



# Molecular and clinical registry of Chilean patients diagnosed with BRAF-mutated colorectal cancer

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**Background:** In recent years, the epidemiological trend of colorectal cancer (CRC) in Chile has become increasingly concerning, particularly due to the high costs associated with managing advanced-stage disease, which places a significant strain on the national healthcare system. In 2012, 2,417 new CRC cases were reported across both sexes in Chile. However, current projections estimate a sharp rise to 5,914 new cases, corresponding to an incidence rate of 20.7 per 100,000 individuals. This study aims to register the presence of *BRAF* mutations in Chilean patients to better characterize the molecular epidemiology of CRC in the local population. *BRAF* mutations are associated with poor prognosis, with affected patients typically exhibiting a median overall survival (OS) of less than 12 months.

**Methods:** We present a cohort study that included 23 Chilean patients newly diagnosed with CRC and recruited up to April 1, 2024. Patients with a history of another malignant neoplasm diagnosed within the previous three years were excluded. *BRAF* mutations were analyzed in all participants treated within the public and private healthcare systems in Santiago, Chile.

**Results:** Despite the rising prevalence of CRC in Chile, the frequency and distribution of *BRAF* mutations in the local population are unknown. Median OS differed by sex, with female patients showing a shorter OS of 24 months compared to 75 months in male patients ( $P=0.16$ ). When stratified by stage at diagnosis, patients with stage II–III disease demonstrated a markedly longer median OS of 134 months, whereas those with stage IV disease had a median OS of only 24 months ( $P=0.12$ ; not significant).

**Conclusions:** Establishing comprehensive records of the most prevalent *BRAF* mutations in Chilean patients with CRC is essential for advancing precision oncology research. This knowledge has the potential to transform clinical management strategies and enhance treatment outcomes and the quality of life of patients. Consequently, an accurate assessment of *BRAF* gene mutations tailored to the molecular and clinical characteristics of each patient is crucial for optimizing treatment outcomes.

**Keywords:** Colorectal cancer (CRC); survival; *BRAF*; precision oncology

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## Introduction

Colorectal cancer (CRC), is the third most common malignancy worldwide and the second cause of death from cancer in both sexes, representing a leading cause of cancer-related mortality and disease burden for public health systems. Moreover, it accounts for approximately one in ten cancer incidence and deaths. Notable geographic disparities in CRC incidence have been observed, possibly explained by differences in healthy lifestyle factors and preventive healthcare measures adopted by some countries. Nevertheless, there is a marked trend towards an increasing incidence of early onset CRC (less than 50 years) which has, unfortunately, obscured the positive outcome shown by the group of more than 50 years of age, which has shown a decrease in its incidence rates (1). In 2020, it was estimated that there were over 1.9 million new CRC cases and 935,000 deaths, making CRC the second leading cause of

cancer mortality, despite ranking third in global incidence. The burden of CRC is disproportionately higher in countries with established transition economies, where incidence rates are approximately four times greater than those currently transitioning to a market-based system. In this scenario, CRC prevention remains the most effective intervention possible to date in terms of disease burden control and regression (1,2). Current CRC management strategies include surgical intervention for resectable cases, and combinations of radiotherapy, chemotherapy, and immunotherapy for non-resectable tumors. Despite these therapeutic advancements, CRC remains incurable in 50% of the cases. However, rapid progress in research methodologies has led to frequent updates in treatment strategies with novel therapeutic agents and approaches emerging to improve patient outcomes (3,4).

Significant advancements in the molecular characterization of CRC have identified numerous oncogenic genes as potential diagnostic and therapeutic targets, thereby advancing personalized treatment options (5). Among these, *BRAF*, which encodes a serine/threonine kinase, is a critical actionable target. Approximately 12% of metastatic CRC cases harbor the *BRAF* V600E mutation, which is associated with poor response to standard chemotherapy and shorter overall survival (OS). Mutations in *BRAF* promote tumor cell proliferation and metastasis, correlating with unfavorable outcomes (6). Over 30 *BRAF* mutations have been identified, with the V600E mutation being the most common, reported in 8–15% of CRC cases, while other mutations occur in approximately 2% of patients (7,8). Patients harboring *BRAF* mutations generally exhibit a poor prognosis, with a median OS of less than 12 months in the metastatic setting, compared with those with wild-type *BRAF* mutations. This underscores the critical necessity of tailoring treatment strategies to the molecular and clinical profiles of individual patients to optimize outcomes. The *BRAF* V600E mutation plays a central role in maintaining the CpG island methylator phenotype (CIMP) in CRC, a hypermethylated epigenetic state that frequently leads to the silencing of *MutL* homolog 1 (*MLH1*) and subsequent mismatch repair (MMR) deficiency, culminating in a microsatellite instability (MSI) phenotype (9).

### Highlight box

#### Key findings

- Overall survival (OS) by *BRAF* mutation, sex, and disease stage and Kaplan-Meier curves were used to assess OS in patients with *BRAF*-altered tumors. When stratified by sex, the median OS was 24 months for females and 75 months for males. Stratification by disease stage showed a median OS of 134 months for localized disease and 24 months for metastatic disease.

#### What is known and what is new?

- Anti-*BRAF*/*EGFR* therapy was recently approved for the treatment of metastatic *BRAF* colorectal cancer.
- Understanding the clinical and molecular characteristics of patients with *BRAF* mutations is essential for improving clinical decision making and advancing personalized treatment strategies. Visualization of the signaling pathways involved further supports the rationale for designing clinical trials that target both *BRAF* and *EGFR*, either alone or in combination with additional targeted therapies.

#### What is the implication, and what should change now?

- These key findings provide a strong rationale for designing clinical trials that concurrently target both *BRAF* and *EGFR*. Such trials have already shown improved clinical outcomes compared to previous standard-of-care treatments, highlighting the need to further develop and integrate advanced therapeutic strategies.

Recent findings have demonstrated that correction of the *BRAF* V600E mutation results in significant loss of DNA methylation in over half of the genes hypermethylated in CIMP-high (CIMP-H) tumors, leading to the derepression of gene expression. This strongly supports the notion that *BRAF* V600E is essential for sustaining the CIMP-H phenotype and the associated global gene repression. Nevertheless, the fact that some genes retain methylation after *BRAF* correction suggests that other factors—such as age-related epigenetic changes or chromatin remodeling events—may also contribute to the maintenance of this epigenetic state.

In Chile, the incidence of CRC has significantly increased in recent years, placing considerable financial strain on the healthcare system, particularly in managing advanced stages of the disease (10,11). In 2012, 2,417 new cases of CRC were recorded in both sexes. By contrast, recent projections estimate 5,914 new CRC cases in both sexes, with an incidence rate of 20.7 per 100,000 individuals (11). Mortality data from GLOBOCAN indicates that CRC was the third leading cause of cancer-related deaths in Chile in 2020, accounting for approximately 3,179 deaths (12). Despite advancements in CRC diagnosis and treatment, factors contributing to the increasing mortality rate in Chile remain unclear. This study aims to document the presence of *BRAF* mutations in Chilean patients to enhance the molecular characterization of CRC within the local population.

Here, we report on 23 Chilean patients diagnosed with CRC, whose tumor samples were primarily analyzed for *BRAF* and *NRAS* mutations using validated real-time quantitative polymerase chain reaction (qPCR) kits. In addition, four patients underwent mutation profiling with next-generation sequencing (NGS) using an extended panel of cancer-related genes (BGI-Sentis™, Shenzhen, China). Variants were subjected to a protein-protein STRING interaction analysis and Gene Ontology (GO) enrichment. Lastly, a cox proportional hazard model-covariate analysis was used to assess survival outcomes in the *BRAF*-mutated CRC subgroup. We present this article in accordance with the STROBE reporting checklist (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-2025-115/rc>).

## Methods

### *Design, setting, and patients with CRC*

This cohort biomarker study included 23 patients newly diagnosed with CRC and recruited up to April 1, 2024.

*BRAF* mutations were analyzed in all participants treated within INDISA Clinic, Providencia; INDISA Clinic, Maipú; San Carlo de Apoquindo Clinic, UC Christus (M.G.'s former affiliation) in Santiago, Chile. Patients with a history of another malignant neoplasm diagnosed within the previous three years were excluded. Survival of the study group was evaluated in R Studio software version 4.4.2 (<https://www.r-project.org/>).

### *Tissue samples*

Tissue samples were collected from patients diagnosed with CRC. Samples were collected during surgical resection and promptly sent to the laboratory for processing.

### *Detection of the BRAF V600E mutation in biopsy samples*

*BRAF* was analyzed using two distinct methodologies. The first methodology employed the *NRAS-BRAF* mutation test (*NRAS-BRAF*/1.0) kit from Idylla (CE-IVD), which detects mutations in codon 600 including V600E, V600D, V600R, and V600K. The second methodology utilized the *BRAF/NRAS* mutation test (LSR) kit from Roche (Santiago, Chile), which is capable of detecting mutations in codons 600 and 601 of exon 15 (V600E, V600E2, V600D, V600R, V600K, and K601E), as well as in codons 466 and 469 of exon 11 (G466A, G466V, G469A, G469R, G469V).

### *Additional genes tested*

The other genes analyzed were as follows—*KRAS* gene: *KRAS* was tested using the *KRAS* mutation test kit (*KRAS* from Idylla, CE-IVD), which detects mutations in the following exons and codons—exon 2: codons 12 (G12A, G12C, G12D, G12R, G12S, G12V) and 13 (G13D). Exon 3: codons 59 (A59E, A59G, A59T) and 61 (Q61H, Q61K, Q61L, Q61R). Exon 4: codons 117 (K117N) and 146 (A146P, A146T, A146V). *NRAS* gene: the *NRAS* gene was tested using the *NRAS-BRAF* mutation test kit (*NRAS-BRAF*/1.0) from Idylla (CE-IVD), which detects mutations as follows—exon 2: codons 12 (G12A, G12C, G12D, G12S, G12V) and 13 (G13D, G13R, G13V). Exon 3: codons 59 (A59T) and 61 (Q61H, Q61K, Q61L, and Q61R). Exon 4: codons 117 (K117N) and 146 (A146T and A146V).

### *Profiling by NGS*

Patients were selected for mutational profiling. The

samples were analyzed using commercially available NGS services with panels of cancer-related genes (BGI-Sentis™). (n=4). A subset of four patients underwent comprehensive mutational profiling using NGS. Analyses were performed using commercially available panels of cancer-associated genes (BGI-Sentis™).

### **Genetic mutation analysis**

Mutational profiles were analyzed based on the expression of *BRAF* and associated gene mutations. Mutation frequency and type for each case were compiled into tabular datasets using the “tibble” R package (v4.3.0). Each dataset included *BRAF* and patient-specific genetic alterations.

### **Protein-protein STRING interaction analysis**

To explore the functional interactions among mutated proteins, the STRING database (v12.0) was used. The mutations were mapped to STRING identifiers using the “STRING db” R package. High-confidence interactions (score threshold  $\geq 400$ ) were visualized using the “igraph” package. Interaction networks were simplified by removing loops and multiple edges, and node labels were assigned based on common gene symbols.

### **GO enrichment**

The GO enrichment analysis was conducted using the “clusterProfiler” R package. Functional enrichment for biological processes, molecular functions, and cellular components was performed using enrichGO. The Benjamini-Hochberg method was applied for P value adjustment, with a q-value cutoff of 0.05. Results were visualized through dot plots.

### **Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis**

Pathway enrichment was performed using the KEGG via enrich KEGG. Significant pathways (q-value  $< 0.05$ ) were plotted using the dot plot function from the “enrichplot” R package. Visualization parameters were adjusted to emphasize clinically relevant pathways and interactions.

### **Sociodemographic background**

The background information is distributed in 84 total data

fields with the last follow-up on April 1<sup>st</sup>, 2024.

### **Cox proportional hazard model-covariate analysis**

This analysis evaluates the survival outcomes of a group of *BRAF* mutated CRC patients using a Cox proportional hazards model. The model incorporates the following covariates: gender (sex) and pathological staging (stage 2). The results are based on a sample size of n=18, with 7 out of 9 unique event times (deaths) included in the analysis. The Aalen additive regression was used with test weights.

### **Statistical analysis**

Analyses were performed using SigmaPlot v.16, Survival curves were created using R software version 4.4.2 (<https://www.r-project.org/>).

### **Ethical statement**

This study was conducted in full accordance with the ethical principles outlined in the Declaration of Helsinki and its subsequent amendments. The Ethics Committee of Mayor University granted IRB waiver to this study in accordance with its guidelines (No. 0391). All participating institutions were also informed and agreed the study. Individual consent for this retrospective analysis was waived.

## **Results**

A total of 23 patients were enrolled in the study, and their demographic and clinical characteristics are summarized in *Table 1*. In the validation cohort, 13 patients (56.5%) were male, and 10 (43.5%) were female, with a mean age at diagnosis of 66.3 years. Additional characteristics of this cohort are presented in the supplementary tables, including familial history of cancer (*Table S1*), diagnostic characteristics of colon cancer (*Table S2*), symptoms at diagnosis (*Table S3*), and mutational status (*Table S4*). Cancer staging revealed that most patients were classified as stage IV (n=16, 69.6%) (*Table S5*). Regarding systemic treatments, 19 patients received some form of intervention, while 5 did not undergo any treatment (*Table S6*). The genes of interest were successfully amplified and analyzed in all 23 samples. The *BRAF* V600E mutation was detected in 22 of the cases, while one sample harbored a mutation in codons 466–469. *KRAS* mutations were observed in two samples, and an *NRAS* mutation was identified in one sample.

### Metabolic pathway profiling in Chilean patients harboring BRAF mutations

The next step in our analysis was to select four cases with varying numbers of mutated genes to explore their relationship with *BRAF* alterations. The first case analyzed involved only two mutated genes: *BRAF* and *NRAS*. Genomic analysis of a subset of patients with CRC harboring *BRAF* mutations, occasionally accompanied by *NRAS* alterations, revealed potential functional interactions

**Table 1** Sociodemographic characteristics of the studied cohort

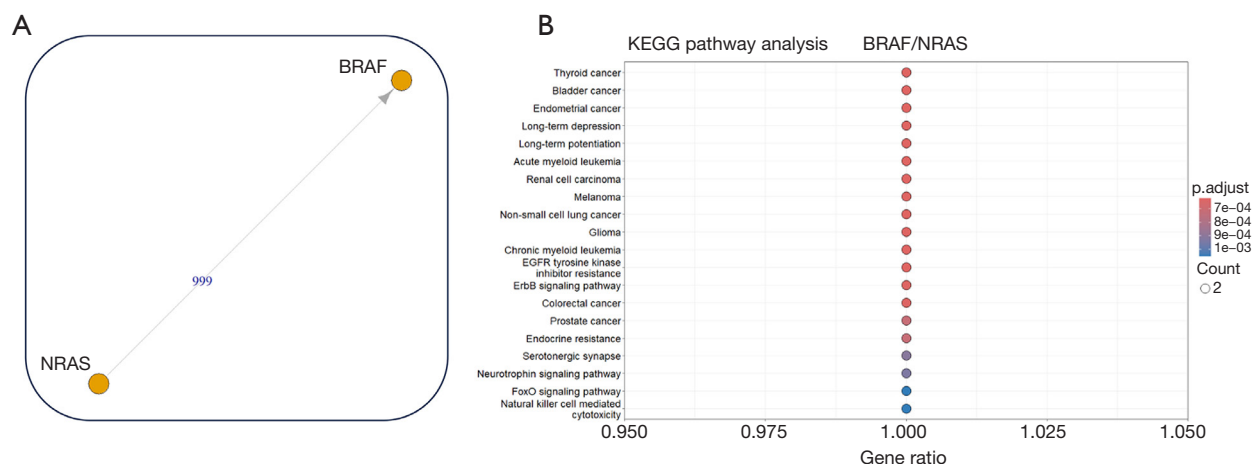
| Characteristic           | Value      |
|--------------------------|------------|
| Gender                   |            |
| Male                     | 13 (56.5)  |
| Female                   | 10 (43.5)  |
| Age at diagnosis (years) | 66.3±12.37 |
| Health insurance         |            |
| Public                   | 7 (30.4)   |
| Private                  | 14 (60.9)  |
| Not reported             | 2 (8.7)    |
| Ethnicity                |            |
| Hispanic                 | 23 (100.0) |

Data are presented as n (%) or mean ± standard deviation.

(Figure 1A). These findings suggest the involvement of other *RAF* isoforms, such as *A-RAF* and *C-RAF*, which, like *BRAF*, are integral components of the MAPK signaling pathway (13). These isoforms may contribute to resistance mechanisms by compensating for the reduced *BRAF* activity, thereby diminishing the efficacy of *BRAF*. Similar mutational patterns and signaling compensations have been observed in other cancer types, as highlighted by the KEGG pathway analysis (Figure 1B).

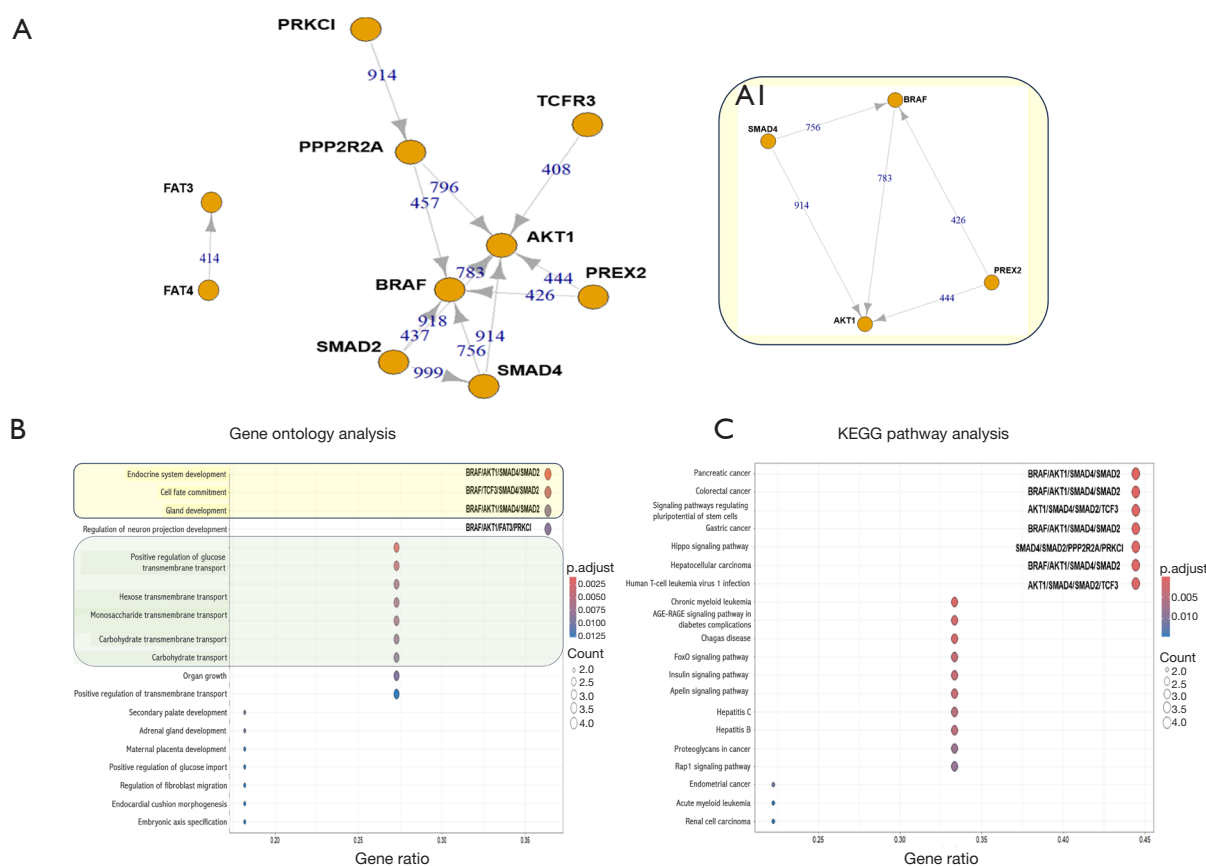
In another case selected, genomic analysis revealed concurrent mutations in eight genes, *BRAF*, *AKT1*, *SMAD4*, and *PREX2*. STRING analysis of protein-protein interactions among these genes (Figure 2A) demonstrated a high degree of interconnectivity, particularly among those classified as pathogenic, most notably *PREX2* has been associated with several malignancies, including prostate and gastric cancers, and is involved in key signaling pathways, such as PIP3 activation of AKT signaling and Signaling by Rho GTPases. Notably, *PREX2* expression is upregulated in antral gastric biopsy samples from patients with gastritis and *Helicobacter pylori*-associated gastric cancer (14).

GO annotations link *PREX2* to GTPase activator activity and guanine nucleotide exchange factor activity. It plays a regulatory role in the insulin signaling pathways, and both its mutation and overexpression have been implicated in tumorigenesis (Figure 2, A1, yellow rectangle). GO analysis of the pathogenic genes identified in this case revealed their involvement in critical biological processes including



**Figure 1** STRING network analysis and functional association between *BRAF* and *NRAS* in colorectal cancer patient. (A) STRING analysis reveals a direct interaction between *BRAF* and *NRAS*, consistent with their classification within the same kinase family. (B) KEGG pathway analysis indicates that mutations in these genes are also implicated in various other cancer types. KEGG, Kyoto Encyclopedia of Genes and Genomes.





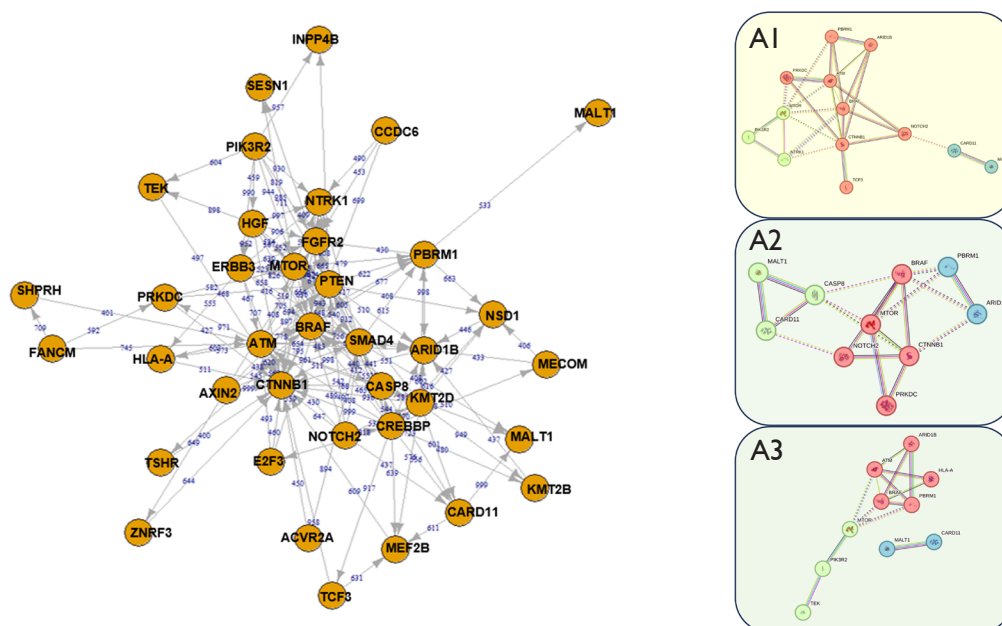
**Figure 2** STRING network analysis and functional associations between BRAF, pathogenic variants, and variants of VUS in colorectal cancer patients. (A) STRING analysis reveals direct interactions between BRAF and several related proteins. Four genes identified through genome-wide analysis exhibited strong associations (A1, highlighted by yellow rectangles). (B) GO analysis indicates that these genes are involved in endocrine system development (light yellow rectangle) and carbohydrate transport (light green rectangle), aligning with their roles within kinase signaling pathways. (C) KEGG pathway analysis demonstrated that mutations in these genes are implicated in multiple cancer types. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; VUS, variants of uncertain significance.

endocrine and glandular system development, carbohydrate transport, and cell fate commitment (*Figure 2B*, yellow rectangle). A second cluster of genes was associated with the positive regulation of glucose and general carbohydrate transport (*Figure 2B*, light green rectangle). Complementary KEGG pathway analysis further supported the association of these mutated genes with various digestive system cancers (*Figure 2C*).

In a third case analyzed, the mutational profile is much more complex, since 47 genes were identified associated with *BRAF* that are involved in various signaling pathways. STRING network analysis revealed significant functional interconnectivity between these genes (*Figure 3A*). A more comprehensive examination uncovered several genes

involved in Variable (V), Diversity (D), and Joining (J) [V(D)J] recombination, which is a crucial mechanism in B and T cell development that generates the diversity of antigen receptors (AgRs) essential for immune recognition (*Figure 3*, A1, yellow rectangle; red nodes), a finding supported by GO analysis. Another gene cluster corresponds to components of the CBM complex (CARD11-BCL10-MALT1), a pivotal regulation of NF- $\kappa$ B activation (*Figure 3*, A1, yellow rectangle, sky blue node). This complex is vital for AgR signaling in lymphocytes, thereby influencing adaptive immunity and inflammatory responses and genes associated with T and B cell activation and differentiation are shown (*Figure 3*, A2, light green rectangle, red nodes). A third group of genes was involved in DNA

A



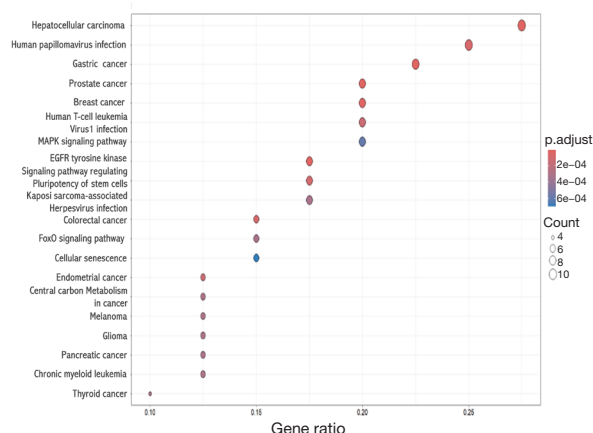
B

Gene ontology analysis



C

KEGG pathway analysis

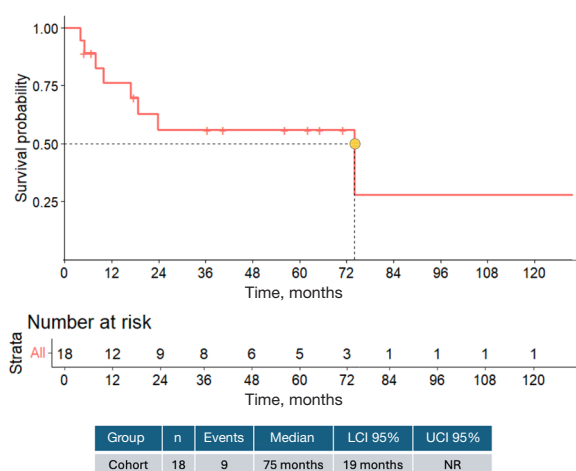


**Figure 3** Mutational landscape and functional network of BRAF and associated proteins. (A) STRING network analysis showing interactions between mutated proteins, BRAF, and co-mutated genes identified in CRC cases. Genes involved in lymphocyte differentiation are highlighted (A1, yellow rectangle, red nodes). Genes associated with T and B cell activation and differentiation are shown (A2, light green rectangle, red nodes). Genes implicated in Tie2 signaling (A3, light green rectangle; blue light node). (B) GO analysis illustrating the biological processes and signaling pathways associated with the identified gene set. (C) KEGG pathway analysis revealed the involvement of these genes in multiple cancer types. CRC, colorectal cancer; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

excision repair mechanisms (Figure 3, A3, light green rectangle; red nodes), along with genes associated with the Tek (Tie2 signaling pathway) (Figure 3, A3, light green rectangle; light green nodes). Tie2 signaling, mediated by the receptor tyrosine kinase Tie2 and its ligands, Ang1 and Ang2, plays a central role in vascular development, maintenance, and remodeling. While Ang1 promotes vessel

stabilization, Ang2 acts antagonistically, facilitating vascular destabilization and angiogenesis. The same CBM complex node was identified within this subgroup (Figure 3, A3, light green rectangle; sky blue nodes).

Alterations were observed in the *PBRM1* and AT-rich interaction domain 1B (*ARID1B*) loci in all identified subgroups. *PBRM1* encodes a subunit of ATP-dependent



**Figure 4** OS of Chilean patients stratified by BRAF mutation status. Kaplan-Meier survival curves were used to estimate OS in patients with BRAF-mutated tumors (n=18). The median follow-up period was 75 months (orange circles). LCI, lower confidence interval; NR, not reached; OS, overall survival; UCI, upper confidence interval.

chromatin remodeling complexes and is an integral component required for ligand-dependent transcriptional activation by nuclear hormone receptors. *ARID1B* encodes a component of the switch/sucrose non-fermenting (SWI/SNF) chromatin remodeling complex, which is implicated in the regulation of cell cycle progression.

GO analysis confirmed the involvement of these genes in these biological processes, such as lymphocyte differentiation (Figure 3B, light green rectangle). Further emphasized their role in the epithelial-mesenchymal transition (EMT), a critical process in cancer metastasis (Figure 3B, beige rectangle). KEGG pathway enrichment analysis demonstrated that these genes and their associated signaling pathways are implicated in the pathogenesis of multiple cancer types (Figure 3C), reinforcing their potential role in CRC progression and therapeutic resistance.

### **Survival analysis in Chilean patients with CRC harboring BRAF mutations**

#### **Overall patient survival**

The first survival curve estimated the OS of the entire cohort without stratification by covariates. The analysis was performed using the Kaplan-Meier method, which appropriately accounts for censored patients who had not experienced the event (death)

by the end of the study period. In total, 18 patients were included, with nine observed events (deaths). The median OS was 75 months, with a 95% confidence interval (CI) ranging from 19 months to not reached (NR).

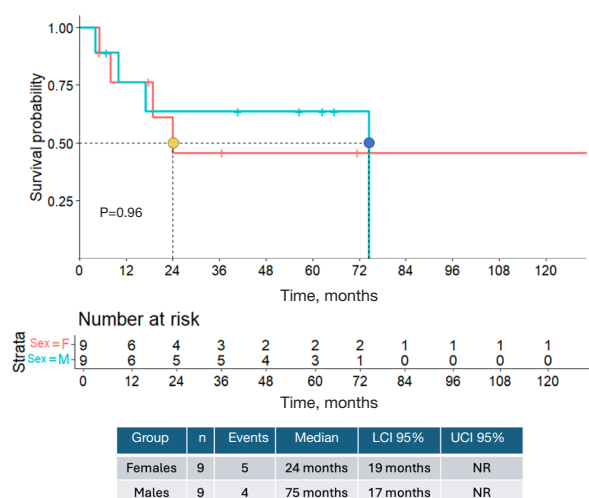
From a statistical perspective, the estimated median OS of 75 months indicates that half of the patients are expected to survive longer than this time, while the other half are expected to survive for a shorter period. The lower bound of the CI (19 months) reflects the uncertainty in the estimate, whereas the NR upper bound is common in survival analyses with small sample sizes or substantial censoring, where the survival function cannot be reliably estimated beyond a certain point. Censoring played an important role in shaping the curve, as some patients had not experienced the event or were lost to follow-up at the study cut-off. The Kaplan-Meier method accounts for these censored observations, ensuring an unbiased estimation of the survival probabilities (Figure 4).

#### **Stratified by gender**

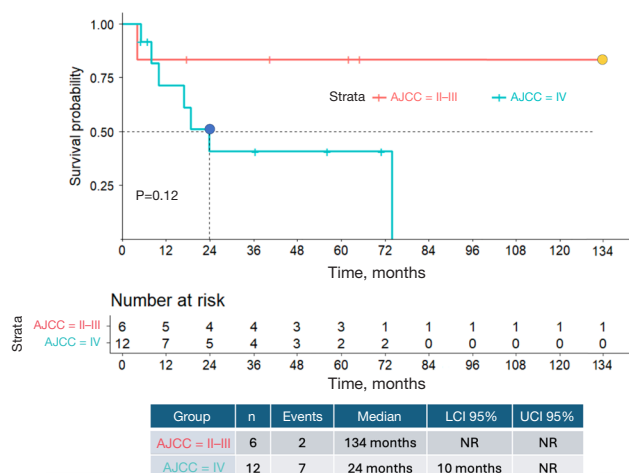
The second survival curve stratified the cohort by sex to explore potential differences in survival outcomes between male and female patients. This stratification provides a more nuanced assessment of how sex influences OS. Among female patients (n=9), there were five events, with a median OS of 24 months (95% CI: 19–NR). In male patients (n=9), four events were observed, with a median OS of 75 months (95% CI: 17–NR). From a statistical perspective, the median OS of 24 months for female patients was notably shorter than the 75 months observed in males, suggesting a possible sex-based survival difference in this cohort. Although this finding is clinically relevant, statistical confirmation would require a formal test, such as the log-rank test. The confidence intervals highlight the uncertainty in these estimates: for females, the lower bound (19 months) is defined, but the upper bound is NR, while for males, the lower bound (17 months) reflects similar uncertainty despite a higher median OS.

The relatively small sample size for each group (n=9) limits the precision of these estimates, as evidenced by the wide confidence intervals and the inability to calculate upper bounds. Furthermore, while sex may play a role, other factors, such as treatment regimens, comorbidities, or genetic differences, could also contribute to survival outcomes. These potential confounders should ideally be addressed in multivariate analyses to strengthen the validity of the findings (Figure 5).





**Figure 5** OS of Chileans patients stratified by BRAF mutation status and gender. Kaplan-Meier survival curves were used to assess OS in patients with BRAF-mutated tumors, stratified by gender: female (n=9) and male (n=9). The median follow-up period was 24 months for females (orange circle) and 75 months for males (blue circle). Line orange: female; line sky blue: male. F, female; LCI, lower confidence interval; M, male; NR, not reached; OS, overall survival; UCI, upper confidence interval.



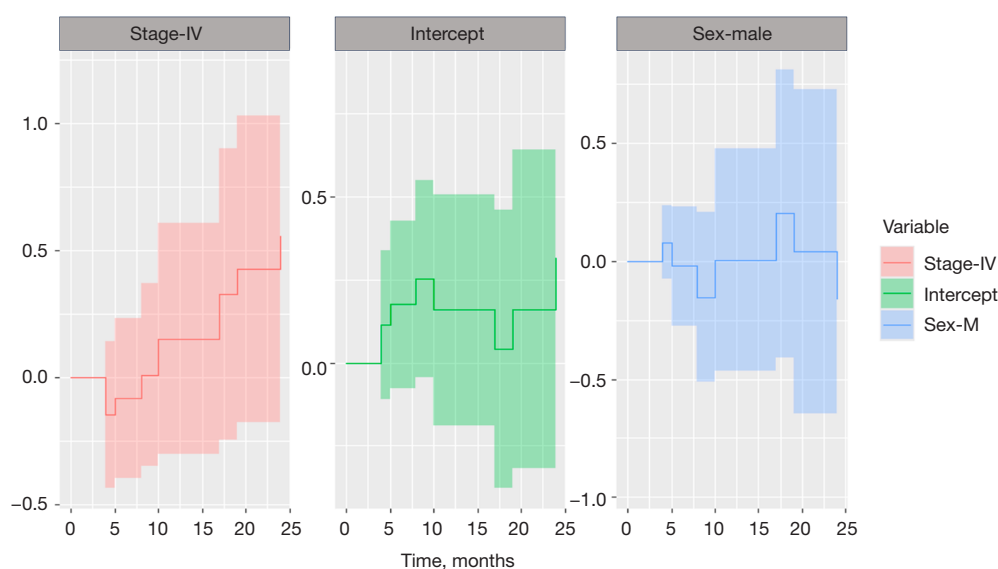
**Figure 6** OS of Chileans patients stratified by BRAF mutation and disease stage. Kaplan-Meier curves were utilized to assess OS in patients with BRAF-mutated tumors, categorized as localized disease (n=6) and metastatic disease (n=12). The median follow-up period was 24 months for metastatic disease (blue circle) and 134 months (orange circle) for localized disease. AJCC II-III: line orange; AJCC IV: line sky blue. AJCC, American Joint Committee on Cancer; LCI, lower confidence interval; NR, not reached; OS, overall survival; UCI, upper confidence interval.

### Stratified by stage at diagnosis

The third survival curve evaluated the OS stratified by the cancer stage at diagnosis. Patients were grouped into two categories: American Joint Committee on Cancer (AJCC) stages II–III (combined) and AJCC stage IV, to assess the impact of disease progression on survival outcomes. Among patients with AJCC stage II–III disease (n=6), there were two events, with a median OS of 134 months (95% CI: NR). In contrast, patients with AJCC stage IV disease (n=12) experienced seven events, with a median OS of 24 months (95% CI: 10–NR). From a statistical perspective, the median OS of 134 months in patients with stage II–III disease was substantially longer than the 24 months observed in patients with stage IV disease, underscoring the strong prognostic influence of disease stage. However, the 95% CI for stage II–III patients was undefined (NR–NR), reflecting the small sample size (n=6) and limited number of events (2 deaths), which introduced considerable uncertainty into the estimate. In contrast, the stage IV group, with a larger sample size and more events, provides a more reliable estimate of survival (median OS 24 months), although the wide 95% CI (10–NR) indicates variability in outcomes among advanced-stage patients. The absence of an upper confidence bound further suggests that the survival data are not mature enough to define long-term survival expectations. Censoring also influenced both groups, particularly stage II–III, where the low number of events resulted in wide confidence intervals. This highlights the need for larger cohorts to improve the precision of survival estimates in this population. Overall, the Kaplan-Meier analyses in this study demonstrated the profound effects of sex and disease stage at diagnosis on patient survival (Figure 6).

### Key findings included

- ❖ Overall patient survival: the median survival time for the entire cohort was 75 months. However, the wide range of survival times and absence of an upper confidence limit underscore the limitations posed by small sample sizes and censored data.
- ❖ Sex-based differences: male patients exhibited a notably longer median survival of 75 months compared to 24 months for female patients. While this suggests potential sex-based differences, statistical validation using tests such as the log-rank test is necessary to determine significance.
- ❖ Stage at diagnosis: patients with stage AJCC (stages II–III) demonstrated a median survival of



| Statistic | Slope   | Coef    | SE (coef) | Z value | P value |
|-----------|---------|---------|-----------|---------|---------|
| Intercept | 0.01440 | 0.03610 | 0.0391    | 0.923   | 0.356   |
| Sex-male  | 0.00149 | 0.00865 | 0.0526    | 0.164   | 0.869   |
| Stage-IV  | 0.02020 | 0.06590 | 0.0489    | 1.350   | 0.178   |

- n=18; 7 out of 9 unique event time used
- Chis q=1.82 on 2 df, P=0.402
- Test weight = Aalen

**Figure 7** Cox proportional hazards model covariate analysis. This analysis assessed survival outcomes in a cohort of colorectal cancer patients harboring BRAF mutations using a Cox proportional hazards model. Sex and pathological stage (stage II) were included as covariates. The model was applied to a sample of 18 patients, incorporating 7 of the 9 distinct event times (deaths). Additionally, the Aalen additive regression model was employed using test-based weights to further evaluate covariate effects over time. M, male; SE, standard error.

134 months, whereas those with advanced disease, stage AJCC (stage IV) exhibited a median survival of 24 months. This contrast emphasizes the critical role of early detection and timely intervention in improving survival outcomes. The limitations of this study include the small sample size, which limits the precision of these estimates, resulting in wide confidence intervals and undefined upper bounds in some cases. Larger datasets are required to validate these observations and provide more robust survival predictions.

- ❖ Future directions: further analyses incorporating additional factors such as treatment modalities and genetic variations are essential to elucidate the independent effects of sex and stage on survival outcomes.

### ***Survival analysis using the cox proportional hazards model in BRAF-mutated CRC Chileans patients***

This analysis assessed the survival outcomes of a cohort of CRC patients with *BRAF* mutations using a Cox proportional hazards model. The results for the covariates and model fit are summarized below. The baseline hazard showed no significant effect on survival, indicating that the hazard rate of the reference group did not differ significantly, over time. This suggests that variability in survival may be better explained by specific covariates or external factors rather than by the baseline hazard itself. Baseline hazard: intercept-slope =0.01440; coefficient =0.03610; standard error (SE) =0.0391; Z=0.923; P=0.36 (Figure 7).

### **Gender: reference level**

No significant association was observed between sex and

survival outcomes in this cohort of patients. This indicates that sex did not exert a primary influence on survival and that other variables, such as treatment modalities or genetic alterations, may have had a greater impact. These findings are consistent with prior studies, suggesting that sex-related survival differences are often more apparent in specific cancer types or larger patient populations but were not detectable in this smaller sample. Sex (male *vs.* female): coefficient =0.00865; slope =0.00149; SE =0.0526; Z=0.164; P=0.87.

#### Disease stage: reference level

Although patients with AJCC stage IV disease demonstrated a trend toward poorer survival than those with AJCC stage II/III disease, this difference did not reach statistical significance. This observation is consistent with the unfavorable prognosis associated with advanced-stage cancers. However, the limited sample size may have reduced the statistical power, making it difficult to detect significant differences, even if they were present. Stage (AJCC IV *vs.* II/III): coefficient =0.06590; slope =0.02020; SE =0.0489; Z=1.350; P=0.18.

#### Model fit: Chi-squared test

The overall model did not significantly explain the variability in the survival outcomes. The included covariates-sex and disease stage-did not fully capture the factors influencing survival in this cohort 1.82 on 2 degrees of freedom (df); P value =0.40 (*Figure 7*).

## Discussion

The *BRAF* V600E mutation represents a distinct molecular subtype of CRC, characterized by unique clinicopathological features and a notably poor prognosis. This underscores the urgent need for deeper clinical and biological insights to inform therapeutic advancements and identify novel response biomarkers (15). A key opportunity lies in the identification, validation, and clinical application of biomarkers that are capable of accurately predicting disease progression, recurrence risk, and survival outcomes. Such biomarkers could enhance prognostic precision and support the development of individualized treatment strategies, ultimately improving the quality of care for CRC patients (16).

Although the cohort analyzed in this study was relatively small and limited to patients harboring *BRAF* mutations, our registry provides a detailed account of the demographic

and clinical characteristics, adding a valuable context to the data in CRC Chileans population. Advances in our understanding of the molecular biology underlying *BRAF*-mutated CRC have led to the identification of several promising biomarker pathways, including MAPK/ERK, PI3K/AKT/mTOR, *TP53*, DNA damage response mechanisms, and the WNT/ $\beta$ -catenin signaling axis. These insights have paved the way for the development of innovative therapeutic strategies tailored to this aggressive CRC subtype (17). Advancements in molecular profiling of metastatic CRC have significantly enhanced the ability to tailor treatments based on tumor-specific biological characteristics, allowing for more personalized care. Although curative outcomes remain rare in metastatic disease, personalized approaches have led to improved survival rates in selected patients. Genomic profiling plays a crucial role in this process by enabling precise treatment selection, ensuring that more patients receive effective therapies, while avoiding unnecessary toxicity from less suitable interventions. These therapeutic strategies aim to enhance antitumor activity and sustain clinical efficacy by combining *BRAF* inhibitors with immune checkpoint inhibitors, anti-VEGF agents, and COX inhibitors (18). Such combination approaches represent a significant advancement in the management of *BRAF*-mutated CRC and offer promising avenues to improve patient outcomes. However, in this complex therapeutic landscape, it is essential to account for multifactorial resistance mechanisms that continue to challenge the treatment efficacy in CRC (19).

Several critical factors must be considered when treating CRC patients, including overall health status, tumor biology and aggressiveness, tumor microenvironment and epithelial mesenchymal transition as targets to overcome tumor multidrug resistance, potential adverse effects of chemotherapy regimens, tumor laterality (left or right-sided), primary tumor location, comorbidities, and the mutational status of key genes, such as *RAS* and *NRAS* (20-22).

Certain antineoplastic agents approved by the World Health Organization (WHO), including encorafenib, 5-fluorouracil, irinotecan, oxaliplatin, trifluridine-tipiracil, and capecitabine, exert antitumor effects by targeting the signaling pathways involved in tumor progression (23-27). Mutations in *RAS* and *BRAF* genes can activate or deregulate cell signaling pathways associated with proliferation and differentiation, contributing to cancer cell resistance to EGFR inhibitors (28-30).

Our genomic analyses further revealed considerable heterogeneity in *BRAF*-associated mutations, with several

notable associations. Among the genes identified, *PREX2* has emerged as particularly relevant and has been implicated in multiple malignancies, including gastric cancer and gastritis in patients with *H. pylori* infection (14). A strong association was also observed with genes involved in carbohydrate transport pathways, highlighting the potential metabolic implications of *BRAF*-mutated tumors and their metabolic signature, which may differ from wild-type tumors.

In one of the analyzed cases, the Tie2 signaling pathway was identified. This gene encodes a receptor that is part of the tyrosine protein kinase (*TEK/Tie2*) family and is predominantly expressed in vascular endothelial cells. Tie2 is crucial for vascular development, angiogenesis, and maintenance of vascular homeostasis, indicating a potential association between tumor progression and angiogenic signaling in this context. Understanding mutations affecting this pathway is vital for optimizing therapeutic strategies, particularly in tumors, where angiogenesis significantly contributes to disease progression. Angiopoietin (Ang), a ligand specific to the Tie2 receptor system, has been linked to tumor growth and progression in patients with incurable stage IV CRC who have undergone primary tumor resection, underscoring the importance of angiogenic signaling in advanced disease (31).

Our survival analysis, using a Cox proportional hazards model, did not find significant associations between sex or stage II disease and OS. Furthermore, the model did not significantly account for variability in survival ("P=0.402"), highlighting the important limitations of the current analysis. To address these constraints, future studies should consider the following improvements—larger sample sizes: expanding the cohort would enhance statistical power and improve the ability to detect meaningful associations. Expanded covariates: incorporating additional variables, such as genetic biomarkers, treatment regimens, performance status, and comorbidities, would allow for a more comprehensive evaluation of survival determinants. These enhancements would support a more nuanced understanding of the multifactorial determinants influencing outcomes in patients with *BRAF*-mutated CRC, thereby facilitating the development of truly personalized treatment strategies and enhancing the precision of clinical decision-making. In conclusion, our study adds to the growing body of evidence advocating for a paradigm shift toward precision oncology in *BRAF*-mutated CRC. By leveraging advanced molecular profiling tools, incorporating diverse clinical and genomic variables, and adopting an integrative approach

to tumor biology, we can advance toward more effective, durable, and patient-centered therapeutic strategies that respond to the complexity and heterogeneity of this aggressive cancer subtype.

## Conclusions

We report 23 Chilean patients diagnosed with CRC, whose tumors samples were primarily analyzed for *BRAF* and *NRAS* mutations using validated qPCR-based detection kits. Four cases underwent NGS with an extended cancer gene panel (BGI-Sentis™). Identified variants were evaluated using STRING protein-protein interaction analysis and GO enrichment. A Cox proportional hazards model was used to assess the survival outcomes within the *BRAF*-mutated subgroup.

Our findings revealed a strong association between *BRAF* mutations and genes involved in carbohydrate transport pathways, suggesting a distinct metabolic profile in these tumors. In one case, alterations in the Tie2 signaling pathway, a key regulator of angiogenesis and vascular homeostasis, were observed, indicating a potential link between *BRAF*-mutated CRC and pro-angiogenic signaling. Additionally, all analyzed subgroups exhibited alterations in *PBRM1* and *ARID1B*, which are involved in chromatin remodeling and cell cycle regulation. This study contributes to the characterization of the molecular landscape of CRC in the Chilean population, highlighting the key pathways that may influence tumor progression and prognosis.

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