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ORIGINAL RESEARCH

hERG1 positivity and Glut-1 negativity identifies high-risk TNM stage I and II colorectal cancer patients, regardless of adjuvant chemotherapy

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Background: The identification of early-stage colorectal cancer (CRC) with high risk of progression is one major clinical challenge, mainly due to lack of validated biomarkers. The aims of the present study were to analyze the prognostic impact of three molecular markers belonging to the ion channels and transporters family: the *ether-à-go-go-related gene 1* (hERG1) and the *calcium-activated* KCa3.1 potassium channels, as well as the *glucose transporter 1* (Glut-1); and to define the impact of adjuvant chemotherapy in conjunction with the abovementioned biomarkers, in a cohort of radically resected stage I–III CRC patients.

Patients and methods: The expressions of hERG1, KCa3.1, and Glut-1 were tested by immunohistochemistry on 162 surgical samples of nonmetastatic, stage I–III CRC patients. The median follow-up was 32 months. The association between biological markers, clinicopathological features, and survival outcomes was investigated by evaluating both disease-free survival and overall survival.

Results: Although no prognostic valence emerged for KCa3.1, evidence of a negative impact of hERG1 expression on survival outcomes was provided. On the contrary, Glut-1 expression had a positive impact. According to the results of the multivariate analysis, patients were stratified in four risk groups, based on TNM stage and hERG1/Glut-1 expression. After adjusting for adjuvant therapy, stage I and II, Glut-1-negative, and hERG1-positive patients showed the worst survival experience.

Conclusion: This study strongly indicates that the combination of hERG1 positivity and Glut-1 negativity behaves as a prognostic biomarker in radically resected CRC patients. This combination identifies a group of stage I and II CRC patients with a bad prognosis, even worse than that of stage III patients, regardless of adjuvant therapy accomplishment.

Keywords: potassium channels, glucose transporter, biomolecular markers, ion channels, prognostic markers

Introduction

Colorectal cancer (CRC) is the world's third most common cancer in men and the second most common in women.[1] Primary treatment for patients without distant metastasis is surgery. Patients with early-stage CRC could expect a long survival with surgery alone, nevertheless ~50% of stage III and 25% of stage II will relapse.[2] Adjuvant chemotherapy is the standard of care for patients with stage III, while the real benefit in stage II is not still clear and the routine use of chemotherapy is not recommended.[3] As no validated biomarker is available for routinely assessing patients' risk stratification, the decision on whether to accomplish chemotherapy or not for stage II CRC patients currently relies on clinical features as T4, number of lymph nodes analyzed, perforation or obstruction, and grading.[4]

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Therefore, the identification and validation of novel biomolecular markers, that could support classical clinicopathological parameters in prognostic definition, is one of the upmost challenges in the management of CRC. Such validated biomarkers would in turn help clinicians to identify patients with highest relapse risk and more susceptible to take advantage from adjuvant therapy. Until now, microsatellite instability (MSI) is an important marker to select patients with stage II CRC for adjuvant chemotherapy. Improved survival from adjuvant therapy has been recently demonstrated for patients with proficient DNA mismatch repair (pMMR) tumors, whereas patients with high-level microsatellite (MSI-H) or defective mismatch repair (dMMR) tumors did not show any benefit from fluorouracil (FU)-based therapy.[5–8] For patients with low-grade MSI (85% of all stage II patients), a promising way to identify groups that could take advantages from adjuvant therapy is ColoPrint (Agendia), an 18-gene expression classifier that identifies early-stage colon cancer patients at higher risk of disease relapse.[9,10] Other similar multigene assays, for example, Oncotype DX colon cancer assay[11] and CoIDx, have been evaluated to support clinicians' decisions, providing prognostic and predictive information. Unfortunately, the information obtained from these tests have only a prognostic value, and there is no evidence of predictive value about the potential benefit of adjuvant therapy. A new frontier in this field is represented by some in-silico studies that, making use of large amounts of data originating from available independent data sets, can help in identifying novel potential biomarkers. The predictive role of the transcription factor CDX2 in stage II CRC was identified with a similar approach. Lack of expression of this marker defines a group of patients with high relapse risk, which seems to take advantage from adjuvant therapy, in terms of survival.[12] More recently, a microRNA-based model was identified, which was capable to enhance in-silico prediction of therapeutic response of individual CRC cases.[13] Finally, an immune-derived *PD-L1* gene expression profile helped to identify a subgroup of stage II and III CRC patients with a favorable prognosis that should not receive chemotherapy.[14]

In this study, the prognostic impact of three potential biomarkers belonging to the "ion channels and transporters" family was evaluated: two potassium channels (the *ether-à-go-go-related gene 1* [Kv11.1 or hERG1] channel and the "intermediate conductance" calcium-activated KCa3.1 channel, encoded by the *KCNN4* gene) and the *glucose transporter 1* (Glut-1). hERG1 is a voltage-activated potassium channel belonging to the *ether à-go-go* (EAG) family, frequently overexpressed in several types of human cancers[15–18] including CRC.[19–22] KCa3.1 has been shown to be expressed at high levels in many human cancers.[23–26] Moreover, the impact of KCa3.1 on cancer cell proliferation, migration, and invasiveness is well described,[25–28] and the use of Kca3.1 blockers has recently shown promising antitumor effects.[29] Recently, the expression of KCa3.1 has also been described in CRC,[30] but its impact as prognostic or predictive value in CRC patients is still unknown. Glut-1 is a carrier protein being part of the hypoxia pathway, which comprises different biomolecular markers (VEGF-A, CA-IX, and EGFR) switched on when the oxygen levels in tumor tissues decrease.[31–33] Moreover, its impact in stage I–III CRC patients has been recently investigated and correlated to hERG1 expression.[34]

The aim of the present study was to analyze the prognostic valence of the above three biomarkers in a cohort of nonmetastatic, TNM stage I–III CRC patients, considering the effects of adjuvant therapy on survival. All the markers were tested by immunohistochemistry (IHC) on surgical samples, and the most relevant clinicopathological features were also included in the study.

Patients and methods

Patients and sample collection

For this study, we enrolled a cohort of 162 patients with pathologically confirmed stages I, II, or III colorectal adenocarcinoma and treated with radical surgery at the Department of General Surgery and Surgical Oncology, Azienda Ospedaliero-Universitaria, Careggi, Florence. Patients' cohort was selected without any bias among a group of patients treated surgically at the department from September 2001 to February 2015, excluding patients with chronic hepatitis C viral infection and those with rectal cancer who had received neoadjuvant radiotherapy or chemotherapy before surgery. After obtaining an informed written consent from each patient, samples of tumor were collected during surgery and treated for IHC analysis, as described later. The study was carried out with approval of the ethics committee of Azienda Ospedaliero-Universitaria Careggi. The classification of adenocarcinomas was conducted under optical microscope, and tumors were staged according to the American Joint Committee on Cancer classification. All patients with disease relapse were treated according to the local guidelines. Moreover, a subgroup of 92 patients (56.8%) with stage II and III CRC received adjuvant chemotherapy after surgery.

IHC

For patients enrolled in the study, formalin-fixed, paraffin-embedded, 7-μm sections were stained by using a commercially

available kit (PicTure Plus kit and DAB; Zymed, Carlsbad, CA, USA), as described previously.[34] Briefly, the antigen retrieval for hERG1 and Glut-1 staining was performed by treatment with proteinase K (5 μg/mL), whereas for KCa3.1 staining the samples are heated in a microwave oven at 600 W in citrate buffer pH 6.0 for 15 minutes. Stainings were performed by using antibodies to hERG1 (1:200; monoclonal antibody produced in our laboratory and distributed by Dival Toscana Srl), KCa3.1 (1:2,000, polyclonal rabbit anti-human KCNN4; Sigma-Aldrich, St Louis, MO, USA), and Glut-1 (1:100, polyclonal rabbit anti-human GLUT1; DakoCytomation, Glostrup, Denmark). Tissue slides were analyzed at a total magnification of 40× field by field, from top left to bottom right, and classified with a scoring system different for each marker. For hERG1, the scoring method described by Lastraioli et al[34] was followed: specimens were classified as "score 0" where no staining was present, specimens with a percentage of neoplastic-stained cells ranging from 1% to 49% were classified as "score 1," and specimens with a percentage of stained cells >50% were classified as "score 2." For the purpose of the analysis, only samples classified with "score 2" (with a high hERG1 expression) were considered "hERG1-positive." For KCa3.1 and Glut-1, only specimens where at least 1% of marked cells were present, without applying any scoring system, were considered positive.[30,34,35] Each specimen was analyzed by two independent investigators, and the interobserver agreement was evaluated according to the simple Cohen κ of concordance and its 95% confidence interval (CI). Images were acquired on a Leica DM 4000B microscope with a Leica DFC 320 Camera using Leica QWin software (Leica Microsystems, Milan, Italy).

Statistical methods

For each patient, the following clinicopathological variables were investigated: age at the intervention, sex, site of tumor, TNM classification, tumor histological grading, mucin content, and adjuvant therapy. Moreover, for each tumor tissue, the expressions of hERG1, KCa3.1, and Glut-1 were also assessed, and each marker was categorized as yes/no with respect to their expression. The association between clinicopathological features and biological markers was evaluated by χ^2 and Fisher's exact tests when appropriate. A two-sided $P \leq 0.05$ was considered significant. The impact of each parameter on survival was analyzed by evaluating two variables: disease-free survival (DFS), defined as the time from intervention to death or recurrence of disease, whatever the cause, and overall survival (OS). The Kaplan–Meier inverse method was applied to establish the median follow-up time.[36] The statistical analysis was performed as described previously.[34] Briefly, DFS and OS were calculated according to the Kaplan–Meier product-limit method,[37] first at the univariate analysis, and the Cox proportional hazard model was used to calculate the hazard ratios (HRs) and appropriate 95% CIs. Subsequently, the independent effect of each parameter on both the survival variables was investigated by a multivariate Cox regression model. As in Lastraioli et al,[34] starting from a model including all the clinicopathological variables and the biological markers, nonsignificant variables were progressively removed, according to a backward stepwise procedure based on the likelihood ratio test. Finally, for each risk group of patients identified, a Cox proportional hazards model (with the average covariate method) was applied to obtain the nonparametric evaluation of the survivor functions and accompanying HRs, adjusted for adjuvant treatment. Data were analyzed using the statistical software SAS 9.2 (SAS Corporation, Cary, NC, USA).

Results

Characteristics of the patients' cohort

Primary tumor samples were collected from 162 patients diagnosed as stage I–III CRC. The clinicopathological features of the patients' cohort are summarized in Table 1. Among 162 patients, 86 (53.1%) were female and 76 (46.9%) were male. Patients' age ranged from 40 to 90 years, with a median age of 69 years. Ninety-two patients (56.8%) received adjuvant chemotherapy after surgery. Tumors were mostly located in the right colon (73), whereas 39 were located in the left, 14 in the transverse colon, and 36 in the rectum.

Analysis of hERG1, KCa3.1, and Glut-1 expressions

In all the samples, the expressions of hERG1 and KCa3.1 potassium channels, as well as that of the glucose transporter Glut-1, by IHC, were investigated using different scoring systems for the three markers (see "Materials and methods"). Figure 1 shows IHC representative pictures relative to samples with different scorings of hERG1 (Figure 1A–C), KCa3.1 (Figure 1D and E), and Glut-1 (Figure 1F and G).

hERG1 turned out to be expressed (ie, score 2) in 40 out of 162 CRC primary tissues (24.7%), KCa3.1 in 56.8% (92/162), and Glut-1 in 34.6% (56/162) of samples (Table 1). The expressions of hERG1 and Glut-1 were significantly associated (P=0.001), whereas no significant association between KCa3.1 and the other two biomarkers emerged.

Table 1 Univariate analysis of clinicopathological and biomolecular markers for DFS and OS

Parameter	Patients, n (%)	DFS			OS		
		3-year DFS	HR (95% CI)	*P*-value	3-year OS	HR (95% CI)	*P*-value
Age				0.98			0.44
<70 years	85 (52.5%)	61.7%	1 (ref)		65.2%	1 (ref)	
>70 years	77 (47.5%)	55.6%	0.99 (0.60–1.63)		59.6%	1.23 (0.72–2.14)	
Sex				0.88			0.59
Female	86 (53.1%)	57.2%	1 (ref)		62.1%	1 (ref)	
Male	76 (46.9%)	59.7%	1.04 (0.62–1.71)		62.5%	1.16 (0.67–2.00)	
Tumor site				0.94			0.43
Right colon	73 (45.1%)	57.8%	1 (ref)		55.7%	1 (ref)	
Transverse colon	14 (8.6%)	51.6%	0.83 (0.34–2.06)		51.6%	0.96 (0.39–2.37)	
Left colon	39 (24.1%)	74.1%	0.83 (0.44–1.58)		80.5%	0.56 (0.26–1.19)	
Rectum	36 (22.2%)	49.5%	0.89 (0.47–1.68)		60.5%	0.63 (0.36–1.45)	
TNM stage				**0.01**			**0.04**
Stage I	32 (19.7%)	70.6%	1 (ref)		73.0%	1 (ref)	
Stage II	57 (35.2%)	67.9%	0.98 (0.42–2.30)		77.0%	0.88 (0.35–2.21)	
Stage III	73 (45.1%)	46.8%	2.08 (0.97–4.47)		47.8%	1.84 (0.81–4.18)	
Mucin				0.69			0.75
No	120 (74.1%)	57.4%	1 (ref)		63.3%	1 (ref)	
Yes	42 (25.9%)	60.6%	0.89 (0.51–1.56)		59.7%	1.10 (0.61–2.00)	
Histological grading				0.47			0.79
G1	16 (9.9%)	57.7%	1 (ref)		57.1%	1 (ref)	
G2–G3	146 (90.1%)	58.6%	0.69 (0.25–1.91)		62.9%	0.87 (0.31–2.43)	
Adjuvant				**0.01**			**0.01**
No	70 (43.2%)	76.0%	1 (ref)		76.4%	1 (ref)	
Yes	92 (56.8%)	45.4%	2.78 (1.58–4.88)		51.6%	2.34 (1.28–4.28)	
hERG1				0.26			0.17
Negative	122 (75.3%)	60.4%	1 (ref)		64.5%	1 (ref)	
Positive	40 (24.7%)	51.2%	1.38 (0.78–2.46)		54.0%	1.54 (0.83–2.85)	
KCa3.1				0.55			0.86
Negative	70 (43.2%)	57.8%	1 (ref)		58.0%	1 (ref)	
Positive	92 (56.8%)	59.3%	0.86 (0.51–1.42)		64.9%	1.05 (0.60–1.84)	
Glut-1				**0.02**			**0.01**
Negative	106 (65.4%)	49.5%	1 (ref)		54.9%	1 (ref)	
Positive	56 (34.6%)	78.0%	0.51 (0.28–0.91)		78.2%	0.410 (0.21–0.80)	

Note: Statistically significant parameters are highlighted in bold.
Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival.

Association analysis

The associations between the expressions of the three biomolecular markers and the clinicopathological characteristics of the patients were analyzed. No significant association emerged between the expressions of the two potassium channels and clinicopathological characteristics such as age, TNM stages, sex, histological grading, and adjuvant therapy. KCa3.1 was mainly expressed in mucinous CRC primary samples (71.4% mucinous tumors vs 52.5% nonmucinous tumors; *P*=0.045), and an association, although not significant, was found between hERG1 and tumor site, with the channel more expressed in transverse and left colon (*P*=0.267).

Glut-1 expressed more in patients aged <70 years (42.4% vs 26%; *P*=0.029) and in left and transverse colon (27.4%, 42.9%, 53.9%, and 25% for right colon, transverse colon, left colon, and rectum, respectively; *P*=0.019). Glut-1 was less frequently detected in mucinous tumors (*P*=0.088).

Impact on survival outcomes

The impact on survival was analyzed evaluating both DFS and OS. Patients were followed up for a median time of 32 months. Thirty of 162 patients (18.5%) had a disease relapse and 32 (19.8%) died during follow-up. The univariate analysis (Table 1) showed that TNM stage, adjuvant therapy, and Glut-1 expression have a significant impact on DFS and OS: TNM stage III and adjuvant therapy emerged as indicators of worse prognosis, whereas Glut-1 had a positive impact on survival.

The multivariate analysis (Table 2) confirmed the trends of stage III TNM, adjuvant therapy, and Glut-1 expression and also showed a significant negative impact of hERG1 expression on both DFS and OS. For KCa3.1, both univariate

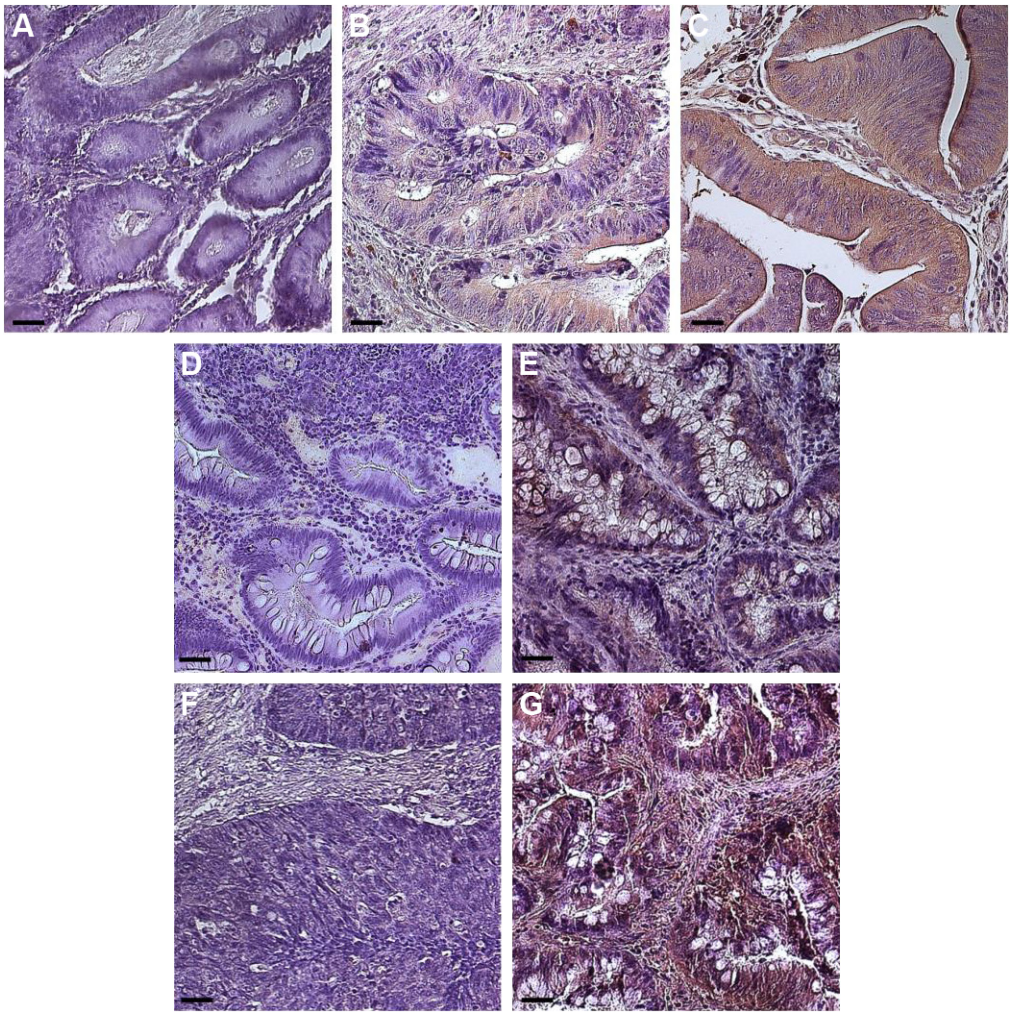

Figure 1 Immunohistochemical scoring for all markers in CRC primary samples.
Notes: (**A**–**C**) Representative examples of hERG1 scoring in CRC specimens using the anti-hERG1 monoclonal antibody: (**A**) score 0 (0% of positive cells), (**B**) score 1 (1%–49% of positive cells per microscopic field), and (**C**) score 2 (>50% of positive cells per microscopic field). Note that only samples belonging to score 2 were considered positive. (**D** and **E**) Representative example of KCa3.1 scoring: negative (**D**) and positive (**E**) representative CRC specimens. (**F** and **G**) Glut-1 scoring in representative CRC specimens: (**F**) negative and (**G**) positive samples. Original magnification: 200×. Scale bars: 50 μm.
Abbreviations: CRC, colorectal cancer; IHC, immunohistochemistry.

and multivariate analyses did not show any statistically relevant association with the survival outcomes. For risk stratification analysis, all the variables that did not show any significant independent effect on DFS and OS were progressively removed. After adjusting for adjuvant treatment, this analysis led to stratification of the patients into four different risk groups, based on TNM stages and hERG1/Glut-1 expression. Three groups encompass TNM stage I and II patients, who are further subdivided on the basis of the expression of hERG1 and Glut-1, and the fourth group comprises stage III patients independently on the molecular phenotype. As shown in Figure 2 and Table 3, the group comprising Glut-1-negative and hERG1-positive stage I and II patients had the worst survival experience.

Discussion

The present study was aimed at identifying novel biomolecular markers to be employed in the clinical practice for the identification of high-risk early-stage CRC patients, for further selection of treatment options. The prognostic impact of two potassium channels hERG1 and KCa3.1, as well as that of Glut-1, in a cohort of 162 surgically resected, stages I–III CRC patients was analyzed. It is evident that, independent of adjuvant treatment effect, 1) three main variables significantly impact on survival (both OS and DFS): TNM (negative when stage III), hERG1 (negative), and Glut-1 (positive); 2) hERG1 positivity and Glut-1 negativity serve to identify a subset of stage I and II CRC patients whose survival curves are worse than those of stage III CRC patients.

Table 2 Multivariate analysis of factors related to DFS and OS (by the Cox's regression model)

Parameter	DFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
TNM stage		0.0110		0.0060
I	1 (ref)		1 (ref)	
II	0.43 (0.17–1.10)		0.39 (0.14–1.08)	
III	1.16 (0.48–2.79)		1.16 (0.46–2.92)	
hERG1		0.0127		0.0058
Negative	1 (ref)		1 (ref)	
Positive	2.57 (1.40–4.73)		2.75 (1.43–5.29)	
KCa3.1		0.0708		0.3109
Negative	1 (ref)		1 (ref)	
Positive	0.59 (0.34–1.04)		0.72 (0.38–1.35)	
Glut-1		0.0004		<0.0001
Negative	1 (ref)		1 (ref)	
Positive	0.32 (0.17–0.60)		0.24 (0.12–0.49)	
Adjuvant		0.0001		0.0012
No	1 (ref)		1 (ref)	
Yes	3.42 (1.75–6.67)		3.03 (1.50–6.13)	

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival.

Nowadays, the identification of novel prognostic biomarkers is mandatory, in order to help clinicians in risk stratification and decision making in the treatment of CRC patients with early-stage, in particular stage II, disease.[38] Indeed, it is well known that a subgroup of stage II patients, usually not treated with adjuvant chemotherapy, recurs after surgery.[39] Those stage II patients whose disease recurs would benefit from adjuvant chemotherapy, like those with a TNM stage III disease. Hence, clinicians continue to grapple with the problem of determining those stage II patients most likely to derive benefits from adjuvant chemotherapy, in order to improve the outcomes in this patient population and, in the meantime, avoid potentially toxic overtreatments. The most important parameters among a handful of prognostic variables that are considered for treatment choices are only tumor stage, tumor grade, and MSI.[40,41]

In the present study, the expression and the prognostic impact of two potassium channels, hERG1 and KCa3.1, whose aberrant expression has been strongly associated to many human cancer, including CRC, were investigated.[15–30] The expression of Glut-1, another cancer biomarker, belonging to the hypoxia signaling pathway was also investigated.[31–33] Moreover, a previous observation, obtained in a pilot study, demonstrated a significant association and prognostic valence for the combined hERG1 and Glut-1 expressions in CRC patients.[34]

The expression of KCa3.1 channel was detected in >50% of CRC primary samples. Unexpectedly, our data did not show any diagnostic or prognostic relevance for KCa3.1 expression, at least in nonmetastatic CRC, even though its role in driving tumor progression and its association to poor prognosis in other types of human cancer are well recognized.[23–30]

On the contrary, hERG1 expression displayed a negative impact on survival outcomes that, although nonsignificant at the univariate analysis, reached significance in the multivariate model. Most parameters losing their valence, when

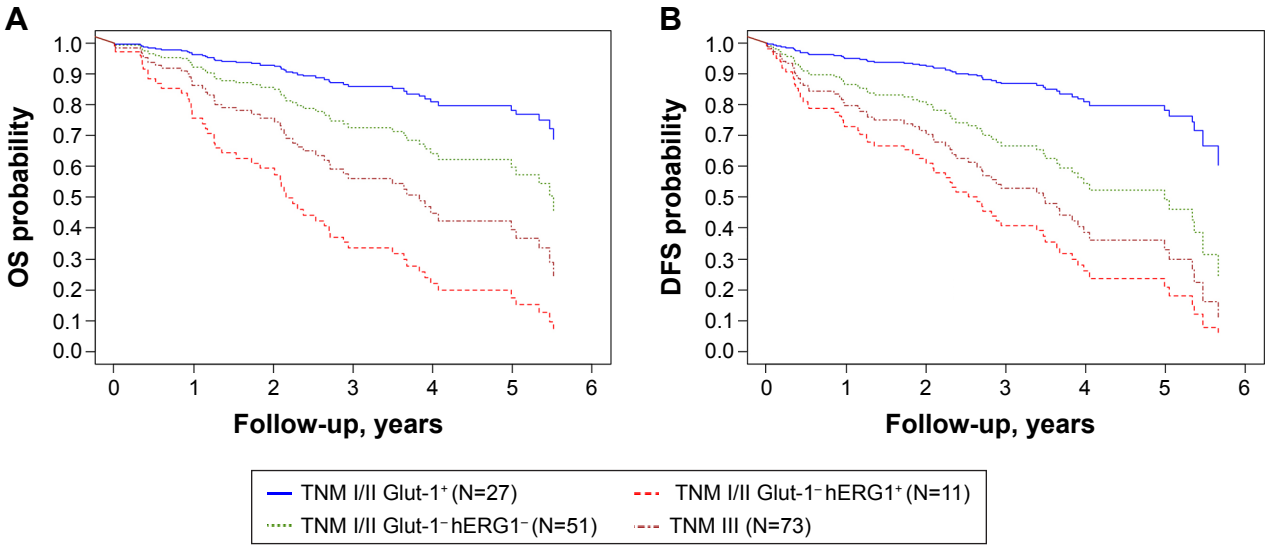

Figure 2 Kaplan–Meier curves of overall and disease-free survival according to different combinations of tumor characteristics (TNM stage, Glut-1, and hERG1 status).
Notes: Kaplan–Meier plots of (**A**) overall survival (OS) and (**B**) disease-free survival (DFS) probabilities for four different groups are reported. Blue curve indicates stage I and II Glut-1-positive samples (27 patients, 16.7%); red curve, stage I and II Glut-1-negative and hERG1-positive samples (eleven patients, 6.8%); green curve, stage I and II Glut-1-negative and hERG1-negative samples (51 patients, 31.5%); brown curve, stage III samples (73 patients, 45.1%).

Table 3 Association between risk groups and survival outcomes adjusted for adjuvant treatment (by means of Cox's proportional hazard model)

Parameter	Patients (n)	DFS			OS		
		3-year DFS	HR (95% CI)	*P*-value	3-year OS	HR (95% CI)	*P*-value
TNM III	73	52.9%	1 (ref)	0.032	56.2%	1 (ref)	0.021
TNM I/II Glut-1$^+$	27	86.8%	0.22 (0.05–0.95)		85.9%	0.26 (0.06–1.14)	
TNM I/II Glut-1$^-$ hERG1$^+$	11	40.8%	1.41 (0.58–3.40)		33.7%	1.89 (0.77–4.70)	
TNM I/II Glut-1$^-$ hERG1$^-$	51	66.6%	0.64 (0.35–1.17)		72.6%	0.56 (0.28–1.11)	

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival.

analyzed under the multivariate models, is an unusual feature. However, the negative impact of hERG1 on prognosis is not surprising, as the impact of hERG1 on tumor progression has been proven by several published papers.[15–22]

Glut-1 showed the strongest correlation with clinicopathological features. In fact, statistically relevant correlations emerged with age (more expressed in <70 years cluster) and tumor site (more expressed in left colon and transverse). Furthermore, Glut-1 positively impacted on the survival at both the univariate and multivariate analyses. Such a positive impact is apparently in contrast with the common view considering Glut-1, being a hypoxia marker, as a tumor progression factor.[31–33,42,43] As discussed in Lastraioli et al,[34] it is believed that the highest Glut-1 expression occurs at a preangiogenic phase of tumor progression, and its disappearance marks the onset of angiogenesis, the true progression step underlying the acquisition of full malignancy in CRC.

Overall, four variables expressed a significant impact on survival: TNM stages, therapy, hERG1, and Glut-1 expressions. The negative impact on the survival of TNM is well defined.[44] The negative prognostic role of adjuvant therapy may appear contradictory, as it was developed with the purpose of improving survival. Until now, this negative impact has been misleading, because it depends more on the clinical characteristics of the group of patients (either stage III or II) who were selected for treatment than on the effects of treatments.

The most novel and relevant result emerging from the present study was that a strong hERG1 expression, combined with the lack of Glut-1 expression, is associated with significant worsening of the prognosis of surgically resectable early stages of CRC patients. The present risk analysis, besides confirming a previous pilot study,[34] led to an even stronger evidence that the outcome of hERG1-positive/Glut-1-negative patients is worse than that of stage III patients.

On the whole, based on the results reported here, it has been proposed that an IHC-based test addressing hERG1 and Glut-1 detection (hERG1/Glut-1 test) could be used in stage II CRC patients to determine the individual risk of cancer recurrence. Alone, or in conjunction with MSI or CDX2 analysis, the hERG1/Glut-1 test could accompany, or even substitute, more complex biomolecular tests. Furthermore, it has been proposed to accomplish an appropriately designed study to confirm the predictive potential of hERG1 positivity and Glut-1 negativity. Once validated in a clinical study, the hERG1/Glut-1 test would contribute to identify those stage II patients with likelihood of benefit from adjuvant chemotherapy.

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Disclosure

The authors report no conflicts of interest in this work.

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