VAV2 expression. VAV2

. 65 expression was divided into two categories, low (H-score <2) and high (H-score ≥2). Ki67

. 66 LI retrieved from patients' pathological records was also categorized into low (<20%) and

. 67 high (≥20%). Clinical and immunohistochemical markers were correlated with progression-

. 68 free (PFS) and overall survival (OS).

. 69 **Results:** VAV2 expression and Ki67 LI were significantly correlated with each other and

. 70 with PFS and OS. Heterogeneity of VAV2 expression inside the same tumor was very low.

. 71 Combined assessment of VAV2 expression and Ki67 LI allowed to improve patient

. 72 stratification to low-risk and high-risk groups.

. 73 **Conclusion:** Combined assessment of Ki67 LI and VAV2 expression improves prognostic

. 74 prediction in ACC.

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. 79 Introduction

. 80 Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with overall poor

. 81 prognosis, limited treatment options when progressed into metastatic stage and

. 82 unsatisfactory response to polychemotherapeutic cytotoxic regimens (1, 2). Hence the

. 83 most efficient method to eradicate the disease consists in complete surgical resection of

. 84 the primary tumor. However, risk of recurrence is high even in this condition. Molecular

studies have identified two subclasses of ACCs with aggressive (C1A) or indolent (C1B)

clinical behavior, respectively (3-6). However, since molecular markers identified by those

studies have not yet found entrance into clinical practice, it would be of particular

importance to stratify patients with ACC into low-or high-risk groups to adequately monitor

disease recurrence and assign them to appropriate therapeutic interventions. The

histological Weiss score, which is commonly used as an established morphometric

criterion for the differential diagnosis in adrenocortical tumors, has limited value as a

prognostic indicator, especially in cases with borderline features (7, 8). Conversely, it was

shown that a number of immunohistochemical markers have a prognostic value in ACC (9-

18). Among those, the most widely used in clinical pathology reports is the Ki67 labeling

index (LI), which is directly related to the proliferative activity of a given tissue (14-18). A

study recently completed by the European Network for the Study of Adrenal Tumors

(ENS@T) could indeed demonstrate that Ki67 LI has a major prognostic role in localized

ACC after complete resection (18). However, Ki67 LI estimates suffer from considerable

intra- and interobserver variability, as highlighted in a recent

study (19). New prognostic

markers are therefore needed to further refine prognostic classification of patients with

ACC as part of a multiparametric analysis.

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The transcription factor Steroidogenic Factor-1 has a pivotal role in regulating

adrenocortical cell proliferation and differentiation (20). Its overexpression is associated to

adrenocortical tumorigenesis through regulation of a specific set of SF-1 dosage-

dependent target genes (21, 22). One of these genes encodes VAV2, a guanine

nucleotide exchange factor (GEF) for small GTPases of the Rho family (23). We have

recently shown that VAV2 overexpression induced by an increased SF-1 dosage in ACC

is an essential factor driving tumor cell invasion (24). Herein, we present the results of a

large study involving ACC cases provided by seven European institutions aimed to assess

the prognostic value of VAV2 expression in ACC and to compare and integrate it with the

Ki67 LI.

Materials and Methods

Immunostaining on formalin-fixed, paraffin embedded ACC samples

. 116 We analyzed a total of 171 adrenocortical tumor tissues from patients with ACC

. 117 provided by seven ENS@T centers (Italy 103, The Netherlands 42, France 20, Germany 6

. 118 samples). 145 samples were previously assembled in 7 tissue microarrays (TMA) with 2 or

. 119 3 cores per sample, interspersed with normal human liver, kidney and placenta tissues,

. 120 and 26 samples were available as full slides. Among the ACC samples, 157 samples

. 121 derived from primary tumors (male/female 59/98, average age±SD 48.7±15.2 years,

. 122 average tumor size±SD 11.2±5.4 cm; for patients' characteristics see Table S1), 6 from

. 123 local recurrences and 8 from distant metastases (liver and lung). The diagnosis of ACC

. 124 was made by established criteria based on clinical, biochemical and morphological data

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. 125 (25). All clinical data were collected through the ENS@T database (registry.ensat.org). All

. 126 patients gave informed consent and the study was approved by ethical committees from

. 127 all participating institutions. Immunohistochemical detection was performed in all samples

. 128 using an indirect immunoperoxidase technique after high temperature antigen retrieval in

. 129 0.01 M citrate buffer (pH 6.5) in a pressure-cooker for 13 minutes. The primary antibody

. 130 was a rabbit monoclonal antibody against the VAV2 protein (clone EP1067Y, ab52640

. 131 Abcam) diluted 1:250 in 25% AB serum in PBS and incubated 1 h at RT. Signal detection

. 132 was performed with the Advance HRP detection system (Dako) and DAB chromogen

. 133 according to the manufacturer's instructions. Nuclei were counterstained with Mayer's

. 134 hematoxylin for 3 minutes. As negative control, universal rabbit negative control (Dako)

. 135 was used. Immunostaining results were analyzed using a light microscope at high

. 136 magnification. VAV2 staining intensity was evaluated independently by two investigators

. 137 blinded to the clinical data (S.S. and I.S.). Cytoplasmic staining intensity was evaluated

. 138 with a grading score of 0, 1, 2 or 3, corresponding to negative, weak, moderate and strong

. 139 intensity, respectively. The proportion of positive tumor cells was calculated for each

. 140 specimen and set up to be scored 0, 0.1, 0.5 or 1, if 0%, 1-9%, 10-49% or >50% of the

. 141 tumor cells were positive for VAV2, respectively. A semi-quantitative H-score was then

. 142 calculated by multiplying the staining intensity grade by the proportion score (12, 24). In all

. 143 cases analyzed, the proportion of VAV2 positive cells was always >50%, so all intensity

. 144 values were multiplied by a factor equal to 1 to yield the H-

score. The cut-off point to

separate samples in high or low VAV2 expression was between H-scores <2 and ≥2. Ki67

LI data assessed by the local pathologists in each expert center were retrieved from the

ENS@T database. The Ki67 LI cut-off value used in this study to separate low LI and high

LI groups was 20%.

Statistical analysis

Correlation analyses were performed using a $|^2$ test for categorical variables. The

inter-observer agreement for the scoring system was evaluated using Cohen’s kappa-

coefficient and confirmed using Pearson’s correlation coefficient. As cutoff for strong

agreement 0.81 was chosen for the kappa-coefficient and 0.75 for Pearson’s coefficient

(26). The comparison of clinical and histopathological characteristics was performed on

GraphPad Prism 6.0 software using non-parametric Mann-Whitney test (for two groups)

and Kruskal-Wallis test with Dunn's correction for multiple testing (for more than two

groups), as appropriate. A p value <0.05 was considered to be statistically significant.

Survival analysis for ACC patients was calculated as

described (24) using the Kaplan-

Meier method and differences between groups were assessed with log-rank and Cox

proportional hazards statistics, using the SPSS software package (version 23.0.0 for Mac),

after adjustment for sex, age and tumor stage. Progression-free survival (PFS) was

defined as time elapsed from primary resection of ACC to the first recurrence, loco-

regional or systemic. Overall survival (OS) was defined as time elapsed from primary

resection of ACC to disease-related death or last follow-up visit. In the group of patients

with R0 resection, OS data were available for 100 (VAV2) and 105 (Ki67 LI) patients,

respectively. 92 of those patients had both VAV2 and Ki67 LI OS data available. Viable

cell data after *VAV2* knockdown were analyzed by 1-way ANOVA with Dunnett's

correction for multiple comparisons.

Results

VAV2 expression is a strong predictor of PFS and OS in ACC patients

Examples of different VAV2 expression patterns in ACC are shown in Fig. 1. A H-

score was assigned to each sample, which took in

consideration both staining intensity

. 175 and the percentage of cells stained by the anti-VAV2 antibody. The inter-observer

. 176 agreement was very good with Cohen's kappa coefficient equal to 0.85 (95%CI: 0.72-

. 177 0.89) and Pearson coefficient r=0.90 (95%CI:0.86-0.93), p<0.001. In contrast to Ki67

. 178 staining, which is usually heterogeneous throughout a tumor, VAV2 expression was fairly

. 179 equally distributed within a given tumor, with all samples presenting a percentage of

. 180 stained cells >50%. H-score heterogeneity among different TMA tissue cores belonging to

. 181 the same tumors was limited, with a residual standard deviation σ=0.14 and an intra-class

. 182 correlation coefficient α=0.95 (95%CI: 0.92-0.97) (Fig. S1). The same homogenous

. 183 distribution was also observed when whole tumor slides were analyzed (Fig. 1). VAV2

. 184 expression in the tumor was strongly correlated to both PFS (Fig. 2A) and OS (Fig. 2B),

. 185 confirming the results of our previous study performed on an independent smaller cohort

. 186 of ACC patients (24). Patients with strong VAV2 expression had a 2.8-fold higher risk to

. 187 experience a recurrence and 1.6-fold increased risk to die. No statistically significant

. 188 difference existed for VAV2 expression in primary tumors and metastatic sites from the

. 189 same patients (p=0.67). The Ki67 LI was also a strong predictor of PFS (Fig. 2C) and OS

. 190 (Fig. 2D), as reported in previous studies (14-18). Both VAV2 expression and Ki67 LI were

. 191 strongly correlated with OS even in patients with R0 resection (Fig. S2). VAV2 expression

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. 192 and Ki67 LI had a similar strong prognostic value for PFS and OS both in univariate and in

. 193 multivariate analysis, taking into account patients' age, sex and tumor stage (Table 1).

194

. 195 **Combined assessment of VAV2 expression and Ki67 LI improves prognostic power**

. 196 In general, a significant correlation existed between Ki67 LI and VAV2 expression

. 197 in our ACC cohort (Fig. S3). A strong correlation also existed when Ki67 LI and VAV2

. 198 expression were considered as categorical (low *vs*. high) variables ($|^2$ = 6.18, p=0.01).

. 199 However, in several cases these two parameters were dissociated with one value being

. 200 elevated and the other low in the same tumor. Remarkably, in those patients PFS and OS

. 201 were intermediate between the high-risk (high VAV2 expression-high Ki67 LI) and the low-

. 202 risk groups (low VAV2 expression-low Ki67 LI) (Fig. 3A, B). Merging the groups with high

203 VAV2-low Ki67 LI and low VAV2-high Ki67 LI and comparing them to the high VAV2-high

204 Ki67 LI and low VAV2-low Ki67 LI groups identified three classes of patients with very

205 different RFS (159.7±23.2, 90.3±15.7 and 20.8±5.8 months, respectively) and OS

206 (203.7±29.6, 130.3±29.6 and 41.6±5.1 months, respectively) (Fig. 3C, D). This type of

207 stratification maintained a strong prognostic value even in R0 patients (Fig. S4).

208 Remarkably, when considering the high-risk group apart from all other patients with ACC,

209 a very strong correlation existed with OS in the whole cohort (Fig. 4A) and with both PFS

210 and OS in R0 patients (Fig. 4B, C). Furthermore, isolated high VAV2 expression or high

211 Ki67 LI showed a prediction value for worse PFS and OS that was slightly lower compared

212 with the combination of both high VAV2 expression + high Ki67 LI [PFS: 22 months,

213 HR=0.67 (VAV2) and 28 months, HR=0.66 (Ki67 LI) *vs*. 9 months for the combination; OS:

214 66 months, HR=0.73 (VAV2) and 40 months, HR=0.82 (Ki67 LI) *vs*. 33 months for the

215 combination].

216

217 Discussion

. 218 The prognosis of ACC patients is variable and poorly predictable. A recent large

. 219 multicentric ENS@T study has shown that the KI67 LI is the most powerful parameter

. 220 predicting disease recurrence and survival in ACC patients after complete tumor resection

. 221 (18). The Ki67 LI has been integrated with the combined evaluation of morphological

. 222 parameters (number of mitoses/presence of necrosis) in the newly introduced Helsinki

. 223 score, which reportedly is able to more accurately predict recurrence in ACC (8, 27).

. 224 However, even if Ki67 LI assessment is routinely performed in diagnostic pathology

. 225 laboratories for a large number of neoplastic disorders, its standardization and

. 226 reproducibility have been questioned for many tumor types, including ACC (19). It is

. 227 therefore important to identify other molecular markers that can complement the Ki67 LI to

. 228 obtain a more accurate stratification of the risk of recurrence in patients with ACC. In this

. 229 perspective, molecular prognostic indicators derived from genomic studies are very

. 230 promising (3, 28, 29), but for routine implementation they suffer from the important

. 231 drawback that, at least at the present state of technology, frozen tumoral material is

. 232 required. On the other hand, prognostic value of circulating

markers of malignancy awaits

. 233 validation in large cohorts of ACC patients (30-33).

. 234 We have recently shown that VAV2 overexpression is an essential driver of cell

. 235 invasion in conditions of increased SF-1 dosage through its GEF activity for the small

. 236 GTPases Rac1 and Cdc42 (24). Those data directly link VAV2 with the potential

. 237 mechanism of malignancy consisting in increased cellular invasiveness. In the present

. 238 study we extended the previous study to a large European cohort of patients with ACC

. 239 and show that the tumor VAV2 H-score is significantly correlated to PFS and OS. The

. 240 combined assessment of VAV2 expression and Ki67 LI improves patient risk stratification,

. 241 with cases presenting high Ki67 LI but low VAV2 expression having significantly longer

. 242 PFS and OS compared to patients with concordant high-risk parameters. In our study,

. 243 VAV2 H-score assessment, which was mainly performed on TMA tissue cores, was

. 244 associated to an excellent intratumoral reproducibility and is then in principle less prone to

. 245 intra- and interobserver variability, although further work is needed to specifically address

. 246 this question on an even larger number of cases. These results show that

. 247 immunohistochemical assessment of VAV2 expression may usefully complement the

. 248 measurement of the Ki67 LI for prognostic stratification of patients with ACC.

249 250

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PFS		Univariate analysis			Multivariate analysis		
	Variables	*HR*	*95% CI*	*p*	*HR*	*95% CI*	*p*
	age (n=113; n=99)	1.00	0.98-1.02	0.77	0.99	0.98-1.01	0.91
	sex						
	female (n=78; n=69)						
	male (n=35; n=30)	1.19	0.69-2.04	0.52	1.17	0.64-2.13	0.59
	Tumor stage						
	I (n=12; n=8)						
	II (n=66; n=61)	7.19	0.98-52.52	0.05	5.32	0.71-39.39	0.10
	III (n=23; n=19)	6.29	0.81-48.65	0.07	5.03	0.64-39.65	0.12
	IV (n=11; n=11)	27.51	3.40-222.11	0.002	14.65	1.74-122.96	0.01
	VAV2 expression						
	VAV2 low (H-score 0-1) (n=52; n=52)						
	VAV2 high(H-score 2-3) (n=48; n=47)	2.80	1.57-4.98	<0.001	2.83	1.54-5.21	0.001
	Ki67 LI						
	Ki67 low (<20%) (n=63; n=62)						
	Ki67 high (≥20%) (n=42; n=42)	2.77	1.58-4.86	<0.001	2.43	1.37-4.31	0.002

OS		Univariate analysis			Multivariate analysis		
	Variables	*HR*	*95% CI*	*p*	*HR*	*95% CI*	*p*
	age (n=156; n=74)	1.01	0.99-1.03	0.14	1.01	0.98-1.03	0.35
	sex						
	female (n=98; n=79)						
	male (n=58; n=45)	1.27	0.76-2.12	0.35	1.39	0.80-2.41	0.24
	Tumor stage						
	I (n=12; n=8)	3.71	0.50-27.60	0.2			
	II (n=72; n=65)	3.71	0.50-27.60	0.2	3.77	0.49-28.84	0.20
	III (n=35; n=29)	4.52	0.59-34.49	0.14	3.85	0.49-29.87	0.19
	IV (n=24; n=22)	19.07	2.52-144.30	0.004	13.74	1.77-106	0.01
	VAV2 expression						
	VAV2 low (H-score 0-1) (n=66; n=60)						
	VAV2 high (H-score 2-3) (n=76; n=64)	1.64	1.01-2.66	0.042	2.03	1.07-3.83	0.02
	Ki67 LI						
	Ki67 low (<20%) (n=77; n=72)						
	Ki67 high (≥20%) (n=68; n=60)	2.94	1.67-5.19	<0.001	2.31	1.24-4.30	0.008

394 395

396

. 397 **Table 1. Analysis of parameters correlated with PFS and OS in univariate and**

. 398 **multivariate analysis.** Numbers of cases taken into account for univariate and

. 399 multivariate analysis, respectively, are indicated in parentheses for each variable.

400

401 402 403

. 404 **Abbreviations**: CI, confidence interval; HR, hazard ratio; PFS, progression free-survival;

. 405 OS, overall survival.

Figure legends

Figure 1. Examples of various intensities of VAV2 staining in ACC specimens. H-score value is indicated for each image, respectively. Scale bar, 400 ⌠m (images in left column); 50 ⌠m (images in right column).

Figure 2. Correlation of VAV2 expression (H-score) and Ki67 LI with PFS and OS in our ACC series. (A) PFS in low VAV2 expression (H-score <2) group (green line) 127±15.9 months; high VAV2 expression (H-score ≥2) group (red line) 25.7±4.1 months. p<0.001, Kaplan-Meier method. **(**B) OS in low VAV2 expression (H-score <2) group (green line) 180±22 months; high VAV2 expression (H-score ≥2) group (red line) 87.4±13 months. p=0.001, Kaplan-Meier method. **(**C) PFS in low Ki67 LI (<20%) group (green line) 137±17.9 months; high Ki67 LI (≥20%) group (red line) 68.5±14.3 months. p<0.001, Kaplan-Meier method. **(**D) OS in low Ki67 LI (<20%) group (green line) 187.5±22.9 months; high Ki67 LI (≥20%) group (red line) 96.2±17 months. p=0.001, Kaplan-Meier method. The numbers of cases analyzed for each group are reported in parentheses.

Figure 3. Correlation of combined VAV2 expression (H-score) and Ki67 LI with PFS and OS in our ACC series. (A) PFS in low VAV2 expression (H-score <2)-low Ki67 LI (<20%) group (green line) 159.7±23.2 months; high VAV2 expression (H-score ≥2)-low Ki67 LI (<20%) group (yellow line) 50.7±8.4 months;

low VAV2 expression (H-score <2)-high Ki67 LI (≥20%) group (pale green line) 96.6±26.3 months; high VAV2 expression (H-score ≥2)-high Ki67 LI (≥20%) group (red line) 20.8±5.8 months. Compared to low VAV2-low Ki67 LI: high VAV2-low Ki67 LI HR=2.55 (1.09-5.97), p=0.030; low VAV2-high Ki67 LI HR=2.46 (0.97-6.23), p=0.058; high VAV2-high Ki67 LI HR=6.75 (2.97-15.31), p<0.001; Kaplan-Meier method. **(**B) OS in low VAV2 expression (H-score <2)-low Ki67 LI (<20%) group (green line) 203.7±29.6 months; high VAV2

expression (H- score ≥2)-low Ki67 LI (<20%) group (yellow line) 120.4±20.5 months; low VAV2 expression (H-score <2)-high Ki67 LI (≥20%) group (pale green line) 126±26.7 months; high VAV2 expression (H-score ≥2)-high Ki67 LI (≥20%) group (red line) 41.6±5.1 months. Compared to low VAV2-low Ki67 LI: high VAV2-low Ki67 LI HR=2.66 (1.08-6.52), p=0.032; low VAV2-high Ki67 LI HR=3.51 (1.38-8.91), p=0.008; high VAV2-high Ki67 LI HR=5.38 (2.33-12.40), p<0.001; Kaplan-Meier method. **(**C) PFS in low VAV2 expression (H-score <2)-low Ki67 LI (<20%) group (green line) 159.7±23.2 months; high VAV2 expression (H-score ≥2)-high Ki67 LI (≥20%) group (red line) 20.8±5.8 months; all other patients with dissociated VAV2 expression-Ki67 LI group (grey line) 90.3±15.7 months. Compared to low VAV2-low Ki67 LI: other HR=2.51 (1.17-5.39), p=0.018; high VAV2-high Ki67 LI HR=6.75 (2.97-15.31), p<0.001; Kaplan-Meier method. **(**D) OS in low VAV2 expression (H-score <2)-low Ki67 LI (<20%) group (green line) 203.7±29.6 months; high VAV2 expression (H-score ≥2)-high Ki67 LI (≥20%) group (red line) 41.6±5.1 months; all other patients with dissociated VAV2 expression-Ki67 LI group (grey line) 130.3±18.1 months. Compared to low VAV2-low Ki67 LI: other

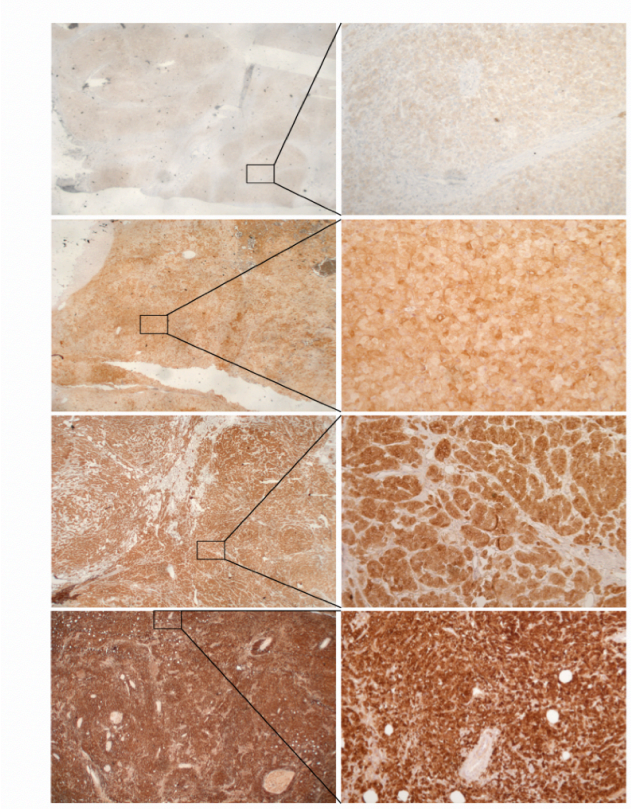
HR=2.99 (1.32-6.73), p=0.008; high VAV2-high Ki67 LI, HR=5.38 (2.33-12.40), p<0.001; Kaplan-Meier method. The numbers of cases analyzed for each group are reported in parentheses.

Figure 4. Prognosis of high-risk (high VAV2 expression-high Ki67 LI) *vs*. other ACC patients. (A) OS in the whole cohort of ACC patients for the high VAV2 expression (H-score ≥2)-high Ki67 LI (≥20%) group (red line) 41.5±5 months; all other patients (green line) 175.5±19.8 months. p<0.001, Kaplan- Meier method. (B) PFS in R0 patients for the high VAV2 expression (H-score ≥2)-high Ki67 LI (≥20%) group (red line) 20.8±5.8 months; all other patients (green line) 127.3±15.7 months. p<0.001, Kaplan-Meier method. (C) OS in R0 patients for the high VAV2 expression (H-score ≥2)-high Ki67 LI (≥20%) group (red line) 47.5±6 months; all other patients (green line) 194.8±21.7 months. p=0.005, Kaplan-Meier method. The numbers of cases analyzed for each group are

reported in parentheses.

21

Figure 1



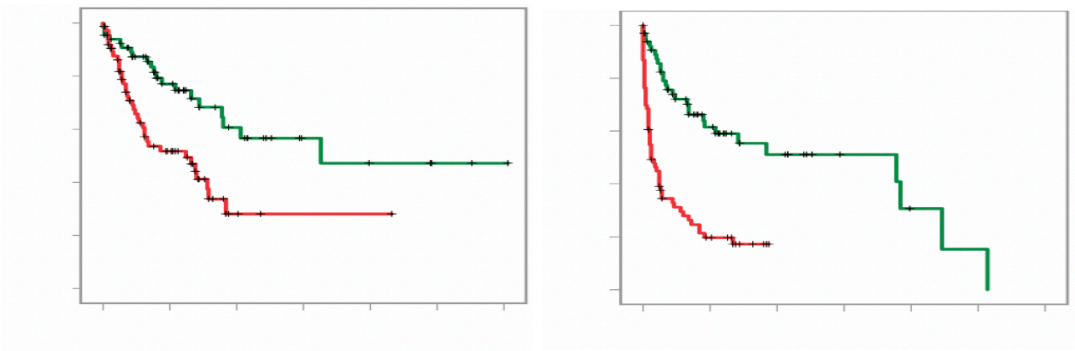
22

H-score=3 H-score=2 H-score=1 H-score=0

Figure 2

AB 100

100 80



80 60 40 20

C 100

80 60 40 20

low VAV2 (n=52) 60 p<0.001 40

low VAV2 (n=66)

p=0.001

high VAV2 (n=76)

50 100 150 200 250 300 Time (months)

low Ki67 LI (n=77)

p=0.001

high Ki67 LI (n=68)

50 100 150 200 250 300 Time (months)

20 00

0

high VAV2 (n=48)

50 100 150 200 250 300 Time (months) D

low Ki67 LI (n=63)

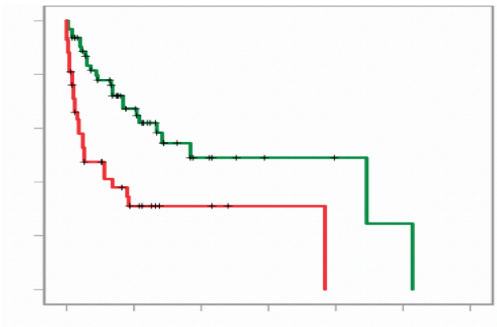
p<0.001

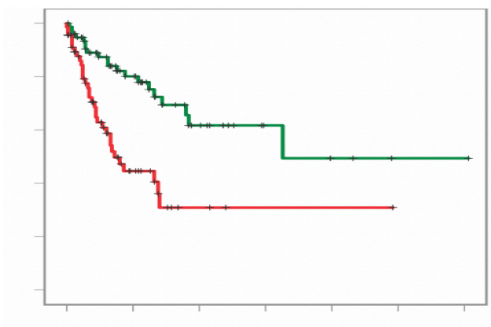
high Ki67 LI (n=42)

00

0 50 100 150 200 250 300 Time (months)

0





100

80

60

40

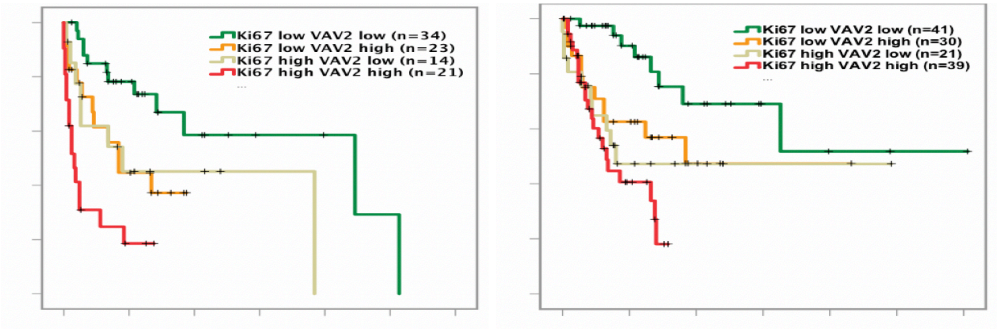
20

0

Progression-free survival (%) Progression-free survival (%)

Overall survival (%) Overall survival (%)

Figure 3 AB



C

100 80 60 40 20

100 80 60 40 20

100 80 60 p=0.058 40 20 00

p=0.032

p<0.001

p=0.008

0

p=0.030 p<0.001

VAV2 low-Ki67 LI low(=34) VAV2 high-Ki67 LI low (=23) VAV2 low-Ki67 LI high (=14) VAV2 high-Ki67 LI high (=21)

50 100 150 200 250 300 Time (months) D

VAV2 low-Ki67 LI low (n=34)

other (n=37)

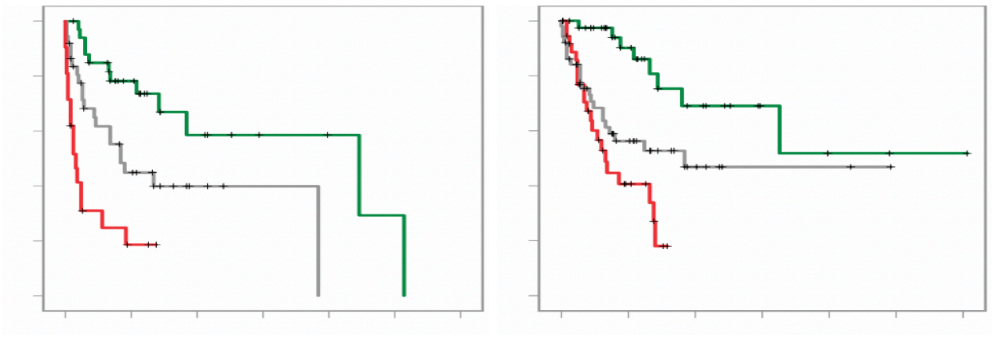
p=0.018

p<0.001

VAV2 high-Ki67 LI high (n=21)

0

50 100 150 200 250 300 Time (months)



00

0 50 100 150 200 250 300 Time (months)

0 50 100 150 200 250 300 Time (months)

24

100

80

60

40

20

VAV2 low-Ki67 LI low (n=41)

p<0.001

VAV2 high-Ki67 LI high (n=39)

other (n=51)

p=0.008

VAV2 low-Ki67 LI low (=41) VAV2 high-Ki67 LI low (=30) VAV2 low-Ki67 LI high (=21) VAV2 high-Ki67 LI high (=39)

Progression-free survival (%) Progression-free survival (%)

Overall survival (%) Overall survival (%)

Figure 4 A

100

80

60

40

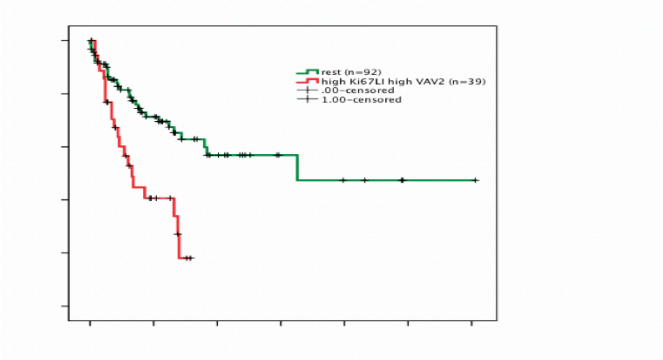
20

0

100 80 60 40 20 0

other (n=92)

p<0.001

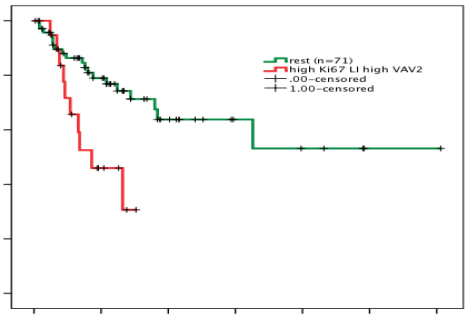


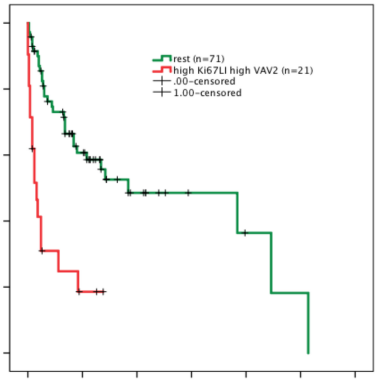
B

0 50 100 150 200 250 300 Time (months)

C

VAV2 high-Ki67 LI high (n=39)





other (n=71)

VAV2 high-Ki67 LI high (n=21)

p<0.001

100 80 60 40 20 0

other (n=71)

p=0.005

0 50 100 150 200 250 300 Time (months)

0 50 100 150 200 250 300 Time (months)

25

VAV2 high-Ki67 LI high (n=21)

Progression-free survival (%) Overall survival (%)

Overall survival (%)