KCNB1 gene polymorphisms and related indel as predictor biomarkers of treatment response for colorectal cancer – toward a personalized medicine

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Abstract
The KCNB1 gene variants were differentially associated with cancers. However, their association with colorectal cancer has not yet been explored. We investigated the contribution of the KCNB1 gene variants rs3331, rs1051295, and indel (insertion/deletion) rs11468831 Polymorphism as predictors of the treatment response in colorectal cancer patients. A retrospective study, which involved 291 Tunisian colorectal cancer patients (aged 60.0 ± 13.1 years), who were stratified into responder and non-responder groups, according to TNM stages and their responsiveness to chemotherapy based on fluorouracil. KCNB1 genotyping was performed with amplification-refractory mutation system–polymerase chain reaction, and was confirmed by Sanger sequencing. Sex-specific response was found and colorectal cancer females are less likely to achieve a positive response during the chemotherapy strategy, compared to males. Weight and body mass index, tumor size, and tumor localization are considered as predictive factors to treatment responsiveness. Carriage of rs11468831 Ins allele was significantly associated with successful therapy achievement (p adjusted < 0.001). Stratification of colorectal cancer patients’ response according to tumor localization and TNM stages reveals negative association of rs3331 Major allele to treatment response among the patients with advanced cancer stages (subgroup G2). The presence of rs3331 (homozygous minor) C/C genotype was positively associated with decline in carcinoembryonic antigen (p = 0.043) and CA19-9 (p = 0.014) serum levels. On the other hand, the presence of rs1051295 (homozygous minor) A/A genotype was correlated with marked decline in CA19-9 serum levels. KCNB1 haplotype did not reveal any association between haplotypes and treatment response. The results obtained suggest that gender-specific strategies for screening treatment and prevention protocols as well as KCNB1 variants may constitute an effective model for ongoing personalization medicine.

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Keywords
CA19-9, CEA, gender, TNM stages, colorectal cancer, KCNB1, personalized medicine, treatment response

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Highlights
- Female patients were less frequent to achieve successful treatment outcome.
- Indel rs11468831 is associated with successful colorectal cancer therapy regardless of TNM stages.
- KCNB1 SNP rs3331 is associated with treatment sustained response.
- CEA and CA19-9 level decline is correlated with CC genotype of rs3331.
- CA19-9 rate decline is correlated with GG genotype of rs1051295.
- KCNB1 haplotype is not associated with treatment response.

Introduction
Colorectal cancer (CRC) is a major cause of mortality. It is classified as the third leading cause of cancer-related deaths worldwide.1,2 According to GLOBOCAN’s estimations, the incidence of CRC is progressively increasing during the recent decades.3 In Tunisia, the age standardized prevalence of CRC is estimated at 6.4/100,000 in 1994, and is expected to rise to 39.3/100.000 by 2024, largely due to the absence of effective disease management.4,5 The etiology of CRC remains elusive and most likely combines environmental and genetics factors.6

The decision-making of treatment in Tunisia is based on established clinical oncology guidelines in light of the uniqueness of Tunisian patients.7,8 Folfox, which is a chemotherapy regimen made up of folinic acid (FA), fluorouracil (5-FU), and Oxaliplatin remains the mainstay of standard care for CRC treatment in Tunisia. Carcino-embryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are two tumor markers widely used for screening disease process and evolution.9–11 However, their role is limited because of their poor sensitivity to specificity.12–15 This prompted the search for alternative biomarkers, especially in light of the emergence of resistance to therapy.7

The clinical heterogeneity of alteration and defective function of an array of genes was shown to influence the disease process. These include TP53, KRAS, and BRAF genes.16–18 Recently, voltage-gated potassium (Kv) channels were associated with numerous neoplasia, principally those affecting the digestive tract.19,20 Kv2.1, a ubiquitous potassium channel subtype, was shown to be expressed in various tumor cells, and to participate in cell proliferation.21,22 Kv2.1 is encoded by the KCNB1 gene, is located on chromosome 20q13.2, and is differentially expressed in several tissues.23 Several KCNB1 variants were implicated in modulating its gene expression.

In spite of the demonstrated association of KCNB1 with cancer, no study has yet investigated the effect of KCNB1 polymorphism on CRC progression, or the response to treatment.

In the present study, we examined the association of the KCNB1 polymorphisms rs3331, rs1051295, and indel related variants rs11468831 on CRC treatment response in Tunisian patients.

Materials and methods
Patient data collection
We performed a longitudinal prospective study that involved 291 CRC patients (family, fCRC and sporadic, sCRC). All cases were followed up in the Salah Azaiz Oncology Institute from February 2016 to January 2018.

The disease stage was assessed according to the TNM classification of the UICC.24 While the treatment responses are correlated to TNM stage’s patients, we stratified the cohort into two groups. The first group (G1) includes patients in stages II and III divided into relapsed and non-relapsed patients and the second group (G2) includes responder and non-responder (stage IV) patients.

Data were collected retrospectively from medical records and personal interviews. Dukes’ classification, modified by Astler–Coller, was used to determine CRC stages.25,26 Patients were followed up after completion of their chemotherapy every 6 months over 3 years, with blood analysis including CBC, CEA, and CA 19-9 levels.

Treatment strategies
The type of the applied treatment depends on the pathological reports and TNM stage. In fact, patients carriers of colon (CC) and rectal cancer (RC) diagnosed with primitive tumor (stage I), undergo surgery directly without being treated with chemotherapy. However, patients diagnosed in stage II with high risk factor were differently treated according to tumor localization. Indeed, CC patients follow an adjuvant
chemotherapy based on fluorouracil (5-FU) and RC patients could undergo neoadjuvant concurrent chemo/radiotherapy as preoperative treatment followed by surgical resection.

All patients with RC and CC in stage III receive postoperative 5-FU-based chemotherapy. Finally, CRC patients with metastatic disease (stage VI) should have first-line chemotherapy according to clinician’s medical decision. They are classified as responder and non-responders according to their treatment responsiveness. All patients have undergone surgery according to NCCN (National Comprehensive Cancer Network) and ESMO (European Society for Medical Oncology) Guidelines.

**Genotyping assays**

Peripheral venous blood was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes. Genomic DNA was prepared using QIAamp® DNA blood Mini Kit. Genotyping of rs3331, rs1051295 was done by amplification-refractory mutation system-polymerase chain reaction (ARMS-PCR), and standard PCR was used for genotyping rs11468831 variant. Only rs11468831 PCR products were separated using capillary electrophoresis on DNA 500 and DNA 1000 Labchips (Agilent 2100 Bio-analyzer, Biotech Vertriebsgesellschaft m.b.H Agilent Technologies, Lithuania, landlocked). While both rs3331 and rs151295 were confirmed by Sanger sequencing ABI PRISM 3100 DNA Analyzer (Applied Biosystems). Results were analyzed via Sequencer 3.1.1 software.

**Radioimmunoassay of CEA and CA 19-9 serum tumor markers**

Serum concentration and kinetics follow-up of CEA and CA19-9 tumor markers were assessed by radioimmunoassay (Beckman Coulter, Brea-Calif).²⁷

**Statistical analysis**

Statistical analysis was performed with R for Windows. Continuous variables were expressed as mean ± SD, while categorical data were expressed as percent of the total. Independent sample t-test was used for inter-group comparisons of continuous data which were normally distributed, and chi-square test for categorical variables.

Logistic regression was used in analyzing the independent contribution of key covariates with sustained treatment response; \( p < 0.05 \) was considered statistically significant. Exhaustive regression was conducted to define the genotype with the real influence on treatments response.

Power of the study was calculated using the following parameters: number of subjects, genotypic relative risk for heterozygous and homozygous minor allele, with assuming the CRC prevalence in Tunisia. Accordingly, the overall power was 88%.

**Results**

**Patients characteristics**

The characteristics of patients included in the study are presented in Table 1. No significant differences were observed between groups in terms of age, body mass index (BMI), smoking history, alcohol consumption, hypertension, anemia, extent of tumor differentiation, and histology. On the other hand, among G1 group, the sex-ratio (SR) was significantly different between relapsed (1.34) and non-relapsed patients (0.81) \( (p = 0.05, \ OR = 0.60, \ 95\% \ CI = [0.35–0.93]). \) Weight was significantly lower in non-relapsed patients \( (p = 0.0035) \) and tumor localization was significantly different between both \( (p = 0.049) \). Indeed, CC patients (68.5%) have better chances to cancer clearance compared to RC patients (31.55%). Patients in stage II with non-relapsed response are statistically higher (55.05%) and tumor size found as indicator factor to positive response among G1 group \( (p = 0.036) \). Regarding G2 groups, no differences were detected on the previously mentioned parameters.

**Clinical responses to therapy**

Stratification factors were essential components of treatment response (i.e. type of treatment and TNM stage). Primary neoadjuvant with Folfox (5-FU + FA) was well established treatment with a high frequency of positive response (non-relapsed) among patients with TNM stage II. In fact, 66.7% of non-relapsed patients were under Folfox treatment versus 33.4% of relapsed cases \( (p = 0.0126) \). However, 40.81% only of patients belonging in G1 group were responders and 59.19% were non-responders \( (p = 0.618) \). In addition, 2.40% of total patients received Cetuximab treatment. We found 66.7% of patients were negative responders and only 33.4% had successfully responded to the treatment.
| Parameters | Group 1 (G1) | Group 2 (G2) | p<sup>1</sup> | p<sup>2</sup> | p<sup>3</sup> |
|------------|-------------|-------------|--------------|--------------|--------------|
|            | Non-relapsed | Relapsed | Total | Non-relapsed | Relapsed | Total | Non-relapsed | Relapsed | Total | Non-relapsed | Relapsed | Total | Non-relapsed | Relapsed | Total | Non-relapsed | Relapsed | Total |
| Gender (male/female) | 67/82 | 51/38 | 118/120 | 0.056 |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Age (years)<sup>2</sup> | 59.20 ± 1.00 | 60.32 ± 1.53 | 59.62 ± 0.85 | 0.524 | 62.04 ± 2.98 | 62.5 ± 2.17 | 62.30 ± 1.76 | 0.795 | 0.41 |
| Weight (kg) | 63.04 ± 13.02 | 69.40 ± 16.68 | 65.35 ± 14.70 | 0.0035 |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| BMI<sup>2</sup> | 24.56 ± 0.38 | 25.25 ± 0.61 | 24.28 ± 0.33 | 0.443 | 26.94 ± 1.49 | 26.14 ± 0.90 | 26.47 ± 0.80 | 0.609 | 0.37 |
| Family history of cancer |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| sCRC | 92 (61.74) | 51 (57.30) | 143 (60.09) | 0.498 |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| fCRC | 57 (38.26) | 38 (42.69) | 95 (39.91) |         | 13 (56.53) | 18 (60.00) | 31 (58.49) |         |         |         |         |         |         |         |         |         |         |         |
| Smoker | 50 (33.55) | 34 (38.20) | 84 (35.29) | 0.468 | 10 (43.47) | 12 (40.01) | 22 (41.50) | 0.515 | 0.46 |
| Alcohol | 41 (27.51) | 27 (30.33) | 88 (38.57) | 0.641 | 7 (30.43) | 7 (23.33) | 14 (26.41) | 0.474 | 0.82 |
| Hypertension | 111 (74.50) | 74 (83.14) | 185 (77.73) | 0.123 | 17 (73.91) | 18 (60.00) | 35 (60.03) | 0.570 | 0.65 |
| Anemia | 36 (24.16) | 23 (25.48) | 59 (24.68) | 0.771 | 5 (21.73) | 8 (26.66) | 13 (24.52) | 0.325 | 0.21 |
| Tumor localization |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Colon | 102 (68.45) | 52 (58.42) | 154 (64.70) | 0.049<sup>a</sup> | 16 (69.56) | 14 (56.53) | 30 (60.37) | 0.984 | 0.044 |
| Rectum | 47 (31.55) | 37 (41.58) | 84 (35.30) |         | 7 (30.43) | 14 (46.67) | 21 (39.62) |         |         |
| Differentiation |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Well | 79 (54.10) | 48 (56.47) | 127 (54.97) | 0.711 | 39 (13.01) | 13 (43.33) | 22 (41.52) | 0.995 | 0.75 |
| Moderate | 55 (37.69) | 30 (35.29) | 85 (36.79) |         | 13 (43.33) | 22 (41.52) | 0.995 | 0.75 |
| Poor | 12 (8.21) | 7 (8.24) | 19 (8.24) | 0.936 | 3 (13.05) | 1 (3.34) | 4 (7.54) | 0.998 | 0.51 |
| Histological type (adenocarcinoma) |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Lieberkuhnian | 119 (84.40) | 71 (83.33) | 190 (84.07) | 0.610 | 20 (86.95) | 24 (88.88) | 44 (88.00) |         |         |
| Tubular | 7 (4.98) | 6 (7.06) | 13 (5.75) | 0.893 | 1 (4.36) | 1 (3.70) | 2 (4.00) | 0.847 | 0.904 |
| Mucinous | 13 (9.21) | 6 (7.06) | 19 (8.40) | 0.488 | 2 (8.69) | 2 (7.42) | 4 (8.00) | 0.998 | 0.571 |
| Signet Ring Cell | 2 (1.41) | 2 (2.35) | 4 (1.78) | 0.554 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0.995 | 0.904 |
| TNM classification<sup>b</sup> |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| II | 72 (48.33) | 49 (55.05) | 121 (50.85) | 0.047<sup>a</sup> |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| III | 77 (51.67) | 40 (44.95) | 117 (49.15) |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| IV |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Tumor size (cm)<sup>2</sup> | 11.58 ± 1.57 | 12.64 ± 2.57 | 12.25 ± 1.72 | 0.036<sup>a</sup> | 8.52 ± 0.98 | 10.54 ± 1.95 | 9.95 ± 1.17 | 0.631 | 0.84 |

CRC: colorectal cancer; BMI: body mass index; sCRC: sporadic CRC; fCRC: familial CRC; TNM: tumor, nodes, metastases according to Dukes' Classification modified by Astler–Coller.

<sup>1</sup>Pearson chi-square (categorical variables), Student's t-test (continuous variables).

<sup>2</sup>Mean ± standard deviation.

<sup>3</sup>p<sub>1</sub>: relapsed versus non-relapsed.

<sup>4</sup>p<sub>2</sub>: responder versus non-responder.

<sup>5</sup>p<sub>3</sub>: total population with positive response (non-relapsed + responder) versus total population with negative response (relapsed + non-responder).

<sup>a</sup>Values marked in bold are inferior to 0.05.

<sup>b</sup>Adjusted according to gender.

<sup>p</sup>1 calculated by comparing TNM I versus TNM II; p<sup>2</sup> was calculated by comparing TNM III versus TNM VI; p<sup>3</sup> was calculated comparing TNM I, II, III, and VI, and only the associated p of TNM VI was written.

*Table 1. Clinicopathological characteristics of the CRC patients.*
**KCNB1 polymorphisms and treatment response among fCRC and sCRC patients**

Results of the association between rs3331, rs1051295, and rs11468831 variants with the treatment response are summarized in Table 2.

Most fCRC and sCRC patients carrying rs11468831 homozygous major allele genotype achieved positive response to treatment as non-relapsed \((p = 2.72 \times 10^{-5})\) and/or as responder cases \((p = 0.021)\), compared to non-Ins/Ins genotype carriers. In fact, homozygous minor allele genotype frequency was 44.9% among relapsed patients and 38.7% among non-responders. This association remained significant after adjustment for tumor localization, gender, tumor size, and body weight.

The association of rs1051295 genotype with treatment response was comparable in both groups (G1 and G2) \((p = 0.79\) and \(p = 0.63)\), respectively. However, our analysis reveals an association between rs3331 genotypes and both groups (G1 and G2). Indeed, the association was marginally noticed among G1 patients. Unlikely, the major genotype of rs3331 was not consistently associated with positive treatment response in G1 and G2. Regarding G1 strata, carriage of the homozygous Major rs3331 allele \((T/T)\) was marginally associated with sustained treatment \((p = 0.07)\).

Interestingly, we found high association of rs3331 with therapy response G2. In fact, responder patients were frequently carriers of non-homozygous major allele (87%). Moreover, patients carrier of the major homozygous allele \((T/T)\) were 4 folds (3.66) more likely for treatment failing during the therapy process \((p = 0.03)\). Consequently, carrying the minor rs3331 allele regardless of the number of copies appears to enhance the positive response for treatment among patients with advanced TNM stages (IV), (Table 2).

**Association between CEA and CA19-9 with KCNB1 polymorphisms and indel mutation and treatment response according to tumor localization**

Subsequently, we evaluated the kinetics of decline in CEA and CA19-9 serum levels among CRC patients over the 39 months of treatment period according to the presence of rs3331, rs1051295, rs11468831 (Figure 1), and tumor site (Figure 2).

The magnitude of the decline varied according to the presence of specific variants-genotype. The minor allele rs3331 \((p = 0.043)\) and rs1051295 \((p = 0.065)\) are associated with pronounced decline in CEA \((p = 0.014)\) and CA19-9 \((p = 0.016)\). There was no correlation between rs11468831 genotypes and changes in CEA and CA19-9 serum levels during treatment or follow-up periods.

Analysis of CC and RC subgroups revealed associations among RC patients (Fig. 2). Significant differences were also detected between the CEA decline and rs3331 \((p = 0.017)\), rs1051295 \((p = 0.006)\), and rs11468831 \((p = 0.023)\). Likewise, there was a decline in CA19-9 level in rs3331 \((p = 0.029)\), rs1051295 \((p = 0.0061)\), and rs11468831 \((p = 0.028)\) genotype carriers.

**Association of KCNB1 polymorphisms and the type of treatment**

This identifies spurious factors via a cross-model chi-square statistic that tests for stability in parameter estimates across models. The results from Table 2 confirmed the association of rs3331 and rs1051295 homozygous minor allele genotypes with positive response when patients were treated with 5-FU-based chemotherapy (Folfox) \((p < 0.001)\), and to a lesser extent when the Irinitecan is used instead Oxaloplatin (Folfri) \((p < 0.01)\). This confirmed the association of the minor genotype of rs3331 and rs1051295 with positive response among patients treated with Folfox \((p < 0.01)\) and Folfiri \((p < 0.05)\).

**Discussion**

Despite the availability of therapeutic regimens for CRC treatment, a large proportion of patients do not adequately respond, and thus they are considered as non-responders and/or relapsed. The standard treatment for CRC in Tunisia is financially demanding, leading to the necessity of assessing predictors of response before initiating and during treatment. We, therefore, evaluated the utility of KCNB1 variants and related polymorphisms as predictors of the treatment response in CRC patients. To the best of our knowledge, this is the first study to examine this aspect of KCNB1 variants in CRC treatment and follow-up.

SR was different among the relapsed and non-relapsed subgroups 1.34 and 0.81, respectively. Indeed, it is well established that women have a higher risk of developing right-sided (proximal) RC, which is associated with a more aggressive form of neoplasia compared to left-sided (distal) RC. Although differences were revealed in tumor location between women and men\(^{28,29}\) and the sex-distinguishable results of the most common cancer screening test (iFOBT)\(^{30}\) colorectal cancer screening guidelines do not distinguish females from males. This may explain the higher observed frequency of more advanced neoplasia when tumors are first detected and false negative results in colonoscopy among females. In addition, the sex-specific response
| Polymorphisms | Group 1 (G1), n = 238 | Non-responders \(^1\) | Responders \(^1\) | Total population | p\(^2\) | OR | CI (95%) | p\(^3\) | OR | CI (95%) |
|---------------|------------------------|------------------------|----------------|----------------|-------|-----|--------|-------|-----|--------|
| rs3331        |                        |                        |                |                |       |     |        |       |     |        |
| T/T           | 32(29.9)               | 10(12.6)               | 0.123          | 0.030          | 1.00  |     |        |       |     |        |
| T/C           | 64(59.8)               | 43(64.2)               | 0.55           | 0.25-1.22      | 1.00  |     |        |       |     |        |
| C/C           | 11(10.3)               | 11(16.4)               | 0.33           | 0.10-1.02      | 1.00  |     |        |       |     |        |
| T/T           | 32(29.9)               | 10(12.6)               | 0.07           | 0.24-0.91      | 0.99  |     |        |       |     |        |
| T/C-C/C       | 75(70.1)               | 69(77.4)               | 0.51           | 0.24-0.91      | 0.99  |     |        |       |     |        |
| rs1051295     |                        |                        |                |                |       |     |        |       |     |        |
| G/G           | 20(16.5)               | 13(18.1)               | 0.964          | 0.047          | 1.00  |     |        |       |     |        |
| A/G           | 81(66.9)               | 47(65.3)               | 1.12           | 0.50-2.50      | 1.00  |     |        |       |     |        |
| A/A           | 20(16.5)               | 12(16.7)               | 0.79           | 0.39-3.06      | 1.00  |     |        |       |     |        |
| G/G           | 20(16.5)               | 13(18.1)               | 0.79           | 0.39-3.06      | 1.00  |     |        |       |     |        |
| A/G-A/A       | 101(83.5)              | 78(81.9)               | 1.11           | 0.51-2.45      | 1.00  |     |        |       |     |        |
| rs11468831    |                        |                        |                |                |       |     |        |       |     |        |
| ins/ins       | 80(63.7)               | 57(77.0)               | 1.39           | 0.047          | 1.00  |     |        |       |     |        |
| ins/del      | 39(26.5)               | 20(22.5)               | 0.75           | 0.39-2.50      | 1.00  |     |        |       |     |        |
| del/del      | 30(20.1)               | 40(44.9)               | 0.28           | 0.15-0.53      | 1.00  |     |        |       |     |        |
| ins/ins       | 80(63.7)               | 57(77.0)               | 1.39           | 0.047          | 1.00  |     |        |       |     |        |
| ins/del      | 39(26.5)               | 20(22.5)               | 0.75           | 0.39-2.50      | 1.00  |     |        |       |     |        |
| del/del      | 30(20.1)               | 40(44.9)               | 0.28           | 0.15-0.53      | 1.00  |     |        |       |     |        |

**Table 2.** KCNB1 rs3331, rs1051295, and rs11468831 genotype frequencies according to treatment response.

**Non-relapsed** includes subjects without CRC, and **responders** include patients with CRC.

**Bold** indicates statistical significance (p < 0.05).

- OR: odds ratio; CI: confidence interval; CRC: colorectal cancer.
- \(^1\) Number of subjects (frequencies: percent total).
- \(^2\) p value adjusted according to the clinic-pathological parameters of the CRC patients.
- \(^3\) p value calculated by comparing total patients with positive response to treatment (non-relapsed + responder) versus total patients with negative response to treatment (relapsed + non-responder).

OR: odds ratio; CI: confidence interval; CRC: colorectal cancer.
could be explained by the anticancer drug used, which can cause toxicity to the reproductive system and does not consider the sex effect. Furthermore, the apparent difference in SR between relapsed and non-relapsed might be, to an extent, due to the socioeconomic barriers in Tunisia within female patients that cause delay screening and diagnosis.

We observed significant differences between non-relapsed and relapsed patients in terms of weight, tumor localization, tumor size, and TNM classification. In fact, results of over 7 million individuals, and more than 93,000 patients indicate that weight and/or BMI of 25.0–27.4 kg/m², 27.5–29.9 kg/m², and >30.0 kg/m² were associated with 19%, 24%, and 41% increased risks of developing CRC, respectively, when compared to normal BMI (≤ 24 kg/m²). The non-relapsed had lower mean weight (63.04 kg) and BMI (24.5 kg/m²) when compared with relapsed (69.40 kg and 24.28 kg/m²).

Figure 1. Kinetics of changes in CEA and CA19-9 serum markers among non-responder versus responder CRC patients, according to KCNB1 genotype status. Polymorphisms. (A, D) rs3331; (B, E) rs1051295; (C, F) rs11468831.

Figure 2. Kinetics of changes in CEA and CA19-9 serum markers among non-responder versus responder RC patients, according to KCNB1 genotype status. Polymorphisms. A, D) rs3331; (B, E) rs1051295; (C, F) rs11468831.
respectively; \( p = 0.0035 \)). Moreover, a significant association between treatment response and TNM stage was detected.

Indeed, among G1 group, most patients with primary TNM stage fulfill positive results since 5-FU-based chemotherapy improves local control of the tumor. There was a systematic effect of diagnostic TNM stage on responsiveness to chemotherapy; patients with TNM stage IV were less likely to respond to the treatment (64.27\%), \( p = 0.041 \). Henceforth, the poorer response of patients in colon cancer is likely to be related to delayed diagnosis. Thus, it is possible that more aggressive treatment and/or dose play a role in better response and clearance among these cases. Another puzzling possibility is that our patients with advanced TNM are the oldest subgroups (62.59 \pm 12.24 years) compared to others (first, second, and third stages) 49 \pm 0.01, 59.25 \pm 14.02, and 59.77 \pm 12.35 years old, respectively, which can limit the treatment outcomes. In fact, the metastatic tumor cells of young patients may be more sensitive to therapy compared to older patients.32–34

In addition to environmental factors, cancer pathogenesis is attributed to mutations in oncogenes or tumor suppressor genes involved in cell division and/or cell death. Increasing evidence demonstrated that ion channel genes contribute to the progression of various carcinomas, and the proliferation of several cell types.35–40 We explored the correlation between rs3331, rs1051295, and rs11468831 variants and the response to chemotherapy among CRC patients.

By stratifying the total cohort according to tumor localization, our results endorse that most RC patients with “Ins/Ins” genotype were treatment responsive unlike “Del/Del” genotype carriers, who were generally non-responders \( p < 0.001 \).

Approximately 45.79\% of total CRC patients were carriers of rs11468831 major allele homozygous, and 73.39\% of them achieved a positive response to treatment as relapsed while 60.86\% was responders from the G2 group.

Roughly 71.2\% of patient carriers of rs3331 Major genotype responded positively to the 5 FU-based chemotherapy treatment. It was unlikely that a significant association was revealed among responder and non-responder patients of G2. Indeed, 76.9\% of non-responders were carriers of T/T genotype and 70\% of responders were patients with minor rs3331 genotype C/C. Consequently, patient carriers of T/T genotype were 3.6-fold more at risk to not fulfilling positive treatment outcomes.

Hence, we hypothesize that both the rs3331C/C genotype and rs11468831 ins/ins genotype constitute favorable genotypes to improve the treatment response.

It is possible that the carriage of these variants could alter the ion channel normal function, causing impairment in pathways involved in tumor exclusion and treatment response. Recently, it was suggested that KCNB1-induced autophagy inhibits tumor growth, and increases CRC survival,41 and that autophagy represents a key point in CRC aggressiveness and progression.39,42–46 Indeed, it is well established that cancer cells can overcome autophagy and survive by avoiding the stress of anticancer drugs.47,48 The cause–effect nature of the association between variants and response to treatment in CRC remains to be understood. Our results and growing body of evidence linking KCNB1, autophagy, cancer progression, and treatment response prompts the speculation that these variants may constitute a promising determinant of the chemotherapy outcomes. Currently, the widely used diagnostic procedure for CRC is endoscopy, which is highly sensitive and specific in identifying CRC.12,49,50 Yet, the high cost, invasive nature, and need for repeated testing resulted in limited implementation of endoscopy for CRC follow-up. Consequently, blood biomarkers constitute efficient indicators for CRC evaluation, and monitoring the treatment response, which are mainly CEA and C19-9.51 However, it lacks the specificity, as it is associated with other cancer types.52

Due to the heterogeneity of CRC phenotypes, a single tumor marker, such as CA19-9, is not considered a stand-alone diagnostic test. Significant associations were found between rs3331 genotypes and kinetics of CEA and CA19-9 among responder patients with the minor genotype being favorable for the decline in CEA and CA19-9 levels, and thus treatment response. Yet, no clear association was seen between rs1051295 genotypes and CEA kinetics among responder patients. Moreover, carriage of Ins/Ins genotype was correlated to a faster decline in CEA and CA19.9 levels, when compared to Del/Del genotype.

Our results recommend gender-specific strategy for screening and treatment. Also, prevention protocols can be established to reduce the treatment failure and improve the quality of life. In addition, as these polymorphisms may modulate treatment response and chemotherapy pathway, its screening, before starting the chemotherapy session may enhance the positive responsiveness.

These results sustain the rational for considering new approaches for safe and effective chemotherapy, among Tunisian CRC patients. Moreover, these polymorphisms appear to be reliable markers for monitoring the disease kinetics, and treatment response, compared to CEA and CA19-9. In fact, in spite of CEA and/or CA19-9 levels are often elevated among patients with gastrointestinal malignancies, patients with conformed cancers frequently have normal levels similar to healthy subjects.53 In addition, elevated CEA levels may be detected in smokers as well as patients with a variety of non-malignant diseases. Hence, proposing new genetics
tools could be effective modalities to surrogatetumor markers and manage, effectively, the asymptomatic individuals for CEA and CA19-9.

The limits of this study are related to the retrospective side. In addition, not all patients have been able to use EGFR target therapy, due to the public healthcare system that does not cover the treatment charges. Nevertheless, this study could serve to extend our understanding of the phenotype aspects of CRC in Tunisia.

Conclusion

The assessment of CRC patients to chemotherapy is crucial. Our results showed that women are more prone to the aggressive form of CRC. Moreover, our analysis supports the notion that screening of the KCNB1 gene and related variants may improve the effectiveness of treatment by implementation of personalized approach for patients with unfavorable genotype in order to monitor the disease in a better way.

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Authors’ contributions
MB carried out the experiments and manuscript preparation, IS conducted statistical analysis and manuscript drafting, SB carried out sample processing, RBA assisted with molecular data acquisition, HK performed bioanalyzer data acquisition, AF did sample processing, RH studied the concept of the original idea, AMO dealt with patient recruitment, AME supervised clinical data acquisition, WAY discussed the results and contributed to the final manuscript, BYW contributed to the final manuscript, and BBZ studied the concept and design, data interpretation, and final manuscript preparation. All authors read and approved the final manuscript.

Availability of data and material
The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Consent for publication
All authors have carefully examined and approved the content of the manuscript, and understand and accept that in the event of its publication, all copyright shall be transferred to the Tumor biology journal.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval
All the patient investigations conformed to the principles outlined in the Declaration of Helsinki and have been performed with the permission of Oncology Department released by the Ethics Committee of Salah Azaiz Oncology Institute (SAI) (Registration No.: ISA/2016/02. All the patients were informed about the purpose of the study, and consented in writing to participate in the study.

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