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Mutaciones del gen KRAS como Factor Predictivo en el Cáncer Colorrectal: Una Revisión Sistemática

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Agradecer en primer lugar a mi tutora Alba Coret por guiarme, aconsejarme y apoyarme durante todo el proceso.

Agradecer especialmente a mis padres, mi hermano y mi abuela por ser mi pilar fundamental. Agradecer al resto de mi familia, a mis amigas de Meliana, a mi familia del agility y a mis amigas de Castellón, sois refugio.

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RESUMEN

INTRODUCCIÓN: Las mutaciones de KRAS bloquean la acción de la GTPasa intrínseca, evitando la degradación de la unión RAS+GTP manteniendola activa, produciendo resistencia intrínseca del CCR a tratamientos con inhibidores de EGFR. El 97% son en el codón 12 o 13.

MÉTODO: El objetivo principal es revisar la relación de la mutación del gen KRAS y el pronóstico del CCR en estadíos iniciales.

METODOLOGÍA: Se realizó una búsqueda en las bases de datos PubMed, Scopus y Registro Cochrane de Ensayos Controlados hasta febrero de 2023 con las palabras clave: *colorectal cáncer, KRAS mutation y prognosis.* Tras excluir los artículos no publicados en los últimos 10 años, aquellos que leyendo el título/*abstrac*t no cumplían nuestro objetivo y los eliminados por criterios de exclusión se obtuvieron un total de 22 artículos. El riesgo de sesgo se analizó con la herramienta QUIPS.

RESULTADOS: La mutación KRAS fue detectada en más del 35% de los participantes en trece estudios, siendo en todos el codón 12 el más prevalente. En dicisiete artículos se asociaron las mutaciones de KRAS con peor pronóstico, menor SLR (cuatro estudios), SLE (cuatro estudios), SCE (cuatro estudios) y SG (cinco estudios).

CONCLUSIONES: En la mayoría de los artículos se ha relacionado la mutación de KRAS con una menor supervivencia pero la heterogeneidad entre ellos no permite extraer conclusiones sólidas. Se necesitan más estudios con mayor similitud entre pacientes y método de medición del pronóstico para comprobar el empeoramiento de la supervivencia que confieren las mutaciones de KRAS en estadíos iniciales

PALABRAS CLAVE: Mutación de KRAS, Cáncer colorectal, pronóstico, biomarcadores tumorales, estadíos iniciales, vía de señalización de EGFR.

ABSTRACT

INTRODUCTION: KRAS mutations block the action of intrinsic GTPase, preventing the degradation of the RAS+GTP binding in CRC, keeping it active, producing intrinsic resistance to treatments with EGFR inhibitors. 97% occur at codon 12 or 13.

METHOD: The main objective is to review the connection between the KRAS gene mutation and the prognosis of CRC in its initial stages.

METHODOLOGY: The PubMed, Scopus, and Cochrane Central Trials Register databases were searched up to February 2023 using the keywords: colorectal cancer, KRAS mutation, and progosis. After excluding articles not published in the last 10 years, those that did not meet our objective by reading the title/abstract, and those eliminated due to exclusion criteria, a total of 22 articles were obtained.The risk of bias was analyzed using the QUIPS tool.

RESULTS: The KRAS mutation was detected in more than 35% of the participants in thirteen studies, with codon 12 being the most prevalent in all of them. In seventeen articles, KRAS mutations were associated with a worse prognosis, lower RFS (four studies), SLE (four studies), SCE (four studies), and OS (five studies).

CONCLUSIONS: In most of the articles, the KRAS mutation has been associated with a lower survival, but the heterogeneity between them does not allow us to draw solid conclusions. Further studies with greater similarity between patients and prognosis measurement method are needed to verify the worsening of survival conferred by KRAS mutations in early stages

KEYWORDS: KRAS mutation, colorectal cancer, prognosis, tumor biomarkers, early stages, EGFR signaling pathway

EXTENDED SUMMARY

INTRODUCTION: The epidermal growth factor receptor (EGFR) proliferative signaling pathway originates two signaling cascades: RAS-BRAF-MAPK and Pl3K-AKT-PTEN-mTOR. The RAS family of genes are involved in the first of the cascades, and of these, the KRAS mutation is the most common, presenting in around 30-40% of CRC (6,8). KRAS mutations block the action of an intrinsic GTPase, preventing the RAS+GTP binding from being degraded, remaining constantly active, and therefore, maintaining the proliferative signal and promoting CRC development. By keeping the signal on EGFR active, KRAS mutations produce intrinsic resistance to treatment with anti-EGFR (cetuximab, panitumab) used in metastatic CRC. The most common mutations occur in exon 2, more specifically in codon 12 or 13 (8).

METHODS: The prognosis confered by KRAS mutations in metastatic CRC is widely studied because they produce resistance to anti-EGFR treatment. On the other hand, the studies that analyze the survival variations in patients with mutated KRAS in the initial stages are scarce and with contradictory results among them. Therefore, the main objective of this review is to establish a relationship between these mutations and the prognosis of CRC in non-metastatic stages. Likewise, as specific objectives, the aim is to review the prevalence of mutations and establish their most aggressive variants.

METHODOLOGY: The PubMed, Scopus, and Cochrane Central Trials Register databases were searched from December 2022 to February 2023 using the keywords: colorectal cancer, KRAS mutation, and prognosis. A total of 1772 articles were obtained, after excluding those not published in the last ten years, those that after reading the title and abstract did not meet our objective and those that were eliminated according to our exclusion criteria, we obtained a total of 22 articles to analyze. The studies included were those on patients with a pathological and genomic diagnosis of CRC, patients with a pathological diagnosis in a non-metastatic stage (stage I to III), and with ages included from 18 to 99 years, observational studies (cohorts, cases and controls) and

standard of care arms in clinical trials and studies establishing the prognostic relationship in terms of OS, DFS, CSS, and RFS. On the other hand, studies that do not include genetic data, carried out in non-human populations or in patients of pediatric age, published in non-investigated journals, editorials and letters to the editors, opinion articles and articles without original data, studies that do not specify the prevalence of the mutation in the sample, which did not specify the median follow-up time or with a median follow-up time of less than 36 months were excluded. The risk of bias was analyzed using the QUIPS tool.

RESULTS: KRAS mutations have been associated with decreased CSS, RFS, and DFS in four articles each one. Regarding DFS, there are certain nuances, since in one of the articles it was only associated in patients with left CRC and in another, only in those who did not receive adjuvant chemotherapy. In addition, in three other studies, KRAS mutations were not associated with decreased DFS, and only in one of the studies, having a KRAS mutation was not associated with decreased RFS. In contrast, OS was lower in patients with KRAS mutations only in five of the fifteen studies that analyzed it, and three studies have also been associated with lower CSS and RFS but not lower OS.

In studies that exclude BRAF mutations because they are an independent prognostic factor, KRAS mutations have been associated with lower DFS, RFS, CSS, and OS. Likewise, in seven studies patients with MSI were excluded for the same reason, in three of them there were differences in survival between patients with KRAS mutated and MSI and those with KRAS mutated but MSE, survival being lower in the first group.

The prevalence of the mutation is greater than 35% in thirteen studies, in all of them codon 12 is the most prevalent. In four studies that analyze the differences in pronostic according to the different mutations. In one of them, lower DFS was associated in patients with codon 12 mutations and in another, lower OS in patients with codon 13 mutations. None of the codon 12 mutations (G12V and G12C and G12D) was associated with worse survival compared to the rest.

DISCUSSION: In seventeen of the twenty-two studies analyzed, the KRAS mutation has been associated with worse survival, but there are differences regarding the way of analyzing the prognosis (DFS, CSS, SG or RFS), the number of patients included, the tumor stage, the sociodemographic characteristics of the patients, and the type of therapy received among the different articles. In addition, not all take into account the coexistence of BRAF or MSI mutations that confer a poor prognosis by themselves. The heterogeneity between studies and the discrepancy between survival results is also collected in other studies such as that of *Amanda K. Arrington et al*. The prevalence of the mutation in our studies is consistent with that observed in other publications, such as that *of Amanda K. Arrington et al* (30-50% prevalence) and that of *Li et al*. (around 50% prevailed), codon 12 being the most common in all of them.

CONCLUSIONS: In various articles, KRAS gene mutations have been associated with a worse prognosis in patients with non-metastatic CRC, but more studies with more homogeneous populations are needed to confirm these results. The prevalence is around 30-40%, coinciding with other published articles. The results in terms of determining the most aggressive variants have been very discrepant among them, so it has not been possible to draw solid conclusions.

ÍNDICE GENERAL

ÍNDICE DE ABREVIATURAS

- **CCR:** Cáncer colorectal
- **CCRm:** Cáncer colorectal metastásico
- **EFGR:** Receptor del factor de crecimiento epidérmico
- **MSE:** Estabilidad de los microsatélites
- **MSI:** Inestabilidad de los microsatélites
- **SG:** Supervivencia global
- **SLE:** Supervivencia libre de enfermedad
- **SLR:** Supervivencia libre de recurrencia
- **SCE:** Supervivencia cáncer específica

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1. INTRODUCCIÓN

1.1 Incidencia del Cáncer colorrectal

El cáncer colorrectal (CCR) es la tercera causa de cáncer más común en hombre y la segunda en mujeres (1), con una incidencia estimada en 2022 en España de 43.370 nuevos casos (28.706 de colon y 14.664 de recto) (2). El diagnóstico y la resección temprana de los pólipos adenomatosos precancerosos ha permitido reducir la incidencia de CCR en los mayores de 50 años (1).

Esta disminución de la incidencia no se ha visto reflejada en un descenso de la incidencia global, se postula que puede ser debido a un aumento de los nuevos casos de CCR en pacientes menores de 50 años (1). Los factores que se han visto asociados a un incremento de la incidencia de CCR están relacionados principalmente con el estilo de vida (dieta occidentalizada rica en grasas, inactividad física, obesidad, estrés, tabaco). De igual forma, las alteraciones de la microbiota por el uso de antibióticos parece jugar un papel importante en la patogenia del CCR (1). Cabe destacar que un 10-20% de los pacientes con CCR tienen antecedentes familiares y un 5% de ellos presentan mutaciones reconocidas de CCR hereditario (1).

1.2 Mutaciones genéticas

De forma general la formación de un tumor consiste en la acumulación de alteraciones en el genoma de las células que lo forman. Estas alteraciones pueden ser cambios en la propia secuencia del ADN o cambios en su expresión (alteraciones epigenéticas) que provocan la pérdida de genes con función reguladora negativa sobre el ciclo celular (genes supresores tumorales) o la sobreexpresión de genes que estimulan el crecimiento celular (oncogenes) (3) .De esta forma, el desarrollo del CCR consiste en una secuencia de cambios mutacionales sobre células previamente sanas que acaban desembocando en células neoplásicas con capacidad de replicación ilimitada.

La alta incidencia del CCR, así como los avances tecnológicos, han permitido el conocimiento de diversas rutas mutacionales implicadas en la patogenia del CCR. A continuación, se detallan más concretamente alguna de estas vías de

carcinogénesis, haciendo especial hincapié en la mutación de la familia de los genes RAS.

1.2.1 Inestabilidad cromosómica

La inestabilidad cromosómica juega un papel importante en la genética del CCR. Concretamente, la **inactivación del gen APC** está presente en 70%-85% de los casos de CCR (4,5,6). La inactivación del gen APC es uno de los pasos iniciales en la vía clásica de la carcinogénesis (involucrada en el paso de adenoma convencional a carcinoma) (7). Esta mutación también se encuentra en los casos de poliposis adenomatosa familiar (PAF) en forma de mutación germinal (mutación presente en los gametos de los progenitores que se incorpora al genoma de todas las células de la descendencia) (7).

La **pérdida de heterogeneidad del cromosoma 18q** también forma parte de las alteraciones genéticas involucradas en la inestabilidad cromosómica. El cromosoma 18q sufre la pérdida de expresión de copias de genes, principalmente SMAD4 y DCC, formando parte de los pasos iniciales de la vía clásica de la carcinogénesis (6).

1.2.2 Inestabilidad de los Microsatélites (MSI)

La inestabilidad de los microsatélites (MSI) se encuentra alterada principalmente en pacientes con CCR hereditario no polipósico o Síndrome de Lynch (6). Asimismo, esta mutación también se puede encontrar en casos de CCR esporádico (hasta el 10%) (6). Los microsatélites son zonas del genoma formadas por secuencias repetitivas que acumulan una gran tasa de mutaciones. La MSI hace referencia a la pérdida de los genes que reparan las mutaciones y el daño en el ADN, estos genes son: MLH1, MSH2, MSH6, PMS2 (4,6).

La inestabilidad de microsatélites ha sido validada como biomarcador de mal pronóstico mediante análisis multivariantes en diversos estudios (6)

1.2.3 Vías de señalización proliferativa EGFR

Existen vías de señalización proliferativa implicadas en el origen y desarrollo del cáncer colorrectal. Concretamente la estimulación al receptor del factor de crecimiento epidérmico (EFGR) origina dos cascadas de señalización: RAS-BRAF-MAPK Y Pl3K-AKT-PTEN-mTOR (1,4,6). Por tanto, en el CCR se producen mutaciones con ganancia de función en las vías de señalización del EFGR que hacen que se mantenga la proliferación y supervivencia de las células cancerosas.

- ✓ KRAS

Dentro de la vía de señalización RAS-BRAF-MAPK encontramos la familia de genes RAS, de los que se conocen tres miembros: H-RAS, N-RAS Y KRAS siendo este último el que con mayor frecuencia se encuentra mutado en el CCR (alrededor del 30-40%) (6,8).

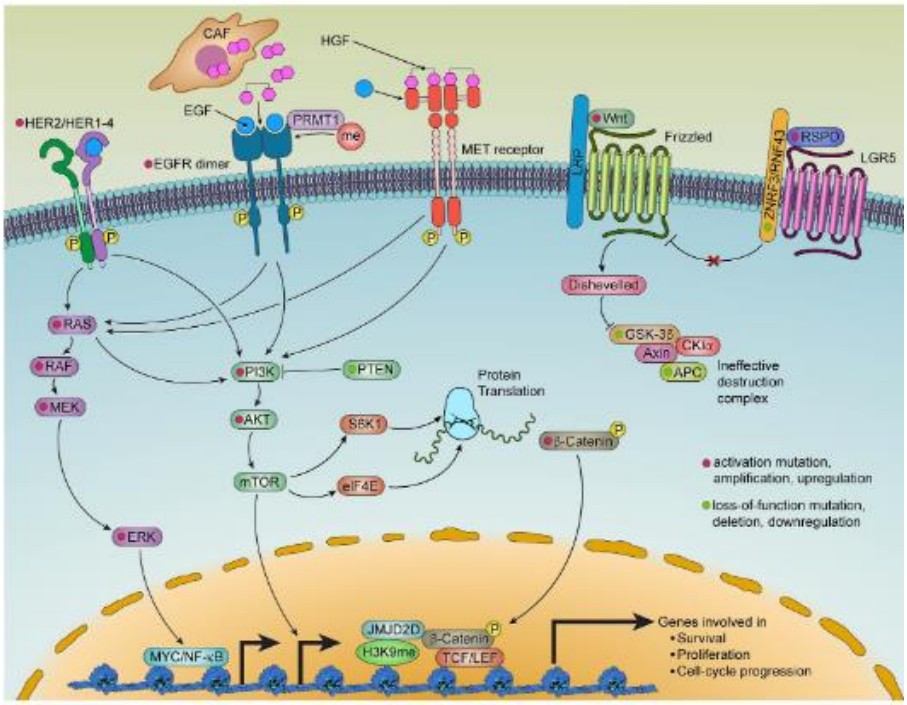

Figura 1: Vías de señalización proliferativa EGFR (1)

En condiciones normales, los genes RAS codifican una serie de proteínas con actividad GTPasa intrínseca que degradan la unión RAS+GTP inactivando la cascada proliferativa. Las mutaciones en KRAS bloquean la acción de la GTPasa, evitando así que se degrade la unión RAS+GTP permaneciendo activa constantemente y por consiguiente manteniendo la señal proliferativa (6,8).

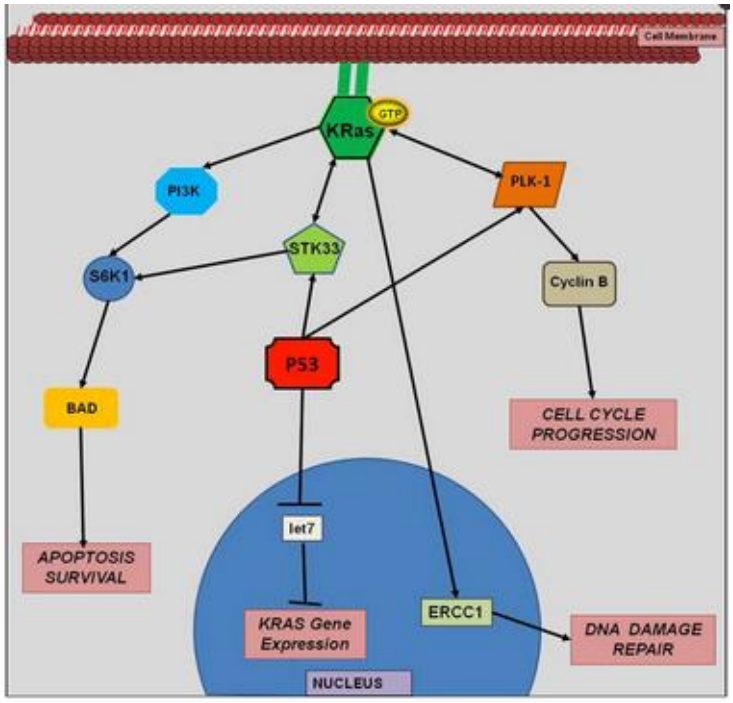

Figura 2: Activación constitucional de KRAS (8)

Debido a que las mutaciones en KRAS mantienen la señal proliferativa activa, en ausencia de señal por parte de EGFR hace que pacientes con KRAS mutado tengan una resistencia intrínseca a tratamientos con inhibidores de EGFR (cetuximab, panitumab) (4–6) tratándose del único biomarcador que se determina actualmente en la práctica clínica y de forma obligatoria antes del tratamiento con anti-EGFR en estadíos avanzados (6).

Las mutaciones más comunes se producen en el codón 12, 13, 59 o 61 (4,6,8), produciéndose cerca del 97% de ellas en los codones 12 o 13. Concretamente, las más comunes en el CCR corresponden a al cambio de la secuencia de nucleótidos GGT por GAT en el codón 12 (8).

- ✓ BRAF

Adicionalmente en la cascada RAS-BRAF-MAPK existen mutaciones en BRAF, el cual se trata de un efector intracelular de KRAS por lo que mutaciones activadoras sobre BRAF mantienen la señal proliferativa en ausencia de estímulo sobre EGFR confiriendo también resistencia al tratamiento con anti-EFGR(4–6). Se trata de una mutación excluyente sobre KRAS (solo puede existir una de las dos mutaciones). Las mutaciones en BRAF, especialmente la sustitución V600E se ha relacionado en diversos estudios con un peor pronóstico en el CCR (1, 6).

1.3 KRAS y CCR no metastático

La relación entre el CCR metastásico y la incidencia de KRAS mutado está ampliamente demostrada en la literatura. Actualmente, la terapia dirigida a estadíos avanzados de colon y recto se basa en el uso de anticuerpos monoclonales (panitumumab, cetuximab), por su capacidad de bloqueo de la activación del EGFR (4,6,8). Debido a la ausencia de respuesta de algunos pacientes a estas terapias, se evidenció que sus celulas tumorales poseían mutaciones activadoras del gen KRAS, responsable del mantenimiento de la la señal proliferativa activa (4,6,8) Actualmente, su uso es predominantemente clínco, pues puede predecir la resistencia de ciertos pacientes a estas terapias (6,8)

Algunos estudios han analizado que mutaciones concretas, como la G12V, se asocian a un peor pronóstico (8), pero a pesar de la importancia de este gen, en la práctica clínica su deteminación únicamente en pacientes con CCR metastásico, no existiendo actualmente amplios estudios que analicen la prevalencia global y su relación con el CCR no metastásico.

2. MÉTODO

2.1 Justificación del estudio

El CCR es el segundo cáncer más frecuente entre las mujeres y el tercero entre los hombres afectando actualmente a casi dos millones de personas a nivel mundial, siendo una causa importante de muerte por cáncer (2)

Los avances en cuanto al conocimiento de la genética y las alteraciones moleculares en la patogenia del CCR ha permitido identificar una serie de biomarcadores involucrados en el desarrollo y evolución del cáncer. Concretamente las mutaciones del gen KRAS están implicadas en alrededor del 30-40% de los CCR (1).

Diversos estudios evalúan el beneficio de asociar cetuximab o panitumab a los tratamientos quimioterápicos estándar del CCR metastásico (FOLFIRI, FOLFOX). En la mayoría de ellos se observa una mejoría de la supervivencia global, supervivencia libre de progresión y respuesta global (Tabla1*)* (6). En cambio, el pronóstico que confiere las mutaciones de KRAS en estadios iniciales no esta igualmente analizado, y en los pocos estudios que lo evlaúan los resultados son contradictorios. En algunos estudios el estado de KRAS no ha demostrado tener valor pronóstico en estadíos II y III (6), en cambio, en otros como en el de *Tanka et al*. o el llevado a cabo por el grupo colaborativo RASCAL sí que se informó la mutación de KRAS como un factor de riesgo independiente en un análisis multivariante (8).

Las mutaciones en el gen KRAS están implicadas en el paso de adenoma convencional a carcinoma, y los estudios realizados sobre el pornóstico que confieren en estadíos no metastásicos son escasos y con resultados contradictorios. Por este motivo, en el presente estudio se pretende realizar una revisión de la prevalencia de la mutación de KRAS en el CCR no metastásico y de su utilidad pronóstica, así como identificar sus variantes mas agresivas y revisar la prevalencia de la mutación en estadíos iniciales, ya que el conocimiento de su implicación pronóstica y terapéutica afectan a la forma en la que entendemos la enfermedad y los algoritmos mediante los que la tratamos.

	RG AC + QT vs. QT	SLP AC + QT vs. QT	SG AC + QT vs. QT
CRYSTAL[33]	57,3% vs. 39,7% HR: 2,069 p < 0,001	9,9 vs. 8,4 meses HR: 0,696 p = 0,0012	23,5 vs. 20,0 meses HR: 0,796; p = 0,0093
OPUS[34]	57,3% vs. 34,0% HR: 2,551 p = 0,0027	8,3 vs. 7,2 meses HR: 0,567 p = 0,0064	22,8 vs. 18,5 meses HR: 0,855 p = 0,39
COIN[37]	64% vs. 57% p = 0,049	8,6 vs. 8,6 meses HR: 0,96 p = 0,60	17,9 vs. 17,0 meses HR: 1,04 p = 0,67
PRIME[40]	57% vs. 48% HR: 1,47 p = 0,018	10,0 vs. 8,6 meses HR: 0,80 p = 0,009	23,9 vs. 19,7 meses HR: 0,88 p = 0,072

AC: anticuerpo; HR: razón de riesgo; QT: quimioterapia; RG: respuesta global; SG: supervivencia global; SLP: supervivencia libre de progresión.

Tabla 1. Impacto del tratamiento con anticuerpos anti-EGFR en pacientes con cáncer colorrectal metastásico y KRAS no mutado (6)

2.2 Objetivos

El objetivo principal de este trabajo es revisar la evidencia actual sobre la relación de la mutación del gen KRAS y el pronóstico del carcinoma colorrectal en estadíos no metastásicos.

Los objetivos específicos se detallan a continuación:

- Revisar la prevalencia de las mutaciones en el gen KRAS en el CCR.
- Establecer una relación entre estas mutaciones genéticas y el pronóstico del CCR.
- Determinar sus variantes más agresivas.

3. METODOLOGÍA

3.1 Criterios de selección de estudios

Para la selección de artículos en nuestro estudio de utilizaron los siguientes criterios:

3.1.1 Criterios de inclusión

- Estudios realizados sobre pacientes con diagnóstico anatomopatológico de cáncer colorectal en estadío no metastásico (estadío del I al III).
- Estudios realizados sobre pacientes con diagnóstico anatomopatológico y genómico de cáncer colorrectal.
- Estudios con pacientes entre los 18 y los 99 años.
- Estudios que relacionen las mutaciones del gen KRAS con la evolución pronóstica de los pacientes: diseños de estudios observacionales (cohortes, casos y controles) y brazos estándar de atención de ensayos clínicos.
- Estudios que establezcan la relación pronóstica en términos de: supervivencia global (SG) supervivencia libre de enfermedad (SLE), supervivencia cáncer específica (SCE) y supervivencia libre de recurrencia (SLR).
- Estudios realizados en los últimos diez años.
- Revisiones sistemáticas como base para la obtención de bibliografía.

3.1.2 Criterios de exclusión

- Artículos que no incluyan datos genéticos.
- Artículos en revistas no indexadas.
- Editoriales y cartas a los editores.
- Estudios en poblaciones no humanas.
- Estudios en edad pediátrica.
- Estudios en CCR hereditario.
- Estudios no publicados en inglés o español.
- Artículos de opinión y artículos sin datos originales.

- Estudios que no especifiquen la prevalencia de la mutación en la muestra.
- Estudios que no especifiquen el tiempo medio de seguimiento.
- Estudios con tiempo medio de seguimiento inferior a 36 meses.

3.2 Fuentes de información y estrategia de búsqueda

Este estudio se realizó de acuerdo con las pautas PRISMA. Se llevó a cabo una revisión sistemática de la literatura en PubMed, Scopus y el Registro Cochrane Central de Ensayos Controlados para los estudios publicados en inglés y español desde diciembre de 2022 hasta el 26 de febrero de 2023. La búsqueda incluía los siguientes términos: *KRAS mutation, colorectal cáncer y prognosis*, con expansión de términos utilizando el diccionario de sinónimos MeSH.

Concretamente, en la base de datos **Pubmed** se utilizaron los términos MeSH de *prognosis, c h ras gene y colorectal neoplasms* combinándolos mediante los operadores boleanos “AND” y “OR” para que se incluyeran tanto como término MeSH como en título o *abstract*. Además se incluyeron distintos sinónimos que hacen referencia al CCR (*colorectal cáncer, colorectal tumors y colorectal adenocarcinoma*) para incluir también los artículos que presentaran estos términos en el título o *abstract.* Con esta búsqueda se obtuvieron un total de **472 artículos**, después de aplicar el filtro de artículos publicados en los últimos diez años siguiendo nuestro criterio de inclusión, se obtuvieron un total de **153 artículos**

En la base de datos **Scopus** se utilizaron los términos: *prognosis, KRAS genes y colorectal neoplasms* y se combinaron con el operador boleano “AND” para que se buscaran en título, *abstract* y palabras clave. Con esta búsqueda se obtuvieron un total de **1256 artículos**, después de aplicar el filtro de artículos publicados en los últimos diez años siguiendo nuestro criterio de inclusión, se obtuvieron un total de **987 artículos**.

Por último en la base de datos **Registro Cochrane Central de Ensayos Controlados** se utilizaron los siguientes descriptores MeSH*: prognosis, genes ras y colorectal neoplasms* que se combinaron mediante los operadores boleanos “AND” y “OR” para que aparecieran tanto como descriptor MeSH como en título, *abstract* y palabras clave. Con esta búsqueda se obtuvieron un total de **44 artículos**, después de aplicar el filtro de artículos publicados en los últimos diez años siguiendo nuestro criterio de inclusión el número de artículos no se modificó.

Las distintas escrituras de búsqueda se muestran a continuación

PUBMED	((prognosis[MeSH Terms]) OR (prognosis[Title/Abstract])) AND ((c h ras gene[MeSH Terms]) OR (kras genes[Title/Abstract])) AND ((colorectal neoplasms[MeSH Terms]) OR (colorectal neoplasms[Title/Abstract]) OR (colorectal cancer[Title/Abstract]) OR (colorectal tumors[Title/Abstract]) OR (colorectal adenocarcinoma[Title/Abstract]))
SCOPUS	TITLE-ABS-KEY (prognosis) AND TITLE-ABS-KEY (kras genes) AND TITLE-ABS-KEY (colorectal neoplasms)
REGISTRO COCHRANE CENTRAL DE ENSAYOS CONTROLADOS	#1MeSH descriptor: [Prognosis] #2 (prognosis):ti,ab,kw #3 MeSH descriptor: [Genes, ras] #4 (kras genes):ti,ab,kw #5 MeSH descriptor: [Colorectal Neoplasms] #6 (colorectal neoplasms):ti,ab,kw (#1 OR #2) AND (#3 OR #4) AND (#5 OR #6)

Tabla 2: Escrituras de búsqueda de las distintas bases de datos

3.3 Selección de estudios y extracción de datos

Los resultados de las búsquedas se importaron al gestor de referencias Mendeley, donde se eliminaron los duplicados y se inició el cribado manual de los artículos. Se realizó un primer cribado mediante la lectura del título y *abstract* en el que se eliminaron los artículos que no correspondían a nuestros objetivos. Posteriormente, se realizó un cribado más detallado mediante la lectura del texto en el que se eliminaron los artículos que no cumplían nuestros criterios de elegibilidad. Por último, se revisaron las referencias de los artículos incluidos por si en la búsqueda bibliográfica no hubiera sido detectado algún artículo de utilidad para nuestro estudio.

La extracción de datos se realizó mediante tres tablas previamente diseñadas en las que se resumió la información sobre las características del estudio y la mutación de KRAS (tipo de estudio y media de seguimiento, variables de confusión incluidas, subtipo de mutación de KRAS analizada y prevalencia de la mutación en la muestra); la información sobre la población incluida (tamaño de la muestra, edad, sexo, nacionalidad, estadío tumoral, localización tumoral y tipo de terapia recibida) y la información sobre los resultados de supervivencia analizados (el tipo de resultado se supervivencia analizado, la comparación que realizan y el resultado obtenido).

3.4 Evaluación del riesgo de sesgos

Para la evaluación del riesgo de sesgos de los artículos seleccionados se utilizó la herramienta **QUIPS,** específica para los estudios sobre factores pronósticos. En ella se clasifica el riesgo de sesgo en seis dominios (participación del estudio, deserción del estudio, medición del factor pronóstico, medición del resultado, estudio de confusión y análisis e informes estadísticos) asignando un valor de sesgo (bajo, moderado o alto) a cada uno de ellos en función de las características del estudio. Sin embargo, esta herramienta no ofrece una valoración del riesgo de sesgo global, por este motivo, utilizaremos la categorización global del riesgo de sesgos empleada por *Wilhelmus Johannes Andreas Grooten et al.* Los autores categorizan como sesgo global bajo (verde) si todos los dominios fueron clasificados como riesgo bajo, o únicamente uno de ellos como riesgo moderado. Se categoriza como riesgo global alto (rojo) si tres o mas dominios se clasifican como riesgo moderado o uno de ellos como riesgo alto. El resto de clasificaciones intermedias se categorizan como riesgo global moderado (amarillo).

La clasificación de sesgo por dominios y global de los artículos se muestra a continuación. El análisis detallado por artículos se adjunta en anexos.

AUTORES	PARTICIPA-CIÓN DEL ESTUDIO	DESERCIÓN DEL ESTUDIO	MEDICIÓN DEL FACTOR PRONÓS-TICO	MEDICIÓN DEL RESULTADO	ESTUDIO DE CONFUSIÓN	ANÁLISIS E INFORMES ESTADÍS-TICOS	GLOBAL
H.Blons et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Luca Reggiani et al.	BAJO	MODERADO	BAJO	BAJO	BAJO	BAJO	BAJO
Jing Chen et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
E.M.V de Cuba et al.	MODERADO	BAJO	MODERADO	BAJO	BAJO	BAJO	MODERADO
Yanhong Deng et al.	BAJO	BAJO	BAJO	MODERADO	BAJO	BAJO	BAJO
V Eklöf et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Tian-An Guo et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Tamuro Hayama et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Shigenori Kadowaki et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Carsten Kamphues et al.	BAJO	BAJO	ALTO	MODERADO	BAJO	BAJO	ALTO
Li li et al.	BAJO	BAJO	BAJO	MODERADO	BAJO	BAJO	BAJO
Oscar Murcia et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Ryota Nakanishi et al.	MODERADO	BAJO	BAJO	MODERADO	BAJO	BAJO	MODERADO

Ehsan Nazemalhosseini-Mojarad et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Shuji Ogino et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Toshiro Ogura et al.	MODERADO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
A I Phipps et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
J. Smeby et al	MODERADO	BAJO	BAJO	BAJO	BAJO	MODERADO	MODERADO
Xiang-Bin Wan et al.	MODERADO	BAJO	BAJO	MODERADO	BAJO	BAJO	MODERADO
Abolfazl Yari et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO
Yuan Zhang et al.	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO	BAJO

Tabla 3: Resumen de valoración de sesgos (QUIPS)

3.5 Análisis y síntesis

Tras la extracción de los resultados de nuestros artículos, se llevó a cabo un síntesis cualitativa los mismos, plasmada en el apartado de síntesis de estudios.

4. **RESULTADOS**

4.1 Resumen del proceso de selección de estudios

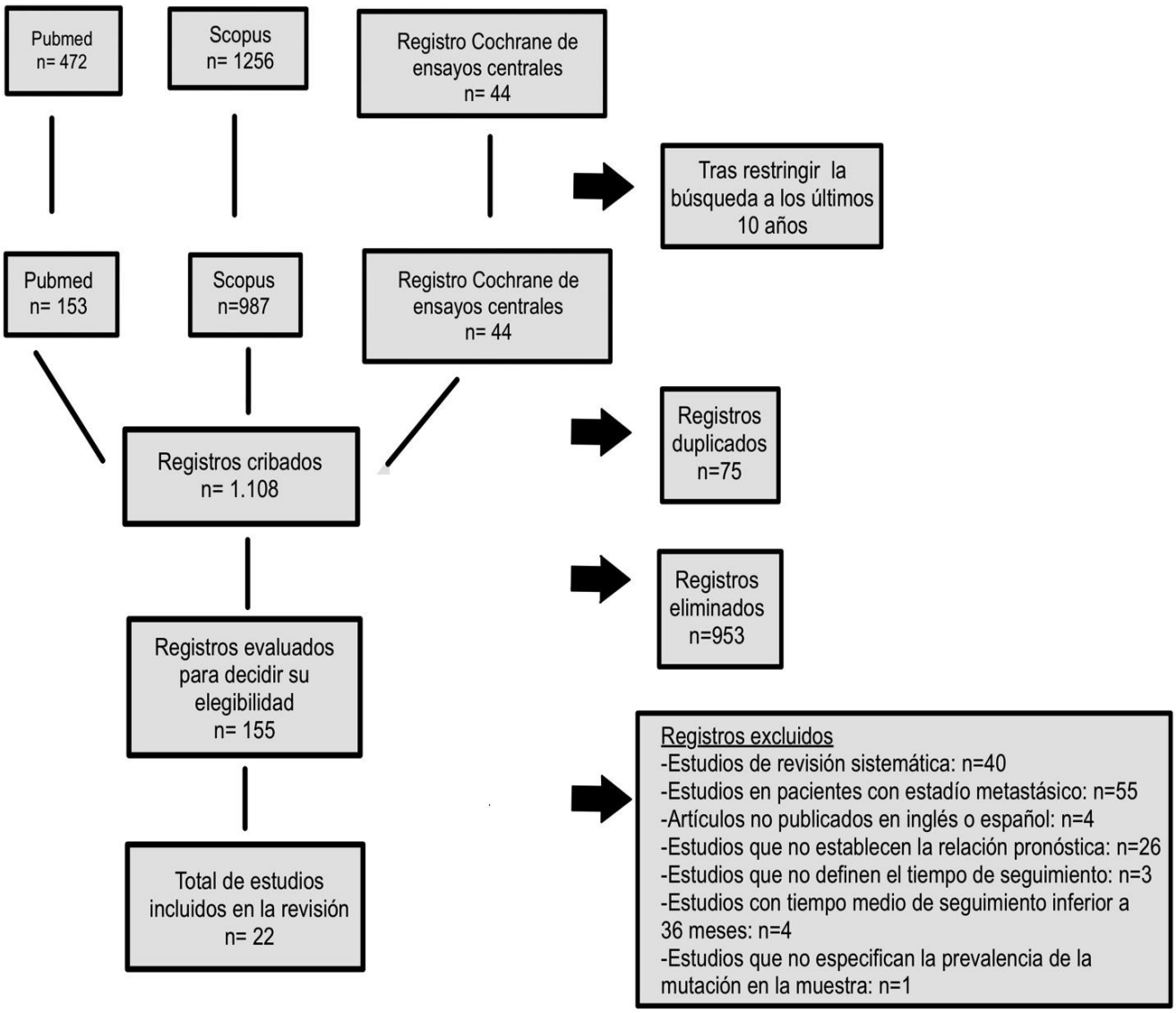

Figura 3: Resumen del proceso de selección de estudios

Tabla 4: Datos sobre las características del estudio y la mutación KRAS

AUTOR AÑO LUGAR	TIPO DE ESTUDIO Y MEDIA DE SEGUIMIENTO	VARIABLES DE CONFUSIÓN (Análisis multivariante)	SUBTIPO DE MÚTACIÓN KRAS ANALIZADA	PREVALENCIA DE LA MUTACIÓN
H. Blons et al 2014	Cohortes retrospectivo KRAS no mutado • 3,4 años KRAS mutado: • 3,8 años	Tumores de alto grado, estadío pN2. estadío pT4, embolia vascular o invasión linfática, obstrucción o perforación	Exón 2: codón 12 (p.G12A p.G12C p.G12D p.G12S p.G12R p.G12V) y codón 13	38,5% Codón 12: 79% Codón 13: 21%
Luca Reggiani et al. 2018 Italia	Cohortes retrospectivo Seguimiento a 5 años	Alto grado de clusters pobremente diferenciados, tumor incipiente, invasión linfovascular, mutaciones KRAS, mutaciones múltiples KRAS y PIK3CA y micormetasiatsis en ganglios linfáticos regionales	Codones 12, 13, 59, 61, 117 y 146	45% G13D n=7 , G12D n=6 G12V n=5
Jing Chen et al. 2014 China	Cohortes retrospectivo Media de seguimiento: 37 meses	Sexo, estadio TNM y mutación BRAF V600E	Exón 2: codón 12 (G12S G12R G12C G12D G12A G12V) y codón 13 (G13C y G13D)	44.9% 32,7% mutación simple Codón 12 (G12D): 35.4% Codón 13 (G13D): 24.0%
E.M.V. de Cuba et al. 2015 Holanda	Cohortes retrospectivo Media de seguimiento: 6.4 años	Edad, sexo, estadio tumoral, localización tumoral, tipo histológico, grado de diferenciación y estado de mutación BRAF/KRAS.	Exón 2: codón 12 y codón 13 Exón 3: codón 59 y codón 61	16% (n=23) Codón 12: n=13 Codón 13: n=6 Codón 12 + Codón 13: n=1 Codón 59: n=2 Codón 61. N=1
Yanhong Denga et al. 2015 China	Cohortes retrospectivo Media de seguimiento: 49 meses (24-78 meses)	Edad, estadio, grado, sito, invasión venosa, niveles de CEA, quimioterapia adyuvante y mutación de KRAS	Exón 2: codón 12 y codón 13	38.3% (n=166) Codón 12 n=123 Codón 13: n=43
V Eklöf et al. 2013 Suecia	Cohortes retrospectivo Cohortes CRUMS: 113 meses. Cohortes NSHDS: 102 meses	Sexo, edad, localización tumoral y estadío tumoral	Exón 2: codón 12 y codón 13	CRUMS: 19.5% NSHDS: 17.9%
Tian-An Guo et al. 2019 China	Cohortes retrospectivo Seguimiento a 5 años	Sexo,edad, localización tumoral, histopatología y metástasis extranodales	Exón 2, exón 3, exón 4 Exón 2: codón 12 (GLY12ALA, GLY12ASP, GLY12ARG, GLY12CYS, GLY12SER, GLY12VAL) y codón 13 (GLY13ASP)	46.4%
Tamuro Hayama et al. 2019, Japón	Cohortes tretrospectivo Seguimiento a 3 años	Estadío T, estadío N y mutación KAS	Exón 2: codón 12 (GLY12ALA, GLY12ASP, GLY12ARG, GLY12CYS, GLY12SER, GLY12VAL) y codón 13 (GLY13ASP)	37,5% Codón 12: 77% • p.G12D:49,2% • p.G12V:28,9% Codón 13: 23%
Shigenori Kadowaki et al. 2015, Japón	Cohortes retrospectivo Media de seguimiento: 87.7 meses	Edad, genero, estadío tumoral, quimioterapia adyuvante, MSI, mutació BRAF y mutación KRAS	Exón 2 y exón 3	38%
Carsten Kamphues et al. 2020	Cohortes retrospectivo Media de seguimiento 73.6 meses	Edad, sexo masculino, estadío T, metástasis nodales en tumor primario, invasión vascular y estado KRAS	No reportado	Colon derecho: 31% Colon izquierdo: 31,8%

Li Li, et al. 2017 China	Cohortes retrospectivo Media de seguimiento 24-56 meses	Grado de diferenciación, estadío tumoral, MMR. Mutación KRAS, BRAF, NRAS y PIK3CA	Exón 2: codón 12 (G12D, G12V, G12C,G12S,G12A,G12R,G12F) y codón 13 (G13D, G12C) Exón 3(A18D,T20M,E31K,Q61H,Q61R,G60D)	45,6% Exón 2: 40% Exón 3: 5.6%
Oscar Murcia et al. 2018	Cohortes retrospectivo Media de seguimiento: 52 meses (16-64)	Edad, sexo, estadío TNM y tratamiento quimioterápico	Exón 2: codón 12 y codón 13	37%
Ryota Nakanishi et al. 2013 Japón	Cohortes retrospectivo Media de seguimiento: 44.1 meses (1.0-189)	Profundidad tumoral (T), grado diferenciación, invasión linfática. Invasión vascular, estado MSI y mutación KRAS	Exón 2: codón 12 y codón 13 Exón 3: Codón 61	33.5% Codón 12: 82%
Ehsan Nazemalhosseini-Mojarad et al. 2019 Irán	Cohortes retrospectivo Media de seguimiento; 5 años	Supervivencia KRAS; mutación BRAF, mutación KRAS, sexo, localización tumoral, diferenciación, estadío TNM y estado MSI Según mutación KRAS en pacientes con MSE; sexo, localizción tumoral. Diferenciación, estadío TNM, historia familiar, mutación KRAS, edad de diagnóstico	Exón 2; codón 12 y codón 13. Exón 3: Codón 61	5.8%
Shuji Ogino et al. 2019	Cohortes retrospectivo Media de seguimiento: 6.2 años	Edad, sexo, IMC, Performance Status. Perforación intestinal, brazo de tratamiento de ensayo clínico, localización tumoral, estado MSI.	Exón 2: codón 12 y codón 13	35%
Toshiro Ogura et al. 2014 Japón	Cohortes retrospectivo Media de seguimiento: 5.6 años (4.1-7.8)	Edad, sexo, localización tumoral, mutación KRAS, mutación BRAF, mutación NRAS, estado de MSI, estadío UICC, grado de diferenciación, componente mucinoso y invasión vascular extramural	Exón 2, exón 3 y exón 4 Exón 2: codón 12 y codón 13	42.4 % Exón 2: 38% Exón 3: 2.0% Exón 4. 2.5%
A I Phipps et al. 2013 Reino Unido	Cohortes retrospectivo Media de seguimiento: 6.5 años (5.3 meses- 17. 3 años)	Edad, sexo, población de estudo, historia de tabaquismo e IMC	Exón 2: codón 12 y codón 13	31% Codón 12: 75% Codón 13: 22%
J. Smeby et al. 2018 Noruega	Cohortes retrospectivo Seguimiento a 5 años	Sexo, edad, estado MSI, localización, estadío, grado de diferenciación, mutación KRAS, mutación BRAF	Exón 2: codón 12 y codón 13 Exón 3: codón 61	31%
Xiang-Bin Wan et al. 2019	Cohortes retrospectivo Seguimiento a 4 años	Mutación KRAS, MEK, ERK, BRAF, estadío T y N	Exón 2: codón 12 y codón 13	31.6% Codón 12: 53 Codón 13: 9
Abolfazl Yari et al. 2020 Irán	Observacional retrospectivo Seguimiento a 5 años	No ajuste	Exón 2: codón 12 y codón 13 Exón 3: codón 61	29% Exón 2: 27% • Codón 12: 77.8% • Codón 13: 22.2% Exón 3: 2%
Ye Yuan et al. 2021 China	Observacional retrospectivo Seguimiento a 69 meses	Edad, grado de diferenciación tumoral, mutación KRAS	Todos los exones	35.17% • G12D: 64,7% • G13D: 29.5% • G14D: 3.9% • Q16H: 2.0%
Meifang Zhang et al. 2020 EEUU	Cohortes retrospectivo Seguimiento a 4 años	Estado de MSI, edad, estadío, grado de diferenciación y mutación KRAS	No reportado	37.9%

Tabla 5: Datos sociodemográficos sobre la población de estudio

AUTOR AÑO LUGAR	TAMAÑO DE LA MUESTRA	CARACTERÍSTICAS DE LA POBLACIÓN (edad, sexo, nacionalidad)		ESTADÍO Y LOCALIZACIÓN TUMORAL		TIPO DE TERAPIA RECIBIDA	
H. Blons et al 2014	nTotal: 1657	Pacientes del ensayo clínico PETACC8 KRAS salvaje • Mujeres: 409 • Hombres: 610 • >70 años: 101 • <o= 70 años: 918	KRAS mutado • Mujeres: 288 (41,3%) • Hombres: 350 (36,5%)	Estadío III KRAS salvaje • Distales: 692 (66,3%) • Proximales: 316 (53,9%) • Ambos lados: 7 (35%)	KRAS mutado • Distales: 351 (33,7%) • Proximales: 270 (46,1%) • Ambos lados: 13 (65%)	KRAS no mutado • Folfox: 513 (61,9%) • Folfox mas cetuximab 506 (61,1%	KRAS mutado • Folfox: 316 (38,1%) • Folfox más cetuximab: 322 (38,9%)
Luca Reggiani et al. 2018 Italia	nTotal: 62	Total • Mujeres: 35 • Hombres 27 • Edad: 69,5 años KRAS salvaje • Mujeres: 10 • Hombres: 24 • >68 años: 18 • < o = 68 años: 16	KRAS mutado • Mujeres: 17 • Hombres: 11 • >68 años: 13 • <o= 68 años: 15	Estadío I Total • Colon derecho: 11 • Colon izquierdo 15 • Recto: 26 KRAS no mutado • Colon derecho: 9 • Colon izquierdo: 9 • Recto: 16	KRAS mutado • Colon derecho: 2 • Colon izquierdo: 6 • Recto: 20	No tratamiento	
Jing Chen et al. 2014 China	nTotal: 214	Población china Total • Mujeres: 87 • Hombres: 127 • Media edad: 68.0 KRAS salvaje • Mujeres: 42 • Hombres: 54 • Media edad: 61.1	KRAS mutado • Mujeres:45 • Hombres: 73 • Media edad: 67	Estadío I: 32 Estadío II: 78 Estadío III: 82 Estadío IV: 19 No datos: 3 Total • Colon: 126 • Recto: 88	KRAS salvaje: • Colon: 73 • Recto: 45 KRAS mutado • Colon: 53 • Recto: 43	No reportado	
E.M.V. de Cuba et al. 2015 Holanda	nTotal: 138	Todos MSI Total • Mujeres: 81 • Hombres: 62 • 30-57 años: 36 • 58-68 años: 36 • 69-78 años:38 • 79-93 años3	KRAS salvaje • Mujeres: 68 • Hombres: 47 • Media de edad: 60 años KRAS mutado • Mujeres: 11 • Hombres: 12 • Media de edad 64 años	Estadío II: 85 Estadío III: 58 KRAS salvaje • Colon derecho: 94 • Colon izquierdo: 20 • No especificado: 1	KRAS mutado • Colon derecho: 17 • Colon izquierdo: 6 • No especificado: 0	Quimioterapia adyuvante: n=36 • KRAS mutado: n=9	

Yanhong Denga et al. 2015 China	nTotal: 433	<u>KRAS salvaje</u> • Mujeres:112 • Hombres:155 • Media de edad: 60.15 <u>KRAS mutado</u> • Mujeres:66 • Hombres:100 • Media de edad:53.9		Estadío II: 218 Estadío III: 215 <u>KRAS salvaje</u> • Colon:200 • Recto: 67 <u>KRAS mutado</u> • Colon: 120 • Recto: 46		FOLFOX n=243 KRAS mutado n=83
V Eklöf et al. 2013 Suecia	CRUMS n=414 NSHDS n=197	Pacientes suecos <u>CRUMS: KRAS salvaje</u> • Mujeres: 143 • Hombres: 188 • <59 años: 55 • 60-69 años: 65 • 70-79 años: 136 • >80 años: 75 <u>CRUMS: KRAS mutado</u> • Mujeres:37 • Hombres: 43 • <59 años: 13 • 60-69 años: 15 • 70-79 años: 26 • >80 años: 26	<u>NSHDS: KRAS salvaje</u> • Mujeres: 81 • Hombres: 66 • <59 años: 36 • 60-69 años: 90 • 70-79 años: 21 • >80 años: 0 <u>NSHDS KRAS mutado</u> • Mujeres: 21 • Hombres: 11 • <59 años: 13 • 60-69 años:14 • 70-79 años:5 • >80 años: 0	<u>CRUMS: KRAS salvaje</u> • Estadío I: 57 • Estadío II: 131 • Estadío III: 67 • Estadío IV: 68 • Colon derecho: 98 • Colon izquierdo: 104 • Recto: 125 <u>CRUMS: KRAS mutado</u> • Estadío I:4 • Estadío II:32 • Estadío III:20 • Estadío IV:23 • Colon derecho: 34 • Colon izquierdo: 21 • Recto: 25	<u>NSHDS: KRAS salvaje</u> • Estadío I: 28 • Estadío II: 54 • Estadío III: 34 • Estadío IV: 31 • Colon derecho: 43 • Colon izquierdo: 40 • Recto: 64 <u>NSHDS: KRAS mutado</u> • Estadío I: 5 • Estadío II: 10 • Estadío III: 8 • Estadío IV: 9 • Colon derecho: 14 • Colon izquierdo: 12 • Recto: 6	No reportado
Tian-An Guo et al. 2019 China	nTotal: 1834	Pacientes chinos <u>Total</u> • Mujeres: 746 • Hombres: 1,088 • Media de edad: 60.2+/-11.9 <u>KRAS salvaje</u> • Mujeres: 372	• Hombres: 611 • Media de edad: 59.5 +/- 11.9 <u>KRAS mutado</u> • Mujeres: 374 • Hombres: 477 • Media de edad. 60.8 +/- 11.9	Estadío I:192 Estadío II: 502 Estadío III: 758 Estadío IV. 382 <u>KRAS salvaje</u> • Ciego. 15 • Colon ascendente: 115 • Flexura hepática. 34 • Colon transverso: 43 • Flexura esplénica; 22 • Colon descendente; 46 • Colon sigmoide: 279 • Recto: 423	<u>KRAS mutado</u> • Ciego: 28 • Colon ascendente: 162 • Flexura hepática. 36 • Colon transverso: 33 • Flexura esplénica; 18 • Colon descendente; 25 • Colon sigmoide; 158 • Recto. 382	No reportado

Tamuro Hayama et al. 2019, Japón	nTotal: 200	KRAS salvaje • Mujeres:42 • Hombres: 84 • Media de edad: 66 KRAS mutado (codón 12) • Mujeres: 28 • Hombres: 29 • Media de edad: 69	KRAS mutado (codón 13) • Mujeres: 6 • Hombres: 11 • Media de edad: 70	Estadío I: 23,5% Estadíos II-III: 76,5% KRAS salvaje • Colon derecho: 34 • Colon izquierdo: 92	KRAS mutado (codón 12) • Colon derecho: 26 • Colon izquierdo: 31 KRAS mutado (codón 13) • Colon derecho: 6 • Colon izquierdo:11	No reportado
Shigenori Kadowaki et al. 2015, Japón	nTotal: 813	Pacientes japoneses KRAS salvaje • Mujeres: 192 • Hombres: 308 • Media de edad: 63.5+/- 10.3	KRAS mutado • Mujeres: 146 • Hombres: 166 • Media de edad: 64.7+/-10.3	KRAS salvaje • Estadío I: 125 • Estadío II: 195 • Estadío III: 180 • Colon Distal: 213 • Colon Proximal: 134 • Recto: 153	KRAS mutado • Estadío I: 58 • Estadío II: 127 • Estadío III: 127 • Colon Distal: 125 • Colon Proximal: 98 • Recto: :89	Quimioterapia adyuvante: • Estadío II: 40% • Estadío III: 76%
Carsten Kamphues et al. 2020	nTotal: 1093	KRAS salvaje (en colon derecho) • Mujeres: 135 • Hombres: 125 • Media de edad: 68 KRAS mutado (en colon derecho) • Mujeres: 57 • Hombres: 60 • Media de edad: 67	KRAS salvaje (en colon izquierdo) • Mujeres: 173 • Hombres: 315 • Media de edad: 64 KRAS mutado (en colon izquierdo) • Mujeres: 106 • Hombres: 121 • Media de edad: 64	KRAS salvaje (en colon derecho) • Estadío I-II: 75 • Estadío III-IV: 186 KRAS mutado (en colon derecho) • Estadío I-II: 23 • Estadío III-IV: 94	KRAS salvaje (en colon izquierdo) • Estadío I-II: 155 • Estadío III-IV: 333 KRAS mutado (en colon izquierdo) • Estadío I-II: 60 • Estadío III-IV: 167	Quimioterapia adyuvante (71.9%: 5-FU) • KRAS mutado: 92,8%
Li Li, et al. 2017 China	nTotal: 160	Total • Mujeres: 56 • Hombres: 104 • </= 35 años: 9 • 36-60 años: 63 • >60 años: 88		Estadío II • IIA:112 • IIB:19 • IIC:29	Total • Colon ascendente: 27 • Colon transverso: 16 • Colon descendente: 35 • Recto: 82	Ningún paciente recibió quimioterapia adyuvante
Oscar Murcia et al. 2018 España	nTotal: 878	KRAS mutado (+MSE) • Mujeres. 84 • Hombres: 134 • Media de edad: 73		KRAS mutado (+MSE) • Estadío I: 45 • Estadío II: 68 • Estadío III: 67 • Estadío IV: 38 • Colon derecho: 69 • Colon izquierdo: 149		Estadío II avanzado (T4N0M0) y estadío III • 5-FU o capecitrabina: 53 • FOLFOX: 34 • No QT: 131

Ryota Nakanishi et al. 2013 Japón	nTotal: 254	Pacientes japoneses <u>KRAS salvaje</u> • Mujeres: 63 • Hombres: 106 • Media de edad: 65.7 +/- 11.6 <u>KRAS mutado</u> • Mujeres: 35 • Hombres: 50 • Media de edad: 63.2 +/- 13.8	<u>KRAS salvaje</u> • Estadío I: 24 • Estadío II: 56 • Estadío III: 72 • Estadío IV: 17 • Colon proximal: 47 • Colon distal o recto: 122	<u>KRAS mutado</u> • Estadío I: 8 • Estadío II: 35 • Estadío III: 25 • Estadío IV: 17 • Colon proximal: 29 • Colon distal o recto: 56	Estadío II y Estadío III QT adyuvante • 42% KRAS mutado
Ehsan Nazemalhosseini-Mojarad et al. 2019 Irán	nTotal: 258	Pacientes iraníes <u>Total</u> • Mujeres: 124 • Hombres: 134 • Media de edad: 56.4 <u>KRAS salvaje</u> • Mujeres: 100 • Hombres: 122 • Media de edad: 56.2 <u>KRAS mutado</u> • Mujeres: 24 • Hombres: 12 • Media de edad: 57.1	<u>Total</u> • Etsadío I: 36 • Estadío II: 117 • Estadío III: 85 • Estadío IV: 20 • Colon derecho: 114 • Colon izquierdo: 144 <u>KRAS salvaje</u> • Etsadío I: 29 • Estadío II: 103 • Estadío III: 73 • Estadío IV: 17 • Colon derecho. 93 • Colon izquierdo: 129	<u>KRAS mutado</u> • Etsadío I: 7 • Estadío II: 14 • Estadío III: 12 • Estadío IV: 3 • Colon derecho: 21 • Colon izquierdo: 15	Solo reportado en pacientes con MSE • KRAS mutado: 6
Shuji Ogino et al. 2019	nTotal: 508	Pacientes procedentes de ensayo clínico multicéntrico de quimioterapia adyuvante (CALGB) <u>Total</u> • Mujeres: 232 • Hombres: 276 • Media de edad: 59.8 +- 11.5 <u>KRAS salvaje</u> • Mujeres: 151 • Hombres: 179 • Media de edad:60.2 +- 11.6 <u>KRAS mutado</u> • Mujeres: 81 • Hombres: 97 • Media de edad: 59.1 +- 11.4	Estadío III <u>Total</u> • Colon derecho: 291 • Colon izquierdo: 212 <u>KRAS salvaje</u> • Colon derecho: 191 • Colon izquierdo: 136 <u>KRAS mutado</u> • Colon derecho: 100 • Colon izquierdo: 76		5-FU + Leucovorina • KRAS mutado: 109 Irinotecan+F-FU+Lecucovorina • KRAS mutado. 157
Toshiro Ogura et al. 2014 Japón	nTotal: 1304	<u>Total</u> • Mujeres: 524 • Hombres : 780 • Media de edad: 63.8+/-10.4 <u>KRAS mutado</u> • Mujeres: 244 • Hombres: 309 • Media de edad: 64.2 +/- 10.4	<u>Total</u> • Estadío 0: 48 • Estadío I: 248 • Estadío II: 407 • Estadío III: 384 • Estadío IV: 217 • Proximal: 379 • Distal: 544	<u>KRAS mutado</u> • Estadío 0: 33 • Estadío I: 89 • Estadío II: 170 • Estadío III: 173 • Estadío IV: 88 • Proximal: 189 • Distal: 209	No reportado

A I Phipps et al. 2013 Reino Unido	nTotal: 2120	KRAS salvaje • Mujeres: 721 • Hombres. 609 • <50 años: 346 • 50-59 años: 291 • 60-69 años: 415 • 70-74 años: 278	KRAS mutado • Mujeres: 329 • Hombres: 264 • <50 años: 147 • 50-59 años: 143 • 60-69 años: 188 • 70-74 años: 115	KRAS salvaje • Estadío localizado: 553 • Estadío regional: 610 • Estadío a distancia 155 • Desconocido: 23 • Colon Proximal: 505 • Colon Distal: 364 • Recto: 424 • Desconocido: 37	KRAS mutado • Estadío localizado: 220 • Estadío regional: 293 • Estadío a distancia. 75 • Desconocido: 5 • Proximal: 255 • Distal: 147 • Rectal: 183 • Desconocido: 89	No reportado
J. Smeby et al. 2018 Noruega	nTotal: 1197	Pacientes noruegos Total • Mujeres: 634 • Hombres: 563 • </= 70 años: 493 • > 70 años: 704	KRAS mutado • Mujeres: 29 • Hombres: 33 • </= 70 años: 28 • > 70 años: 33	Total • Estadío I: 195 • Estadío II. 475 • Estadío III. 327 • Estadío IV: 198 • Colon derecho. 493 • Colon izquierdo: 369 • Recto: 312 • Síncrono: 23	KRAS mutado • Estadío I: 27 • Estadío II. 29 • Estadío III. 35 • Estadío IV. 33 • Colon derecho: 33 • Colon izquierdo. 29 • Recto: 29 • Síncrono. 35	No reportado
Xiang-Bin Wan et al. 2019	nTotal: 220	Total • Mujeres: 107 • Hombres: 113 • <56 años: 17 • >/= 56 años: 76	KRAS mutado • Mujeres: 64.5 • Hombres: 74.3 • <56 años: 71 • >/= 56 años: 65.9	Total • Estadío II: 62 • Estadío III: 73 • Colon derecho: 45 • Colon izquierdo: 175	KRAS mutado • Estadío II: 71.9 • Estadío III: 72.63	No reportado
Abolfazl Yari et al. 2020 Irán	nTotal: 100	Población Iraní Total • Mujeres: 36 • Hombres: 64 • Media de edad: 59.60 +/-15.24 KRAS salvaje • Mujeres: 26 • Hombres: 45 • Media de edad: 57.7 +/-16.09	KRAS mutado • Mujeres: 10 • Hombres: 19 • Media de edad: 64.20 +/- 11.96	Total • Estadío I: 11 • Estadí II: 17 • Estadío III: 59 • Estadío IV: 13 • Colon derecho: 29 • Colon izquierdo: 30 • Recto: 41 KRAS salvaje • Estadío I: 7 • Estadí II: 12 • Estadío III: 41	• Estadío IV: 11 • Colon derecho: 19 • Colon izquierdo: 23 • Recto: 29 KRAS mutado • Estadío I: 4 • Estadí II: 5 • Estadío III: 18 • Estadío IV: 2 • Colon derecho: 10 • Colon izquierdo: 7 • Recto: 12	No reportado

Ye Yuan et al. 2021 China	nTotal: 145	Población China <u>KRAS salvaje</u> • Mujeres: 39 • Hombres: 55 • >= 65 años. 34 • <65 años. 60	<u>KRAS mutado</u> • Mujeres. 16 • Hombres. 35 • >= 65 años. 25 • <65 años. 26	<u>KRAS salvaje</u> • Estadío II: 26 • Estadío III: 43 • Estadío IV: 25 • Recto: 53 • Colon sigmoide. 7 • Colon transverso. 10 • Colon descendente. 8 • Colon ascendente: 15 • Ciego: 0	<u>KRAS mutado</u> • Estadío II: 13 • Estadío III: 22 • Estadío IV. 16 • Recto. 24 • Colon sigmoide. 3 • Colon transverso. 6 • Colon descendente. 7 • Colon ascendente. 9 • Ciego: 2	No reportado
Meifang Zhang et al. 2020 EEUU	nTotal: 45,761	Población estadounidense <u>KRAS salvaje</u> • Mujeres: 3,264 • Hombres: 15,159 • <65 años: 14,931 • >65 años: 13,492 <u>KRAS mutado</u> • Mujeres: 8,470 • Hombres: 8,868 • <65 años: 9,309 • >65 años: 8,029		<u>KRAS salvaje</u> • Estadío I-II: 8,806 • Estadío III-IV: 17, 837 • Colon: 24,778 • Recto: 3,645 <u>KRAS mutado</u> • Estadío I-II: 4,575 • Estadío III-IV: 11,722 • Colon: 15,266 • Recto: 2,072		QT adyuvante: • KRAS mutado: 12, 013 RT adyuvante • KRAS mutado: 2,133

Tabla 6: Datos sobre resultados de supervivencia

AUTOR AÑO LUGAR	RESULTADO DE SUPREVIVENCIA ANALIZADO	COMPARACIÓN	RESULTADOS (significación estadística p-valor:<0.05)	CONCLUSIONES
H. Blons et al 2014	Supervivencia libre de recurrencia	KRAS mutado/BRAF salvaje vs KRAS y BRAF salvaje	p-valor: <0.0001 • Codón 12: p-valor <0.01 • Codón 13: p-valor =0.26 Multivariante HR: 1,58 CI95%: (1.28-1.94) p-valor <0.001	KRAS confiere peor pronóstico (menor supervivencia libre recurrencia) en pacientes sin mutación de BRAF. Desglosando por codones solo la mutación del codón 12 confiere menor supervivencia libre de recurrencia KRAS es un factor de riesgo de menor supervivencia libre de progresión de forma independiente
Luca Reggiani et al. 2018 Italia	Supervivencia cáncer específica	KRAS mutado vs KRAS salvaje	p-valor: 0,019 Univariante HR: 2.4 CI (95%): (2,6-13,2) Multivariante: no factor pronóstico independiente (no datos)	KRAS confiere peor pronóstico (menor supervivencia cáncer específica) KRAS es un factor de riesgo (menor tiempo supervivencia cáncer específica) en análisis univariante, pero no de forma independiente
Jing Chen et al. 2014 China	Supervivencia general	KRAS mutado vs KRAS no mutado KRAS mutado/BRAF salvaje vs KRAS y BRAF salvaje	p-valor: 0.133 p-valor: 0.035 Codón 13: p-valor 0.0011 Codón 12: p-valor: 0.247 Multivariante HR: 1.1 CI95%: (0.5-2.2)	KRAS confiere peor pronóstico (menor supervivencia general) únicamente en el grupo de pacientes con BRAF no mutado. Desglosando por codones, únicamente el codón 13 confiere menor supervivencia general. KRAS no es un factor de riesgo en pacientes con BRAF no mutado de forma independiente
E.M.V. de Cuba et al. 2015 Holanda	Supervivencia general Supervivencia cáncer específica	KRAS mut. Vs KRAS y BRAF salvaje KRAS mut o BRAF mutado vs KRAS y BRAF salvaje	SCE: p-valor: 0.07 SG: p-valor: 0.33 SCE: p-valor: 0.03 SCE estadío II: p-valor: 0.03 SCE estadío III: p-valor: 0.3 SCE Multivariante HR 3,65 95%CI (1.09-12.44) p-valor: 0.04 SG: p-valor: 0.11	KRAS confiere peor pronóstico en términos de supervivencia cáncer específica pero no en términos de supervivencia general en pacientes con MSI. La mutación de KRAS o BRAF confieren peor pronóstico (menor supervivencia cáncer específica) en pacientes con MSI. Desglosando por estadíos KRAS confiere peor supervivencia cáncer específica solo en el estadío II. La mutación de KRAS o BRAF es un factor de riesgo de menos supervivencia cácner específica de forma independiente La mutación de KRAS o BRAF no influye en la supervivencia general
Yanhong Denga et al. 2015 China	Supervivencia libre de enfermedad	KRAS mutado vs KRAS salvaje en pacientes sin FOLFOX adyuvante KRAS mutado vs KRAS salvaje en pacientes con FOLFOX adyuvante	p-valor: 0.027 p-valor: 0.781 Multivariante: HR: 1.572 95%CI (1.058-2.335) p-valor: 0.025	KRAS confiere peor pronóstico (menor supervivencia libre de enfermedad) únicamente en el grupo de pacientes sin quimioterapia adyuvante. KRAS es un factor de riesgo independiente de menor supervivencia cáncer específica.

V Eklöf et al. 2013 Suecia	Supervivencia cáncer específica	KRAS mutado vs KRAS salvaje (en ambas cohortes)	CRUMS: p-valor: 0.002 NSHDS: p-valor: 0.305 CRUMS: Multivariante HR: 1.458 95%CI (1.023-2.155) NSHDS: Multivariante HR: : 0.789 95%CI (0.443-1.438)	KRAS confiere peor pronóstico (menor supervivencia cáncer específica) únicamente en la cohorte de pacientes CRUMS. KRAS es factor de riesgo de menor supervivencia cáncer específica de forma independiente sólo en la cohorte CRUMS.
		KRAS mutado vs KRAS salvaje en pacientes con MSI (solo en cohorte CRUMS)	CRUMS: p-valor: 0.009	KRAS confiere peor pronóstico (menor supervivencia cáncer específica) en pacientes con MSI en la cohorte CRUMS
		KRAS mutado vs KRAS salvaje en pacientes con MSE (en ambas cohortes)	CRUMS: p-valor: 0.042 NSHDS: p-valor: 0.519	KRAS confiere peor pronóstico (menor supervivencia cáncer específica) en pacientes sin MSI únicamente en la cohorte CRUMS
		Cuádruple índice positivo (mutaciones en KRAS o BRAF o PIK3CA o pérdida de expresión de PTEN) VS Cuádruple índice negativo (en ambas cohortes)	CRUMS: p-valor: 0.230 NSHDS: p-valor: 0.003 CRUMS: Multivariante HR: 1.157 95%CI (0.827-1.619) NSHDS: Multivariante HR: 1.308 95%CI (0.787-2.174)	El cuádruple índice positivo confiere peor pronóstico (menor supervivencia cáncer específica) únicamente en la cohorte NSHDS. El cuádruple índice positivo no es un factor de riesgo independiente de menor supervivencia cáncer específica
Tian-An Guo et al. 2019 China	Supervivencia general	KRAS mutado vs KRAS salvaje	p-valor: 0.007	KRAS confiere peor pronóstico (menor supervivencia general) de forma global.
		KRAS mutado vs KRAS salvaje en estadío I-II	p-valor: 0.762 Multivariante HR: 1.20 95%CI (0.37-3.91) p-valor: 0-76	KRAS confiere peor pronóstico (menor supervivencia general) únicamente en los estadíos III y IV.
		KRAS mutado vs KRAS salvaje en estadío III	p-valor: 0.029 Multivariante HR. 1.47 95% CI (0.89-2.42) p-valor: 0.13	KRAS es un factor de riesgo de menor supervivencia general de forma independiente únicamente en el estadío IV.
		KRAS mutado vs KRAS salvaje en estadío IV	p-valor: 0.038 Multivariante HR: 1.60 95%CI (1.07-2.40) p-valor: 0.022	
Tamuro Hayama et al. 2019, Japón	Supervivencia libre de recurrencia	KRAS mutado vs KRAS salvaje	p-valor: 0.0172 Multivariante HR (mutaciones codón 12): 2.05 95%CI (1.08-3.85) p-valor: 0.031 Multivariante HR (G12V): 3.77 95% CI (1.54-8.59) p-valor: 0.002 Multivariante HR (G12C): 6.57 95%CI (1.90-17.7) p-valor: <0.001	KRAS confiere peor pronóstico (menor supervivencia libre de recurrencia). KRAS es un factor de riesgo de menor supervivencia libre de recurrencia de forma independiente en todas las mutaciones del codón 12.

Shigenori Kadowaki et al. 2015, Japón	Supervivencia libre de enfermedad Supervivencia general	KRAS mutado vs KRAS salvaje	SLE: p-valor: 0.02 SLE HR: 1.35 95%CI (1.03-1.75) p-valor: 0.03 SG: p-valor: 0.01 SG HR: 1.46 95%CI (1.09-1.97) p-valor: 0.01	KRAS confiere peor pronóstico (menor supervivencia libre de enfermedad y menor supervivencia general) KRAS es un factor de riesgo de menor supervivencia libre de enfermedad y menor supervivencia general de forma independiente
		KRAS mutado en pacientes con MSI vs KRAS mutado en pacientes sin MSI	SLE p-valor: 0.95 SLE HR (NO MSI): 1.37 95%CI (1.05-1.80) SLE HR (SÍ MSI): 1.34 95%CI (0.34-5.24) SG: p-valor 0.70 SG HR (NO MSI): 1.49 (1,10-2.02) SG HR (SÍ MSI). 1.39 (0.33-5.78)	No existen diferencias de supervivencia en pacientes con y sin MSI y KRAS mutado. KRAS es un factor de riesgo de menor supervivencia libre de enfermedad y menor supervivencia general de forma independiente únicamente en pacientes sin MSI.
Carsten Kamphues et al. 2020	Supervivencia libre de enfermedad Supervivencia general	KRAS mutado vs KRAS salvaje en pacientes con tumores primarios en colon derecho	SLE: p-valor: 0.215 SLE Univariante HR 1.29 95%CI (0.86-1,95) p-valor: 0.22 SG: p-valor: 0.43 Univariante HR 1.19 95% (0.77-1.86) p-valor: 0.43 Excluyendo pacientes con: • Mutación BRAF: SG: p-valor: 0.256, SLE p-valor: 0.18 • MSI: pone que no se asocia pero no p-valores	KRAS no confiere peor pronóstico en pacientes con tumores primarios de colon derecho. KRAS tampoco confiere peor pronóstico tras excluir pacientes con mutación en BRAF o MSI en pacientes con tumores primarios de colon derecho
		KRAS mutado vs KRAS salvaje en pacientes con tumores primarios en colon izquierdo	SLE: p-valor: 0.005 SLE Multivariante HR: 1.45 95%CI (0.99-2.07) p-valor: 0.05 SG Multivariante HR: 1.52 95%CI(1.07-2.15) p-valor: 0.019 Excluyendo pacientes con • Mutación BRAF: SG: p-valor: 0.01 y SLE p-valor: 0.006 • MSI: SG: p-valor: 0.02 y SLE: p-valor: 0.03	KRAS confiere peor pronóstico (menor supervivencia libre de enfermedad) en pacientes con tumores primarios de colon izquierdo. KRAS es un factor de riesgo de menor supervivencia general de forma independiente en pacientes con tumores primarios de colon izquierdo. KRAS confiere peor pronóstico (menor supervivencia general y menor supervivencia libre de enfermedad) tras excluir pacientes con mutación en BRAF y MSI en pacientes con tumores primarios de colon izquierdo.

Li Li, et al. 2017 China	Supervivencia libre de enfermedad Supervivencia general	KRAS mutado vs KRAS salvaje	SLE: p-valor: 0.108 SG: p-valor: 0.372 SLE Multivariante HR: 2.153 95%CI(1.204-3.848) p-valor: 0.010 SG Multivariante HR: 2.729 95%CI (0.99-7.522) p-valor: 0.052	KRAS no confiere peor pronóstico. KRAS es un factor de riesgo de menor supervivencia libre de enfermedad de forma independiente. KRAS no es un factor de riesgo de menor supervivencia general de forma independiente pero esta muy cerca de serlo.
Oscar Murcia et al. 2018	Supervivencia libre de enfermedad	Subtipo KRAS y MSE es un factor de riesgo independiente en la supervivencia	Multivariante HR: 1.21 95%CI(0.96-1.52) p-valor:0.109	KRAS no es un factor de riesgo de menor supervivencia libre de enfermedad de forma independiente en pacientes sin MSI.
		KRAS mutado y MSE que no recibieron QT adyuvante vs KRAS mutado y MSE que recibieron QT adyuvante	p-valor: 0.003 Multivariante HR: 1.93 95%CI(0.86-4.34) p-valor:0.111	Existe un beneficio con la quimioterapia adyuvante en pacientes con KRAS mutado y MSE (mayor supervivencia libre de enfermedad). El tratamiento con quimioterapia adyuvante no es un factor protector de forma independiente en pacientes con KRAS mutado y MSE.
Ryota Nakanishi et al. 2013 Japón	Supervivencia libre de recurrencia Supervivencia general	KRAS mutado vs KRAS salvaje	SLR: p-valor: 0.043 SG: p-valor: 0.45 SLR estadío II: p-valor: 0.007 SLR estadío III: p-valor: 0.83 Estadío II; Multivariante HR: 2.65 95%CI (1.23-6,33) p-valor: 0.01	KRAS confiere peor pronóstico en términos de supervivencia libre de recurrencia pero no en la supervivencia general KRAS confiere peor pronóstico (menor supervivencia libre de recurrencia) únicamente en pacientes con estadío II. KRAS es un factor de riesgo de menor supervivencia libre de recurrencia de forma independiente en pacientes con estadío II
Ehsan Nazemalhosseini-Mojarad et al. 2019 Irán	Supervivencia general	KRAS mutado vs KRAS salvaje	p-valor >0.05 Multivariante HR: 1.222 95%CI(0.600-2.487) p-valor: 0.580	KRAS no confiere peor pronóstico ni es un factor de riesgo independiente
		KRAS mutado vs KRAS salvaje en pacientes con MSE	p-valor: 0.046 Multivariante HR. 8.435 (2.921-10.357) p-valor; 0.012	KRAS confiere peor pronóstico (menor supervivencia general) en pacientes sin MSI. KRAS es un factor de riesgo de menor supervivencia general de forma independiente
Shuji Ogino et al. 2019	Supervivencia libre de enfermedad Supervivencia libre de recurrencia Supervivencia general	KRAS mutado vs KRAS salvaje	SLE: p-valor: 0.89 SLE Multivariante HR: 0.95 95%CI(0.70-1.28) SLR: p-valor: 0.84 SLR Multivariante HR: 0.93 95%CI(0.68-1.28) SG: p-valor: 0.56 SG Multivariante HR: 0.86 95%CI (0.60-1.23)	KRAS no confiere peor pronóstico ni es un factor de riesgo independiente, ni para supervivencia libre de enfermedad, ni supervivencia libre de recurrencia, ni supervivencia general
		KRAS mutado vs KRAS salvaje en pacientes tratados con 5-FU+leocuvorina	SLE Multivariante HR: 1.07 95%CI (0.75-1.55) SLR Multivariante HR; 1.16 95%CI (0.79-1.69) SG: Multivariante HR: 0.94 95%CI (0.62-1.41)	KRAS no es un factor de riesgo de menor supervivencia libre de enfermedad, supervivencia libre de recurrencia o supervivencia general en pacientes tratados con 5-FU+leocuvorina
		KRAS mutado vs KRAS salvaje en pacientes tratados con 5-FU+Leucovorina+Irinotecan	SLE Multivariante HR: 0.88 95%CI (0.53-1.60) SLR: Multivariante HR: 0.97 95%CI (0.57-1.65)	KRAS no es un factor de riesgo de menor supervivencia libre de enfermedad, supervivencia libre de recurrencia o supervivencia general en pacientes tratados con 5-FU+leocuvorina+Irinotecan

Toshiro Ogura et al. 2014 Japón	Supervivencia general	KRAS mutado factor de riesgo independiente en la supervivencia	Multivariante HR: 1.44 (1.18-1.76) p-valor: <0.001	KRAS es un factor de riesgo de menor supervivencia general de forma independiente
A l Phipps et al. 2013 Reino Unido	Supervivencia cáncer específica Supervivencia general	KRAS mutado como factor de riesgo independiente en la supervivencia	SCE: Multivariante HR: 1.37 95%CI (1.13-1.66) SG: Multivariante HR: 1.24 95% CI (1.06-1.45)	KRAS es un factor de riesgo de menor supervivencia cáncer específica y menor supervivencia general de forma independiente
		KRAS mutado como factor de riesgo independiente en la supervivencia en pacientes con BRAF salvaje	SCE: Multivariante HR: 1.40 95%CI (1.14-1.72) SG: Multivariante HR: 1.27 (1.08-1.50)	KRAS es un factor de riesgo de menor supervivencia cáncer especifica y menor supervivencia general de forma independiente en pacientes sin mutación de BRAF.
		KRAS mut codón 12 vs KRAS mut codón 13	SCE: p-valor: 0.54 SG: p-valor: 0.30	No hay diferencias de supervivencia cáncer específica ni supervivencia general entre las mutaciones del codón 12 y las mutaciones del codón 13
		KRAS mutado como factor de riesgo independiente en pacientes con MSI.	SCE: Multivariante HR 0.77 95%CI (0.42-1.41) • Solo en pacientes con BRAF salvaje: HR: 0.87 95%CI (0.47-1.60) SG: Multivariante HR 0.87 (0.53-1.42) • Solo en pacientes con BRAF salvaje HR: 0.87 95%CI (0.47-1.60)	KRAS no es un factor de riesgo de menor supervivencia cáncer específica ni supervivencia general en pacientes con MSI. Tampoco tras excluir a los pacientes con BRAF mutado
		KRAS mutado como factor de riesgo independiente en pacientes con MSE	SLE: Multivariante HR: 1.24 95%CI (1.01-1.52) • Solo en pacientes con BRAF salvaje: 1.36 95%CI (1.09-1.68) SG: Multivariante HR 1.21 95%CI (1.02-1.43) • Solo en pacientes con BRAF salvaje: HR: 1.27	KRAS es un factor de riesgo de menor supervivencia libre de enfermedad y supervivencia general de forma independiente en pacientes sin MSI. También tras excluir los pacientes con BRAF mutado.
J. Smeby et al. 2018 Noruega	Supervivencia general	KRAS mutado vs KRAS salvaje	p-valor: <0.001 Univariante HR: 1.28 95%CI (1.05-1.56) p-valor: 0.016 Multivariante HR: 1.21 95%CI (0.98-1.49) p-valor: 0.08	KRAS confiere peor pronóstico (menor supervivencia general) KRAS se ha asociado con menor supervivencia general pero no de forma independiente
		KRAS mutado vs KRAS y BRAF salvaje en MSE	Univariante HR: 1.30 95%CI (1.06-1.59) p-valor=0.013	KRAS se ha asociado con menor supervivencia general en pacientes sin MSI
		KRAS mutado vs KRAS y BRAF salvaje en MSI	Univariante HR: 0.84 95%CI (0.30-2.38) p-valor: 0.742	KRAS no se ha asociado con menor supervivencia general en pacientes con MSI

Xiang-Bin Wan et al. 2019	Supervivencia libre de recurrencia Supervivencia general	KRAS mutado vs KRAS salvaje	SLR: p-valor: 0.0411 SLR: Multivariante HR: 3.319 95%ci (1.231-8.944) SG: p-valor: 0.4555 SG: Multivariante HR: 1.434 95% CI(0.501-4.101	KRAS confiere peor pronóstico en términos de menor supervivencia libre de recurrenca pero no en menor supervivencia general KRAS es un factor de riesgo de menor supervivencia libre de recurrencia de forma independiente
Abolfazl Yari et al. 2020 Irán	Supervivencia general	KRAS mutado vs KRAS salvaje	p- valor: 0.543	KRAS no confiere peor pronóstico
Ye Yuan et al. 2021 China	Supervivencia libre de enfermedad Supervivencia general	KRAS mutado vs KRAS salvaje	SLE; Multivariante HR 2.19 95%CI (1.372-3.395) p-valor: 0.001 SG: Multivariante HR: 1.897 95%CI (1.309-2.747) p-valor: 0.001 SG: KRASG12D Univariante HR: 2.17 95%CI (1.31-3.58) p-valor: <0.0001	KRAS es un factor de riesgo de menor supervivencia libre de enfermedad y supervivencia general de forma independiente. La mutación KRASG12D se ha asociado con menor supervivencia general
Meifang Zhang et al. **2020**	Supervivencia general	KRAS mutado vs KRAS salvaje	p-valor: <0.001	KRAS confiere peor pronóstico (menor supervivencia general)
		KRAS mutado vs KRAS salvaje en estadíos I-II	Multivariante HR: 1.32 95%CI(1.14-1.54) p-valor: <0.001	KRAS es un factor de riesgo de menor supervivencia general de forma independiente en pacientes sin resección del tumor
		KRAS mutado vs KRAS salvaje en estadíos III-IV	Multivariante HR: 1.18 95%CI (1.10-1.27) p-valor: <0.001	KRAS es un factor de riesgo de menor supervivencia general de forma independiente tanto en los estadíos I-II, como en los estadíos III y IV.

4.3 Síntesis de estudios

En la siguiente tabla se resumen las principales características y resultados de los estudios incluidos en esta revisión, para facilitar la comprensión de resultados y síntesis de información obtenida.

Primer autor	Año	País	Estadío	Casos mutación/casos	Método de detección mutación	Media seguimiento	Codones	Otras mutaciones	Conclusiones
H. Blons et al. (9)	2014		III	638/1657	PCR	3.4 a. KRAS salvaje 3.8 a. KRAS mutado	12 y 13	BRAF excluido	Menor SLR.
Luca Reggiani et al. (10)	2018	Italia	I	28/62	Plataforma genotipa-do alto rendimiento	Seguimiento a 5a.	12, 13, 59, 61, 117 y 146	NR	Menor SCE.
Jing Chen et al. (11)	2014	China	I, II, III, IV	96/214	PCR y secuencia-ción directa	37 m.	12 y 13	BRAF excluido	Menor SG únicamente tras excluir BRAF.
E.M.V. de Cuba et al. (12)	2015	Holan da	II, III	23/138	Análisis de fusión de alta resolución y secuencia-ción	6.4 a.	12, 13, 59,61	MSI BRAF excluido y BRAF mutado	Menor SCE. NO menor SG.
Yanhong Denga et al. (13)	2015	China	II,III	166/433	PCR y secuencia-ción Sanger	49m	12 y 13	NR	Menor SLE en pacientes sin QT adyuvante
V Eklöf et al. (14)	2013	Suecia	I, II, III, IV	CRUMS: 80/414 NSHDS: 32/197	PCR y secuencia-ción	CRUMS: 113m NSHDS: 102m	12 y 13	MSI Cuádruple índice	Menor SCE
Tian-An Guo et al. (15)	2019	China	I, II, III, IV	851/1834	Secuencia-ción bidireccion-al	Seguimiento s 5a.	12, exón 3 y 4	NR	Menor SG
Tamuro Hayama et al. (16)	2019	Japón	I, II, III	74/200	Secuencia-ción directa o ensayo Luminex	Seguimiento a 3 a.	12 y 13	NR	Menor SLR
Shigenori Kadowaki et al. (17)	2015	Japón	I, II, III	312/813	Electroforé-sis en gel de gradiente desnaturali-zante	87.7m	Exón 2 y 3	MSI BRAF (como ajuste)	Menor SLE y SG.
Carsten Kamphues et al. (18)	2020	NR	I, II, III, IV	Colon iz: 227/715 Colon de: 117/378	NR	73.6m	No reportado	BRAF y MSI excluido	Menor SLE únicamente en tumores primarios colon iz.
Li Li, et al. (19)	2017	China	II	73/160	Secuencia-ción Sanger	24-56m	12 y 13 Exón 3	MSI (ajuste) BRAF (ajuste)	NO menor SLE ni SG Sí factor de riesgo indep. de menor SLE
Oscar Murcia et al. (20)	2018	Españ a	I, II,I II, IV	218/878	PCR y secuencia-ción directa	52m	12 y 13	MSI excluido	NO menor SLE Mayor SLE en pacientes con QT adyuvante
Ryota Nakanishi et al. (21)	2013	Japón	I, II, III, IV	85/254	PCR y secuencia-ción directa	44.1m	12,13 Y 61	MSI (ajuste)	Menor SLR NO menor SG
Ehsan Nazemalh osseini-Mojarad et al. (22)	2019	Irán	I, II, III, IV	15/258	Pirosecuen-ciación y Cast-PCR	5a	12,13 y 61	MSI BRAF (ajuste)	Menor SG sólo en pacientes sin MSI
Shuji Ogino et al (23)	2019	NR	III	178/508	Pirosecuen-ciación	6.2a	12 y 13	MSI(ajuste)	NO menor SLE, SLR, ni SG.
Toshiro Ogura et al.(24)	2014	Japón	I, II, III, IV	553/1304	Electroforé-sis en gel de gradiente de desnaturali-zación	5.6 a	12, 13 Exón 3 y 4	BRAF (ajuste) MSI (ajuste)	Menor SG
A I Phipps et al. (25)	2013	Reino Unido	Local.. Regio. Distan.	593/2120	Secuencia-ción directa e inversa	6.5a	12 y 13	BRAF excluido MSI	Menor SCE y SG

J. Smeby et al. (26)	2018	Noruega.	I, II, III, IV	1097/1197	Secuencia-ción Sanger	Seguimiento a 5a	12, 13 y 61	BRAF (excluido) MSI	Menor SG
Xiang-Bin Wan et al. (27)	2019	NR	II, III	62/220	PCR cuantitativa	Seguimiento a 4a	12 y 13	BRAF (ajuste)	Menor SLR NO menor SG
Abolfazl Yari et al. (28)	2020	Irán	I, II, III, IV	29/100	PCR y secuencia-ción directa	Seguimiento a 5a	12, 13 y 61	NR	NO menor SG
Ye Yuan et al. (29)	2021	China	II, III, IV	51/145	Secuencia-ción de nueva generación	69m	Todos los exones	NR	Menor SLE y SG
Meifang Zhang et al. (30)	2020	EEUU	I, II, III, IV	17338/45761		Seguimiento a 4a	NR	MSI (ajuste)	Menor SG

Tabla 7: Principales características y resultados de los estudios elegibles que evalúan la asociación entre las mutaciones del gen KRAS y la supervivencia en pacientes con cáncer colorectal. SCE: Supervivencia cáncer específica. SG: Supervivencia general. SLR: Supervivencia libre de recurrencia SLE: Supervivencia libre de enfermedad. NR: No reportado

4.3.1 Características del los estudios incluidos

Todos los artículos incluidos son estudios de cohortes retrospectivos, en los que se recopilan datos ya generados de cohortes de pacientes diagnosticados de CCR en un periodo determinado. En ellos, el periodo de seguimiento, siguiendo nuestro criterio de inclusión, es mayor de 36 meses, con ocho estudios con un tiempo de seguimiento mayor de 5 años (12,14,17,18,23,24,25).

4.3.2 Datos sociodemográficos sobre la población a estudio

Respecto al tamaño muestral de los estudios incluidos, ocho artículos superan los mil participantes (9,15,17,18,24,25,26), destacando el estudio de *Meifang Zhang et al.* con 45,761 participantes. El estudio con menor tamaño muestral corresponde al artículo de *Luca Reggiani et al.* con únicamente 62 participantes.

Cinco de los estudios incluidos corresponden a población asiática (11,15,17,21,29), dos estudios a población iraní (22,28), otros dos estudios a población sueca y noruega (14, 26) y un estudio a población estadounidense (30). En el resto de artículos no se específica la nacionalidad de los participantes.

En la mayoría de los estudios existe una proporción mayor de hombres, excepto en los estudios de *Luca Reggiani et al*, *E.M.V de Cuba et al,* y la cohorte NSHDS del artículo de *V Eklöf et al*, donde predominan las mujeres.

La media de edad de los pacientes se comprende en un rango entre los 60-75 años excepto en el estudio de *Ehsan Nazemalhosseini-Mojarad et al* y el subgrupo de pacientes con KRAS mutado del estudio de *Yanhong Denga et al*., donde la media de edad es inferior a los 60 años. Un detalle que es necesario destacar es que en el estudio de *E.M.V de Cuba et al.* únicamente se incluyeron pacientes que presentan inestabilidad de microsatélites.

4.3.3 Presencia de la mutación KRAS

La mutación KRAS fue detectada en más del 35% de los participantes en 13 estudios (9,10,11,13,15,16,17,19,20,23,24,29,30). Tres artículos incluyen poblaciones con una prevalencia de la mutación inferior al 20% (12,14,22), destacando el estudio de *Ehsan Nazamalhosseono-Mojarad et al.* con únicamente un 5.8%. En los estudios que desglosan la prevalencia según los codones, en todos los casos el codón 12 es el más prevalente.

El exón 2 de KRAS, que incluye el codón 12 y el codón 13, se analiza en todos los estudios, excepto en el artículo de *Melfang Zhang et al*. en el que no se reporta el subtipo de mutación de KRAS analizado. Adicionalmente, el exón 3 se analiza en diez artículos (10, 12, 15, 17, 19, 21, 22, 24, 26, 28, 29). Asimismo, el exón 4 se analiza en tres estudios (15, 24, 29), y el estudio de *Luca Reggiani et al* también analiza los codones 117 y 146. Es necesario puntualizar que el estudio de *Ye Yuan et al.* analiza todos los exones de KRAS.

4.3.4 Localización y estadío tumoral

La localización tumoral descrita más frecuente corresponde al colon izquierdo o distal, entendiéndose como aquellos tumores situados a partir de la flexura esplénica, que son mayoritarios en once estudios (9, 16, 17, 19, 20, 21, 22, 23, 24, 27, 28). En tres estudios solo se clasifica la localización en tumores

situados en recto o en colon, siendo en todos ellos la localización más frecuente la de colon (11, 13, 30). Únicamente en los estudios *de Luca Reggiani et al. y Tian-Guo et al* la localización rectal es la más frecuente. Por último, en el artículo de *Carsten Kamphues et al*. no reporta datos sobre localización tumoral.

Respecto al estadio tumoral de los pacientes, el mayor porcentaje de estudios corresponde a aquellos que incluyen pacientes en los cuatro estadíos, concretamente lo hacen once estudios (11, 14, 15, 18, 20, 22, 24, 25, 26, 28, 30). De ellos, el mayor número de participantes corresponde a los estadíos II y III. Cuatro estudios analizan los estadíos II y III únicamente (12, 13, 21, 27), dos estudios analizan los estadíos I, II y III (16, 17), otros dos estudios únicamente analizan el estadío III (9,23) el estudio de *Luca Reggiani et al.* solamente analiza el estadío I igual que el estudio de *Li Li et al*. analiza únicamente el estadío II.

4.3.5 Tratamiento adyuvante

De los artículos que reportan datos sobre el tratamiento adyuvante recibido tras la cirugía, en dos estudios los pacientes no recibieron ningún tipo de tratamiento (10,19), que corresponden a los únicos dos estudios que solo analizan el estadío I y el estadío II. En los que sí que recibieron tratamiento, todos lo hicieron mediante quimioterapia con distintos regímenes, excepto en el estudio de *Meifang Zhang et al.* en el que 2,133 participantes con KRAS mutado recibieron radioterapia adyuvante.

4.3.6 Datos sobre resultados de supervivencia

En el análisis de la supervivencia, las variables del estudio que se incluyen como factores de confusión, son aquellas que mostrando significación estadística en un análisis univariante posteriormente son incluidas en un análisis mulitivariante para comprobar su relación pronóstica de forma independiente. De ellas, ocho estudios (11, 12,17,19, 22, 24, 26, 27) incluyen la

mutación de BRAF y siete estudios incluyen la inestabilidad de microsatélites (17, 19, 21, 22, 23, 24, 26) como variables en el análisis multivariante. Por último, el artículo de *Abolfazi Yari et al*. no analiza la significación pronóstica de las variables en un análisis multivariante.

Los resultados de supervivencia analizados se expresan en términos de supervivencia cáncer específica (SCE) supervivencia libre de enfermedad (SLE) supervivencia libre de recurrencia (SLR) y supervivencia global (SG). La SCE se define como la duración de la supervivencia desde el diagnóstico hasta la muerte por CCR; la SLE se define como el tiempo entre la fecha de cirugía y la fecha de recurrencia local o metastásica, o la aparición de un nuevo tumor primario de colon o la muerte por cualquier causa; la SLR se define como el tiempo entre la cirugía hasta la recurrencia local o metastásica del tumor (no incluyendo la muerte) y la SG se define como el tiempo entre el diagnóstico hasta la muerte por cualquier causa.

En cuatro artículos se analiza la SCE y en todos ellos se relaciona la mutación de KRAS con una peor supervivencia (10, 12, 14, 25*).* Únicamente en uno de los estudios (10) la mutación de KRAS se ha asociado con una menor SCE en un análisis univariante, pero no de forma independiente en el análisis multivariante.

En otros cuatro artículos, se asocia la presencia de la mutación de KRAS con una menor SLR (9, 16, 21, 29*)*. Por el contrario, en uno de los estudios no se relaciona la presencia de KRAS mutado con una peor SLR (23).

Asimismo, las mutaciones en el gen KRAS se han relacionado con una menor SLE en otros cuatro estudios (13, 17, 18, 29). Aunque existen ciertos matices, en uno de ellos únicamente se ha relacionado en el subgrupo de pacientes con CRR izquierdo (18), y en otro, únicamente ha existido una menor SLE en los pacientes que no han recibido quimioterapia adyuvante (13). En tres de los artículos analizados, no se asoció una menor SLE en los pacientes con mutación de KRAS (19, 20, 23), aunque en uno de ellos al incluir la mutación

KRAS en el análisis multivariante, sí que se evidenció que era un factor de riesgo HR: 2.153 95%CI (1.204-3.848) p-valor: 0.010 (19).

En cuanto a la SG, de los quince artículos que la analizan, únicamente en cinco de ellos se ha relacionado con una menor SG (15, 17, 24, 25, 26, 29, 30). Además, en tres estudios pese a asociarse a una menor SCE (12) y SLR (21,27) la mutación de KRAS no se ha relacionado con una peor SG.

De forma más específica, en varios de los estudios se analiza las diferencias de pronóstico en pacientes con KRAS mutado pero BRAF salvaje, ya que se ha demostrado que este último confiere peor pronóstico de forma independiente. En ellos, poseer KRAS mutado se ha asociado a una menor supervivencia libre de recurrencia (9), a una menor supervivencia cáncer específica (12), a peor supervivencia libre de enfermedad (18) y a una menor supervivencia general (11,18,25,26). Especialmente relevante es el estudio de *Jing Chen et al*. en el que la mutación de KRAS se ha asociado a una menor SG únicamente al excluir los pacientes con BRAF mutado. Por último, en uno de los estudios (14) se analiza el impacto en la supervivencia de la mutación KRAS junto a BRAF, PIK3CA o pérdida de expresión de PTEN (cuádruple índice positivo), y estas se han asociado a una menor SCE.

A colación de lo comentado anteriormente, siete estudios analizan el pronóstico que confiere la mutación de KRAS en relación a la inestabilidad de los microsatélites, ya que igual que la mutación de BRAF, también se ha relacionado con un peor pronóstico de forma independiente. En tres de los estudios se ha asociado KRAS a un peor pronóstico pero no existen diferencias entre los pacientes con y sin inestabilidad de microsatélistes, es decir, KRAS confiere peor pronóstico en los dos subgrupos (14, 17, 18). En cambio, en otros tres estudios se ha observado que KRAS no confiere peor pronóstico específicamente en pacientes con MSI (25, 26) o de forma global (22) pero tras excluir a los pacientes con MSI, sí que se ha asociado la mutación del gen KRAS con una menor supervivencia.

En cuanto a la determinación de las variantes más agresivas, cuatro estudios desglosan las diferencias de supervivencia respecto a las distintas mutaciones (9, 16, 25, 29). De ellos, en dos estudios se encontraron diferencias de supervivencia entre codones. Menor SLE únicamente en pacientes con el codón 12 en uno de los estudios (9*)*, mientras que en el otro, se asoció menor SG únicamente con mutaciones del codón 13 (11). En los estudios que analizan las mutaciones del codón 12 específicamente, G12V y G12C (16)*y* G12D (29), todas ellas se han asociado con una menor supervivencia.

En trece estudios se analiza la supervivencia incluyendo los cuatro estadíos tumorales, en diez de ellos KRAS se ha asociado con un peor pronóstico, pero la mayoría no analizan individualmente las diferencias de supervivencia según estadíos, únicamente lo hacen dos estudios (15, 30). En uno de ellos (15) únicamente se han asociado las mutaciones de KRAS con menor SG en estadíos III y IV, siendo un factor de riesgo independiente en el análisis multivariante solamente el estadío IV (HR: 1.60 95%CI (1.07-2.40) p-valor: 0.022). En cambio, en el segundo de ellos (30), las mutaciones de KRAS son un factor de riesgo de menor SG en todos los estadíos. En los nueve estudíos que analizan los estadíos precoces (excluyendo el estadío IV), en siete artículos KRAS se ha asociado con peor pronóstico, con menor SLR (9, 16, 27), SCE (10,12), SLE (13, 17) y SG (17). En dos estudíos, la mutación de KRAS no se ha relacionado con menor supervivencia, en uno de ellos en pacientes con estadío II (19) y en el otro con pacienes en estadío III (23). Solamente el estudio de *E.M.V de Cuba et al.* analiza individualmente las diferencias de pronóstico según el estadío, asociando menor SCE únicamente en pacientes con estadío II.

Adicionalmente, tres estudios analizan la relación de la mutación de KRAS con respecto a la respuesta a la quimioterapia. En dos de ellos, se ha observado que los pacientes con KRAS mutado que recibieron quimioterapia adyuvante tienen una mayor SLE (13, 20). Apoyando estos resultados, en el tercer estudio en el que todos los pacientes habían sido tratados mediante quimioterapia

adyuvante no se ha podido asociar la mutación de KRAS con una menor supervivencia (23).

4.4 Evaluación del riesgo de sesgos (QUIPS)

La mayoría de estudios incluidos presentan un riesgo de sesgo bajo, a excepción de cinco estudios. Cuatro de ellos tienen un riesgo de sesgo moderado (12, 21, 26, 27), todos se categorizaron como riesgo moderado en participación del estudio, tres de ellos también en medición del factor pronóstico (12, 21, 27) y el otro (26), en análisis e informe estadísitco. Solamente uno de los estudios presenta un riesgo de sesgo alto (18), ya que la medición del factor pronóstico se categorizó como riesgo alto debido a que no especificaba los subtipos de mutación de KRAS analizados. Todos los resultados se plasman en la Tabla 3.

5. DISCUSIÓN

El CCR constituye un problema de salud pública por su alta incidencia (1). Resulta primordial identificar factores pronósticos que permitan conocer mejor el desarrollo de la enfermedad y así, en un futuro poder crear nuevas terapias dirigidas a controlar el impacto que generan estos factores sobre la supervivencia de los pacientes. Por ello, nuestro objetivo principal era identificar el pronóstico que confería la mutación de KRAS en los estadíos no metastásicos del CCR .

En diecisiete artículos de los veintidós analizados se ha relacionado la mutación de KRAS con un peor pronóstico, tanto en términos de SLE, SCE, SG y SLR. A pesar de que en la mayoría de los estudios sí que se ha relacionado la mutación de KRAS con una peor supervivencia, existen diferencias en cuanto a la forma de analizar el pronóstico (SLE, SCE, SG o SLR), el número de pacientes incluidos, el estadío tumoral, las características sociodemográficas de los pacientes, y el tipo de terapia recibida. Esta heterogeneidad entre los distintos artículos también se mantiene en los estudios que no han relacionado la mutación de KRAS con una menor supervivencia.

A pesar de que en nuestro medio no se analiza el KRAS en estadios iniciales, en otros países como China, la determinación del KRAS se realiza de forma rutinaria en todos los pacientes con CCR. De los 9 estudios que analizan los estadíos precoces (no metastásicos), siete relacionan la mutación KRAS con un peor pronóstico, con menor SLR (9, 16, 27), SCE (10,12), SLE (13, 17) y SG (17). Debido a este mayor riesgo de recurrencia o metástasis, recomiendan su determinación de forma rutinaria, así como un seguimiento más cercano o incluso quimioterapia activa en algunos casos (9). En dos estudíos, la mutación de KRAS no se ha relacionado con menor supervivencia, uno de ellos en pacientes con estadío II (19) y en el otro con pacienes en estadío III (23). Únicamente uno de los estudios (12) analiza individualmente las diferencias de pronóstico según el estadío, asociando menor SCE solamente pacientes con estadío II.

El tamaño muestral varía desde 62 pacientes en el estudio con menor participación (10) hasta 45,761 en el que más población fue incluída (30). Asimismo, el estadío tumoral en el que se encuentran los pacientes difiere considerablemente entre estudios, desde artículos que analizan los cuatro estadíos, solamente uno o excluyen el estadío mestastásico. En sólo uno de ellos (12) la diferencia de pronóstico se analiza de forma individual en estadíos no metastásicos. La terapia adyuvante recibida por los pacientes varía tanto en la administración o no de tratamiento como en los distintos regímenes quimioterápicos elegidos. Por último, en cuanto a las características sociodemográficas de los pacientes se incluyen participantes de nacionalidades muy distintas entre ellas (iraníes, asiáticos, estadounidenses etc).

La discrepancia entre resultados respecto al pronóstico que confiere la mutación de KRAS y la heterogeneidad entre los distintos artículos también se recoge en otros estudios como el de *Amanda K. Arrington et al.* En otros estudios consultados, sí que se ha relacionado la mutación de KRAS con un peor pronóstico, pero únicamente en pacientes con estadíos metastásicos (31) (32).

En cuanto a la prevalencia de la mutación de KRAS, sí que existe cierta similitud entre los estudios analizados, en la mayoría de ellos la prevalencia oscila entre el 30-45%, con mayor prevalencia del codón 12 en todos ellos. Únicamente en tres artículos la prevalencia es menor del 30% (12, 22, 28). Estos resultados coinciden con los obtenidos en otras revisiones sistemáticas como la de *Amanda K. Arrington et al* con una prevalencia de la mutación del 30-50%, siendo en todos ellos el codón 12 la mutación más frecuente de KRAS. O el del *Li et al.* con una prevalencia de las mutaciones activadoras de los genes KRAS, NRAS o HRAS alrededor del 50%, siendo las mutaciones en los codones 12 y 13 las más prevalentes.

En cambio, en cuanto a determinar las variantes más agresivas la variación entre resultados es máxima, en los cuatro estudios que analizan las diferencias

entre codones cada uno a mostrado un resultado distinto (no diferencias entre supervivencia, supervivencia menor con mutaciones del codón 12, supervivencia menor con mutaciones del codón 13 y supervivencia menor con todas los tipos de mutación del codón 12)

A pesar de que sí que se han relacionado las mutaciones de KRAS con una menor supervivencia, la heterogeneidad entre los distintos estudios analizados dificulta la extracción de conclusiones sólidas sobre el pronóstico que confiere la mutación de KRAS. Son necesarios más estudios con más homogeneidad entre ellos que confirmen el peor pronóstico que confiere KRAS en estadíos no metastásicos.

Asimismo, una de las posibles limitaciones de los estudios analizados es que todos se tratan de cohortes retrospectivos, estudios observacionales en los que se depende de la información recogida previamente, con distintos tiempos de seguimiento entre ellos y con grupos de pacientes muy heterogéneos siendo por tanto las muestras difícilmente comparables.

De igual forma, no en todos los estudios se tiene en cuenta la coexistencia de otras mutaciones que se han demostrado que confieren mal pronóstico por si mismas (MSI, BRAF) (6). Concretamente, en cinco de los artículos que han relacionado la mutación de KRAS con una menor supervivencia no se analizan conjuntamente ni se tienen en cuenta como variable de ajuste las mutaciones de MSI y BRAF, constituyendo un posible sesgo a la hora de interpretar los resultados (10,13,15,16,29).

6. CONCLUSIONES

En la literatura publicada existen numerosos artículos que relacionan las mutaciones de KRAS con la supervivencia y la falta de respuesta al tratamiento anti-EGFR en el estadío metastásico. En cambio, no son tantos los estudios que recopilan la información sobre el pronóstico que confieren las mutaciones de KRAS en estadíos no metastásicos. La identificación de una peor supervivencia con las mutaciones en el gen KRAS supondría un avance en cuanto al conocimiento del pronóstico individualizado de los pacientes en base a sus marcadores genéticos, el desarrollo de nuevas terapias dirigidas e incluso la clasificación de los pacientes en grupos pronósticos en base a las mutaciones que presenten. Por tanto, nuestro objetivo principal era revisar los estudios publicados sobre el pronóstico que confiere la mutación de KRAS en los estadíos no metastásicos.

En la mayoría de los artículos se ha relacionado la mutación de KRAS con una menor supervivencia, pero la heterogeneidad entre ellos no permite extraer conclusiones sólidas. Las diferencias entre la población estudiada, los estadíos tumorales tan distintos, así como el tamaño de grupos y los distintos regímenes de tratamientos aplicados, generan gran discrepancia en los resultados. Por ello, se necesitan más estudios con mayor similitud entre pacientes y método de medición del pronóstico para comprobar más fielmente el empeoramiento del pronóstico que confieren las mutaciones de KRAS.

La principal conclusión sólida que hemos podido extraer del análisis de los estudios es respecto a nuestro objetivo de revisar la prevalencia de las mutaciones de KRAS en el CCR no metastásico. La prevalencia de la mutación de KRAS se estima entre el 30-45% de los pacientes con CRR, coincidiendo con estudios previos publicados. El exón dos (codón 12 y codón 13) es el más prevalente en todos los casos, con predominio del codón 12.

El objetivo del análisis de las variantes mas agresivas no ha mostrado resultados concluyentes, con distintos resultados en cada uno de los estudios analizados.

Las limitaciones de esta revisión sistemática son la inclusión únicamente de artículos publicados en inglés o español y publicados en los últimos diez años. Asimismo el proceso de selección de estudios, la revisión del riesgo de sesgo y la extracción de datos no se ha realizado por pares. Solamente se consultaron tres bases de datos (Pubmed, Scopus y Registro Cochrane de Ensayos Controlados) y no se ha buscado la evidencia no publicada pudiendo caer en el sesgo de publicación (únicamente se publican los estudios con resultados concluyentes).

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8. ANEXOS

Anexo 1: Cuestionario de riesgo de sesgo QUIPS para cada artículo

Author and year of publication	**H.Blons et al** AÑO PUBICACIÓN: 2014			
Study identifier	doi:10.1093/annonc/mdu464			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Patients from the PETACC8 trial had completely resected, histologically proven stage III colon adenocarcinoma and were randomized to receive, as adjuvant treatment, either 6 months of FOLFOX 4 or FOLFOX 4-cetuximab [16].	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	no	no	
Recruitment period	Period of recruitment is adequately described	The trial started in December 2005, it was amended in June 2008,	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	No	no	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	810 met all the criteria for molecular analysis (informed consent and available FFPE sample, no technical failure for KRAS/BRAF status determination), 153 were BRAF-mutated and excluded because of the prognostic impact of BRAF muta- tions	yes	

		(Figure 1), 1 tumor was KRAS- and BRAF-mutated and was also excluded of the analysis.		
Adequate study participation	There is adequate participation in the study by eligible individuals	1657 patients	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: Demographic and clinical characteristics (treatment grupo, gender, age, missing WHO performance, tumor location, hystopathology grade, pn classification, PT calssification, bowel obstruction, VELI	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Among the 2559 patients included in the PETACC8 phase III study, 1810 met all the criteria for molecular analysis (informed consent and available FFPE sample, no technical failure for KRAS/BRAF status determination), 153 were BRAF-mutated and excluded because of the prognostic impact of BRAF muta- tions (Figure 1), 1 tumor was KRAS- and BRAF-mutated and was also excluded of the analysis.	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	Demographic and clinical characteristics of the patients in the KRAS molecular study 1657) were not significantly different from those of the excluded population.	yes	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	Demographic and clinical characteristics of the patients in the KRAS molecular	partial	

		study 1657) were not significantly different from those of the excluded population.Supplementary table		
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	Demographic and clinical characteristics of the patients in the KRAS molecular study 1657) were not significantly different from those of the excluded population.	partial	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS (c.34G > A/p.G12S, c.34G > C/p.G12R, c.34G > T/p.G12C, c.35G > A/ p.G12D, c.35G > C/p.G12A, c.35G > T/p.G12V and c.38G > A p.G13D) a	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	NAs were extracted from formalin-fixed and paraffin-embedded (FFPE) tissues using the QIAamp® DNA Mini Kit (Qiagen®). Molecular an lysis was centralized and carried out retrospectively for 2096 patientsincluded before trial amendment, and prospectively for the other 463 patients, by real-time PCR using TaqMan® probes (Applied Biosystems)	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	Continuous variables are presented as the mean (SD) and median interquartile range.	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	Of the 1657 tumors, 38.5% had a KRAS mutation,	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No	no	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit**			low

	potential bias.			
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	TR was defined as the time between the date of randomization and the date of local or metastatic recurrence. DFS was defined as the time between the date of randomization and the date of local or metastatic recurrence	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observacional retrospective. Median follow-up was 3.4 years (95% CI 3.3–3.4) and 3.8 years (95% CI 3.8–3.9) for patients with wild-type and mutated tumors, respectively.	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Factors included in the multivariate analyses were the treatment group, baseline variables imbalanced between the two PETACC8 arms, and prognostic factors identified in univariate analyses.	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes; treatment group (folfox vs folfox+cetuximab), mutation wild-type versus mutated, female versus male. <70 years versus >70 yeats, hystopatologycal grade G1-G2 versus G3-G4, tumor location; distal cancer versis proximal, PT, Pn bowel obstruction and perforation and VELI.	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of	Yes: obsrvational retrospective study	yes	

	information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).			
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	In the PETACC8 trial, KRAS-mutated tumors were equally numerous in both treatment arms. Moreover, an interaction test was carried out between KRAS status (WT, codon 12 and codon13) and treatment (TTR P = 0.37; DFS P = 0.32) leading to the conclusion that both arms could be pooled to study the impact of KRAS mutations on TTR and DFS.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Factors included in the multivariate analyses were the treatment group, baseline variables imbalanced between the two PETACC8 arms, and prognostic factors identified in univariate analyses.	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	TR and DFS curves were estimated with the Kaplan–Meier method. Differences between groups of patients were analyzed using unstratified log-rank tests. An unstratified Cox regression model was used to estimate hazard ratios (HRs), 95% confidence intervals (CIs) and P values for candidate prognostic factors. Factors included in the multivariate analyses were the treatment group,	yes	

		baseline variables imbalanced between the two PETACC8 arms, and prognostic factors identified in univariate analyses.		
	The selected statistical model is adequate for the design of the study.	Yes. Long-rank tests and multivariate cox regresion model	yes	
Reporting of results	There is no selective reporting of results.	no selective reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Luca Reggiani Bonetti et al.** . AÑO PUBICACIÓN: 2014			
Study identifier	https://doi.org/10.1155/2018/2959801			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	pTNM stage I CRCs	yes	

Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	By a specialized Colorectal Cancer Registry instituted in Modena in 1984 [9], we identified all patients with stage I CRCs diagnosed between January 1984 and December 2004 (518 cases) and, among them, we selected those who died of disease (DOD) during the follow-up (37 cases). Paraffin blocks of the tumors and the relative haematoxylin and eosin- (H&E-) stained slides, stored in the archives of the Pathologic Anatomy of the University of Modena and Reggio Emilia, were available for only 25 of 32 patients (group A). This group of patients was matched with a group of 32 patients with stage I CRCs who were alive or who died of independent diseases (DOID) after a follow-up time longer than sixty months (group B). Cases in group B were consecutive stage I CRCs that fulfilled the inclusion criteria (at least 60-month follow-up) and with available paraffin blocks. All cases were anonymously collected. Pathological features, including tumor size (maximum diameter in centimeters), tumor border configuration (expanding or infiltrating), WHO histological grade [10], pTNM stage [11], TB, LVI, grading based on the counting of PDC [12], and the presence of lymph node micrometas- tases (MM) [13], were available in all cases.	yes	
Recruitment period	Period of recruitment is adequately described	between January 1984 and December 2004	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Colorectal Cancer Registry instituted in Modena	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	he inclusion criteria (at least 60-month follow-up)	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	62 tumors included in the study	partial	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Clinicopathological characteristics: gender, age, riht, left colon, rectum, mean size of the tumor. Size range of the tumor, pT1/pT2, micrometastases, tumorborder configuration, WHO grading, PDC grading, Tumor buddign and LVI.	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low

2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Clinical and pathological features of 62 tumors included in the study	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no.	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	partial	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	No loss of follow-up	partial	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	partial	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			moderate
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS (codons 12, 13, 59, 61, 117, and 146)	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	DNA was extracted from repre- sentative 10 μm-thick sections cut from formalin-fixed and paraffin-embedded blocks of each tumor sample con- taining at least 50% tumor cells. Extraction was performed with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and DNA was quantified with Xpose-NGS (Trinean NV, Gentbrugge, Belgium). Mutations were detected in genome-amplified DNA using the high-throughput genotyping platform Sequenom MassARRAY System (Sequenom, San Diego, CA, USA) and the Myriapod Colon Status Kit (Diatech Pharmacogenetics, Italy) following the manufac-turer's protocol.	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF	The method and setting of	Yes	yes	

Measurement	measurement of PF is the same for all study participants.			
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	28/62 cases (45%) had mutations in the KRAS gene. A	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No	unsure	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	CSS was characterized as the length of survival to death from CRC or to the last follow-up date.	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective Folow-up a 5 years	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariate analysis: Alto grado de clusters pobremente diferenciados, tumor incipiente, invasión linfovascular, mutaciones KRAS, mutaciones multiples KRAS y PIK3CA y micormetasiatsis en ganglios linfáticos regionaes	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes. Pathological features, including tumor size (maximum diameter in centimeters), tumor border configuration (expanding or infiltrating), WHO histological grade [10], pTNM stage [11], TB, LVI, grading based on the counting of PDC [12], and the presence of lymph node micrometas- tases (MM) [13], were available in all cases.	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and	Observational retrospective	yes	

	limited reliance on recall).			
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	The Mantel-Cox log-rank test was applied to assess the strength of association between CSS and each of the parame- ters (age and gender of the patient, size of the tumor, WHO histological grade, PDC grade, pT stage, tumor border configuration, TB, LVI, and MM) as a single variable. Subsequently, a stepwise multivariate analysis (Cox regression model) was utilized to determine the independent effect of each variable on survival. Multivariate analysis was carried out by using stepwise method and including only clinicopathological variables with significant prognostic value at univariate analyses.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Multivariate Cox regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Cancer specific survival (CSS) was assessed by the Kaplan-Meier method, with the date of primary surgery as the entry date. CSS was characterized as the length of survival to death from CRC or to the last follow-up date. The Mantel-Cox log-rank test was applied to assess the strength of association between CSS and each of the parameters (age and gender of the patient, size of the tumor, WHO histological grade, PDC grade, pT stage, tumor border configuration, TB, LVI, and MM) as a single variable. Subsequently, a stepwise multivariate analysis (Cox regression model) was utilized to determine the independent effect of each variable on survival. Multivariate analysis was	yes	

		carried out by using stepwise method and including only clinicopathological variables with significant prognostic value at univariate analyses.		
	The selected statistical model is adequate for the design of the study.	Yes. Long rank-tests and multivariate analysis for survival analysis	yes	
Reporting of results	There is no selective reporting of results.	no selective reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Jing Chen et al.** Año de publicación: 2014			
Study identifier	http://www.biomedcentral.com/1471-2407/14/802			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Chinese patients with CRC primary tumors	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	436 consecutive patients diagnosed with colo- rectal cancer at Zhongda Hospital Affiliated to Southeast University (Nanjing, China) from 2007 to 2012	yes	
Recruitment period	Period of recruitment is adequately described	from 2007 to 2012,	yes	
Place of recruitment	Place of recruitment (setting	at Zhongda Hospital Affiliated to	yes	

	and geographic location) are adequately described	Southeast University (Nanjing, China)		
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	35 were excluded because no surgery was performed. An add-itional 140 patients were excluded, as they were lost during follow-up period. Among the 261 patients eligible for the genetic testing, 38 patients were excluded because no tissue blocks were available. An extra 9 patients were excluded from the remaining 223 patients because of poor DNA quality.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	At last 214 patients were included in our study	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: Demographic and clinical characteristics (gender, age,tumor size (maximum diameter in centimeters), tumor border configuration (expanding or infiltrating), WHO histological grade [10], pTNM stage [11], TB, LVI, grading based on the counting of PDC [12], and the presence of lymph node micrometas- tases (MM)	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	140 patients were excluded, as they were lost during follow-up period.At last 214 patients were included in our study	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	summary table of the major clinicopathological characteristics of the patients included and excluded in this study.	yes	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	summary table of the major clinicopathological characteristics of the patients included and excluded in this study. Sex, age, location, differentation, tumor diameter, TMN-stage, synchronous and metacrhonous metastases	yes	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	There was no difference in the major clinicopathological characteristics between the included and excluded patients:	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately**			low

	represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.			
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS exon 2 werw analyzed 34G > A 34G>C 34G>T 35G>A 35G>C 35G>T 35G>T & 35G 37G>T 38G>A	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Genomic DNA was extracted from 5 sections of 10 μm thickness of macro-dissected formalin-fixed paraffin- embedded (FFPE) tumor samples, containing at least 50% tumor epithelium, as determined by an experiencedpathologist in H&E-stained paraffin sections. The QIAmp DNA Mini Kits (Qiagen GmbH, Hilden, Germany) was used according to the manufacturer's instructions. For each sample, exons 9 and 20 of PIK3CA, exon 2 of KRAS, and exon 15 of BRAF were amplified by PCR. The pre- sence of mutations was detected by direct sequencing at Beijing Genomic Institute (BGI, ABI 3730xL Genetic analyzer, Shenzhen, China) using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems). For all PCR products with sequence variants, both forward and reverse sequence reactions were repeated for confirmation.	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	KRAS mutation status in exon 2 was detected in 96 out of 214 (44.9%) tumor samples, of which 70 (32.7%) had a single mutation	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No	unsure	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			

Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Overall survival (OS) was defined as the period from the date of surgery until death from any cause or last follow-up. .	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Folow-up patients.The median follow-up time of surviving patients was 34 months.	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	To correct for significant prognostic factors, variables including age, sex, differentiation grade, tumor dia- meter, number of lymph nodes examined, TNM stage and KRAS/BRAF/PIK3CA genotype were first exam-ined in colon cancer patients with the univariate Cox regression model (Table 6).	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: Age<=65>65 Sex Female Male Differentiation well moderate poor Lymphnode examined >12<=12Tumor diameter <5 cm> = 5 cm TNM-stage I II III IV KRAS status wt mutant BRAF V600E status wt mutant PIK3CA status wt mutant	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes, clinical data of table 1.	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no	no	

Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	To identify factors associated with OS, we evaluated the following clinicopathological variables in a univariate Cox regression model: age (>65 vs ≤65), sex (male vs female), tumor location (colon vs rectum), tumor differentiation grade, tumor diameter (<5 cm vs ≥5 cm), number of lymph nodes examined (<12 vs ≥12), TNM stage, KRAS status (mutant vs wild-type (wt)), BRAF status (mutant vs wt) and PIK3CA status (mutant vs wt). All variables associated with OS with P < 0.1 in the univariate analysis were entered into a Cox multivariate regression model with backward elimination. A two-sided P value of ≤0.05 was considered statistically significant.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.		yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Patients were divided into two groups: group 1 with mutant KRAS/BRAF and group 2 with wild-type KRAS/BRAF. Comparisons of patients with specific mutations versus the wild-type population only concerned mutations representing more than 10% of all mutations detected this study. The end points for these analyses were TTR and DFS	yes	
	The selected statistical model is adequate for the design of the study.	TTR and DFS curves were estimated with the Kaplan–Meier method. Differences between groups of patients were analyzed using unstratified log- rank testsTo identify factors associated with OS, we evaluated the following clinicopathological variables in a univariate Cox regression model: age (>65 vs ≤65), sex (male vs female), tumor location (colon vs rectum), tumor differentiation grade, tumor diameter (<5 cm vs ≥5 cm), number of lymph nodes examined (<12 vs ≥12), TNM stage, KRAS status (mutant vs wild-type (wt)), BRAF status (mutant vs wt) and PIK3CA status (mutant vs wt). All variables associated with OS with P < 0.1 in the univariate analysis were entered into a Cox multivariate regression model with backward elimination. A two-sided P value of	yes	

		≤0.05 was considered statistically significant.		
Reporting of results	There is no selective reporting of results.	Similar results were obtained for DFS. No results of DFS	partial	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**E.M.V. de Cuba et al.** AÑO PUBICACIÓN: 2015			
Study identifier	DOI: 10.1002/ijc.29855			
Reviewer	Elena Chinchilla Ruiz			
	-			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	stage II and III MSI colon cancers.	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	In total, 143 MSI cancer samples from patients diagnosed between 1987 and 2008 with stage II and III MSI colon can- cers were collected. Rectal cancers were not included in the study. of 332 had a MSI cancer.18 Furthermore, 20 patients out of 196 stage II and III CRC patients from an	yes	

		immunotherapy trial were included.19 Finally, 58 stage II and III archival MSI cases from VU University Medical Center were included.		
Recruitment period	Period of recruitment is adequately described	between 1987 and 2008	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	VU University Medical Center	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	no	no	
Adequate study participation	There is adequate participation in the study by eligible individuals	In total, 143 MSI cancer samples	partial	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: Patient and MSI tumor characteristics: gender, age, tumor location, histological type, grade of differentiation, stage, adjuvant chemoteraphy	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			moderate
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Retrospective study, all patents complete the sudy	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	No patients were lost to follow-up.	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	No patients were lost to follow-up.	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	No patients were lost to follow-up.	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	No patients were lost to follow-up.	no	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low

3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS (exon 2 and 3 that include codons 12/13 and 59/61, respectively)	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	DNA isolation, MSI testing, high reso- lution melting and sequencing for BRAF (exon 15 that includes the V600E mutation) and KRAS (exon 2 and 3 that include codons 12/13 and 59/61, respectively) were per- formed according to diagnostic standards	partial	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	KRAS mutations were observed 16% (n : 23) of cases,	partial	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No	unsure	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			moderate
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	CSS was determined from the date of diagnosis to either the date of colon cancer related death or censorship.	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective. Median follow-up time was 6.4 years	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in**			low

	study participants to sufficiently limit potential bias.			
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Input variables for multivariate analysis were: age, gen- der, tumour stage, tumour location, histological type, grade of differentiation and BRAF/KRAS mutation status.	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes, clinical variables at table 1	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Observational retrospective.	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Uni- and multivariate anal- yses were carried out for stage II and III combined and stage-stratified	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Five-year CSS and OS rates were obtained by means of Kaplan–Meier analysis. Sur- vival curves were compared using the log-rank test. The Cox's proportional hazards regression model was used to study the association between survival and	yes	

		the clinicopatho- logical variables in uni- and multivariate analyses.		
	The selected statistical model is adequate for the design of the study.	long-rank test and multivariate analysis	yes	
Reporting of results	There is no selective reporting of results.	no selective reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	Yanhong Deng et al. AÑO PUBICACIÓN: 2015			
Study identifier	http://dx.doi.org/10.1016/j.molonc.2015.03.006			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Patients with stage II or III CRC who underwent a radical resection surgery	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	Patients with stage II or III CRC who underwent a radical resection surgery between January 2007 and April 2012 were consecutively selected from the Gastrointestinal Hospital of Sun Yat-sen University database. All participants provided informed written consent and the study was approved by the Medical Ethics Board of Gastrointestinal Hospital, Sun Yat-sen University	yes	
Recruitment period	Period of recruitment is adequately described	January 2007 and April 2012	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are	Gastrointestinal Hospital of Sun Yat-sen University database	yes	

	adequately described			
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Patients with the following conditions were excluded from the analysis in the present study: (A) presence of other malignancies, (B)underwent single agent chemotherapy, (C) underwent neo-chemoradiotherapy before surgery, (D) died of complications or other diseases during the same hospitalization of the sur- gery, or (E) tumor recurrence within 3 months.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	473 patients with eligible tumor specimens,	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: Patient demographics and disease charecteristics: age, stage. Tstage, N stage, site, grade, CEA	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Among the 453 patients with an available KRAS status, 433 (95.6%) had follow-up data.	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who sropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement**			

	of PF related to the level of outcome).			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS gene exon 2	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Polymerase chain reaction (PCR) was performed using 100 ng of genomic DNA as a template.. Each mixture contained 10 pmol of each primer. The reactions were performed in a total volume of 31.5 mL. The amplification reaction were as follows: an initialdenaturing cycle of 95 C for 5 min; 45 cycles of 94 C for 25 s, 58 Cfor25s,72 Cfor25s;andafinalextensioncycleat72 C for 10 min. The PCR products were then purified and subjected to direct sequencing using an automatic sequencer (ABI-3730 DNA Sequencer; Life Technologies, CA).	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	(38.3%) demonstrated a KRAS mutation (123 patients in codon 12, 43 in codon 13	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	unsure	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	3-years Desease free survival	partial	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective. 3-years DFS	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement	***Outcome of interest* is adequately measured in**			moderate

Summary	**study participants to sufficiently limit potential bias.**			
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	multivariate anal- ysis adjusted for age, stage, grade, site, vessel invasion, CEA level, and adjuvant chemotherapy	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables at table 1	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables at table 1	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).		yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Survival curves were generated using the KaplaneMeier method, while univariate survival distributions were compared using the log-rank test. Hazard ratios and 95% con- fidence intervals for uni- and multivariate models were computed using Cox proportional hazards regression. The chi-square test was used to evaluate	yes	

		categorical variables.		
	The selected statistical model is adequate for the design of the study.	Long rank tests and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	V EklÖet al. AÑO PUBICACIÓN: 2013			
Study identifier	doi: 10.1038/bjc.2013.212			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Colorectal cancer cases from two separateswedish patient groups	yes	

Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	Archival paraffin-embedded CRC tissue samples from a total 414 patients were included from the Colorectal Cancer in Umeå Study (CRUMS), all collected during primary tumour surgery over the period 1995–2003 at Umeå University Hospital, SwedenClinical data were obtained by reviewing the patient records and survival data were collected from the Swedish population registry during autumn 2012 with a median follow-up time of 113 months for patients still alive at the end of follow-up.From the Northern Sweden Health Disease Study (NSHDS), archival paraffin-embedded CRC tissue from a total of 197 patients was included. The NSHDS cohort consists of three separate cohorts: the Va¨sterbotten Intervention Project (VIP), the Northern Sweden WHO Monitoring of Trends and Cardiovascular Disease Study (MONICA) and the local Mammography Screening Project (MSP) (Hallmans et al, 2003). The CRC cases in the NSHDS cohort, protocols and selection principles used in the present study have previously been described in detail (Van Guelpen et al, 2006)NSHDS patients were followed up until January 2008 with a median follow-up time of 102 months for patients still alive at the end of follow-up.	yes	
Recruitment period	Period of recruitment is adequately described	CRUMS cohrt period 1995–2003 NSHAD cohort followed up until January 2008	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Umeå University Hospital, Sweden.	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	no	no	
Adequate study participation	There is adequate participation in the study by eligible individuals	CRUMS cohort: 414. NSHAD cohort: 197	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1a y 1b: clinical caracterísitcs of colorectal cancers: age, sex, tumor site, stage, hystological type,	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low

2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Al ppacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who sropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS gene exon 2	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	CR conditions for KRAS: 50 ng DNA, 0.5 mg primer, 10 mM dNTP, 1 mM MgCl2 and 0.4U JumpStart Taq (Sigma, Stockholm, Sweden) in a total volume of 20 ml. PCR were run at 95 1C 10 min, 95 1C 15 s, 65–55 1C (□ 1 1C/cycle) 72 1C 30 s (touchdown for 10 cycles); 951C 15s, 551C 15s, 721C 30s for 35 cycles and 721C 10 min. Primers used: forward: 50-tgtaaaacgacggccagtgagtttgtattaaaaggtactgg-30. reverse: 50-caggaaacagctatgacctctgtatcaaagaatggtcct-30.	yes	
	Continuous variables are	yes	yes	

	reported or appropriate cut-points (i.e., not data-dependent) are used.			
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	17,9% KRAS mutated at NSHD cohort and 19,5% KRAS mutated in CRUSM cohort	partial	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	unsure	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	ancer-specific events were defined as death with known disseminated or recurrent disease, and cases were censored at the end of follow-up or at time of death by other causes.	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective. median follow-up time of 113 months for CRUMS cohort and median follow-up time of 102 months for NSHD cohort	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	The final multivariate model included sex, age at diagnosis, stage and tumour site	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables at table 1	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind	Yes: obsrvational study, clinical variables at table 1	yes	

	measurement and limited reliance on recall).			
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	To take into consideration other clinico-pathological factors, multivariate Cox proportional hazard models were used. For multivariate analyses, we analysed Quadruple index, KRAS and BRAF and not PIK3CA and PTEN, as the latter two were not significantly associated with prognosis in univariate analyses. The adjusting variables were selected if they affected the risk estimates for KRAS and BRAF 410% in bivariate analyses. The final multivariate model included sex, age at diagnosis, stage and tumour site. Other factors tested, but not meeting the criteria for inclusion in the multivariate analyses were aberrant p53 protein expression, mucinous histologic tumour type, preoperative radiotherapy and adjuvant chemotherapy. Micro-satellite instability screening status and CIMP status were also tested but excluded due to small subgroups and thereby loss of statistical power	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	For cancer-specific survival analyses, Kaplan–Meier plots were used, and differences between groups were tested by log-rank tests. . To take into consideration other clinico-pathological factors, multivariate Cox proportional hazard models were used	yes	

	The selected statistical model is adequate for the design of the study.	Long rank tests and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	Tian-An Guo al. AÑO PUBICACIÓN: 2019			
Study identifier	DOI: 10.1002/ijc.32489			
Reviewer	Elena Chinchilla Ruiz			
	-			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	cases of KRAS, NRAS, BRAF and MMR data at Fudan University Shanghai Cancer Center over the past 5 years to explore clinicopathologic features and prognosis.	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	A database of patients underwent surgical treatment at the Department of Colorectal Surgery at the Shanghai Cancer Center from January 2013 to June 2018 was retrospectively reviewed. Gene information was found in 2,340 patients and 506 of them were confirmed with incomplete information of gene detection or clinicopathologic features. In total, 1,834 patients were included in the analysis.	yes	
Recruitment period	Period of recruitment is adequately described	from January 2013 to June 2018	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are	Fudan University Shanghai Cancer Center	yes	

	adequately described			
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	one case of both KRAS and NRAS mutations, two cases of KRAS and BRAF mutations and three cases of NRAS and BRAF mutations were excluded from the prognostic analysis.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	1,834 patients were included in the analysis.	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: Clinical characteristics: sex, tummor, site, Tumor size, TNM stage, histological, pathology, differentation, lymphovascular invasion, perineural invasion, estranodal tumor, KRAS mutant, NRAS mutant, BRAf mutant, dMMR	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Al ppacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who sropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low

3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS exons 2–4,	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Sequencing was performed in 1,374 cases. KRAS exons 2–4, NRAS exons 2–4 and BRAF exon 15 were evaluated by bidirectional sequence using ABI 3730XL and a BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA)DNA from the other 460 patients was tested using the AmoyDx KRAS/NRAS/BRAF Mutations Detection Kit (Amoy Diagnostics, Xiamen, China) under the principle of the amplifica-tion refractory mutation system (ARMS), covering the detection of KRAS mutations (exons 2–4), NRAS mutations (exons 2–4) and BRAF V600 mutations (exon 15).	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	46,4% KRAS mutant	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	unsure	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Overall survival (OS) was defined as the period of time between the first surgery and death from any cau	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias	Observational retrospective. 5 years of follow-up	yes	

	(e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).			
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Analiis multivariante: Sexo,edad, localización tumoral, histopatología y metástasis extranodales	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables at table 1	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables at table 1	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Ten to fifteen predictors are necessary to proceed with multivariate survival analysis, whereby the selection for independent factors in the mul- tivariate model was based on the univariate results.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and	**Goal: To judge the risk of bias related to the statistical analysis and**			

Reporting	**presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	nalyses identifying prognostic predictors are performed using Cox proportional hazard models. Ten to fifteen predictors are necessary to proceed with multivariate survival analysis, whereby the selection for independent factors in the mul- tivariate model was based on the univariate results. Log-rank tests were employed to identify the associations between OS and pre- dictors and all results are visualized by survival curves using the Kaplan–Meier method.	yes	
	The selected statistical model is adequate for the design of the study.	Long rank tests and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	Tamuro Hayama et al. AÑO PUBICACIÓN: 2019			
Study identifier	DOI: 10.1002/ijc.32489			
Reviewer	Elena Chinchilla Ruiz			
	-			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			

Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Only patients identified as having stage I–III CRC according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	A total of 200 individuals comprising part of a cohort of con- secutive patients with CRC treated via curative resection at the Teikyo University Hospital, Japan, from 2014 through 2016 were included.Standard demographic and clinicopathologic data were col- lected on each patient	yes	
Recruitment period	Period of recruitment is adequately described	from 2014 through 2016	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Teikyo University Hospital, Japan	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	he exclusion criteria were (1) patient received adjuvant chemotherapy, (2) history of familial adenomatous polyposis or Lynch syndrome, and (3) multiple primary malignancies.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	A total of 200 individuals	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 3: Standard demographic and clinicopathologic data were col- lected on each patient, including sex, age, tumor characteris- tics, date of last follow-up, date and type of recurrence, and date of death; other recorded characteristics included AJCC tumor (T) and necrosis (N) stages, tumor site (right vs. left), and nodal status	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Al pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	

Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS codons 12 and 13	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	DNA was isolated using a QIAamp DNA FFPE Tissue Kit (Qiagen, Manchester, UK) and quantified on a Nano Drop c2000 (Thermo Fisher Scientific, Waltham, MA, USA). An assay kit (KRAS RGQ PCR kit; Qiagen) utilizing the Scorpions and Amplification Refractory Mutation system to detect wild-type (control) and specific mutant forms	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	74 KRAS mutations (37%; 74/200) were detected,	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential**			

	measurement of outcome related to the baseline level of PF).			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Recurrence-free survival (RFS) was calculated from the date of surgery to that recurrence	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective.median 850-day-postoperative follow-up period.	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariate model: T stage, N stage and mutation status	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Cox regression analysis was used to identifyfactors significantly associated with RFS. Factors found to be statistically significant in the log-rank test were en- tered into the stepwise Cox regression model to produce the final model of independent prognostic factors.	yes	

	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Comparisons between groups were made with the chi- squared test or Fisher's exact test for proportions, and the Mann–Whitney U test for continuous variables. Recurrence-free survival (RFS) was calculated from the date of surgery to that recurrence using the Kaplan–Meier method. Cox regression analysis was used to identifyactors significantly associated with RFS. Factors found to be statistically significant in the log-rank test were en- tered into the stepwise Cox regression model to produce the final model of independent prognostic factors. P ≤ 0.05 was considered statistically significant.	yes	
	The selected statistical model is adequate for the design of the study.	Long rank tests and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Shigenori Kadowaki et al.** AÑO PUBICACIÓN: 2015			
Study identifier	DOI: 10.3748/wjg.v21.i4.1275			
Reviewer	Elena Chinchilla Ruiz			
	-			
Biases	**Issues to consider for**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**

	judging overall rating of "Risk of bias"			
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Japanese cohort of patients with curatively resected CRC.	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	A total of 813 consecutive stageI-III CRC patients undergoing curative resection at Saitama Cancer Center between July 1999 and May 2006 were in- cluded. Patients were followed-up until death or February 2012, whichever came first	yes	
Recruitment period	Period of recruitment is adequately described	Between July 1999 and May 2006	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Saitama Cancer Cente	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Patients with the following conditions were excluded: (1) history of radiotherapy or che-motherapy preoperatively; (2) inflammatory bowel disease; or (3) history of familial adenomatous polyposis..	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	A total of 813 individuals	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: Patients caractheristics: age, gender, tumor location, histological grade, T stage, LN metastasos, TNM stage, adjuvant chemotherapy, MSI status	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between**			

	***PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Al pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	Exons 2 and 3 of KRAS	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Genomic DNA was extracted from fresh frozen specimens using the standard phenol-chloroform extraction method. Exons 2 and 3 of KRAS were examined for mutations by denaturing gradient gel electrophoresis, as described previously	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	

Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	KRAS mutations were detected in 38%	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	OS was defined as the interval from the date of resection until death due to any cause or until the censor date of February 1, 2012. DFS was defined as the time from the date of resection to tumor recurrence, occurrence of a new primary colorectal tumor, or death due to any cause.	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospectiveThe median follow-up time was 87.7 mo	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Factors for which the multivariate models were adjusted are age (≥ 65 vs < 65), gender (male vs female), tumor stage (III vs II vsI), adjuvant chemotherapy (Yes vs No), and status of MSI and BRAF or KRAS mutations (Yes vs No).	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level,	Yes: clinical variables at table 1	yes	

	and duration of exposures).			
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables at table 1	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Cox proportional hazards models were used to estimate uni- and multivariate adjusted hazard ratios for DFS and OS according to mutation status	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Survival probability was estimated using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards models were used to estimate uni- and multivariate adjusted hazard ratios for DFS and OS according to mutation statuso further evaluate the potential heterogeneity of the impact of KRAS and BRAF mutations according to MSI status and other covariates [age (≥ 65 vs < 65), gender (male vs female), tumor location (distal/rectum vs proximal), and stage (III vsI/II)], we tested the models that included interaction terms, cross-products of gene mutation status, and another variable of interest in a multivariate Cox model	yes	
	The selected statistical model is adequate for the	Long rank tests and multivariate regresion models	yes	

	design of the study.			
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Carsten Kamphues et al.** AÑO PUBICACIÓN: 2020			
Study identifier	DOI: 10.1002/jso.26352			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Patients with non-metastatic CRC (stages I–III)	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	Patients with non-metastatic CRC (stages I–III) who were surgically treated between January 2000 and December 2018 and with known KRAS mutation status were retrospectively identified from institutional databases at four academic tertiary centers in Europe and two in Japan. Participating centers included Charite—University of Berlin (Berlin, Germany), Erasmus Medical Center (Rotterdam, Netherlands), Attiko Hospital (Athens, Greece), Hippokrateion Hospital (Athens, Greece), Saitama Cancer Center (Saitama, Japan), and Graduate School of Medical Sciences, Kumamoto University (Kumamoto, Japan)	yes	
Recruitment period	Period of recruitment is adequately	between January 2000	yes	

	described	and December 2018		
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Participating centers included Charite—University of Berlin (Berlin, Germany), Erasmus Medical Center (Rotterdam, Netherlands), Attiko Hospital (Athens, Greece), Hippokrateion Hospital (Athens, Greece), Saitama Cancer Center (Saitama, Japan), and Graduate School of Medical Sciences, Kumamoto University (Kumamoto, Japan).	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Patients with unknown BRAF mutation status, unknown microsatellite stability (MSI) status, double KRAS/BRAF mutations, as well as those with unknown follow-up were excluded from the study cohort.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	A total of 1093 individuals	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: Patients characteristics: age at the time of diagnosis, sex, neoadjuvant systemic treatments (for those with rectal tumors), primary tumor laterality, tumor category (T) nodal disease category, tumor grade, lymphovascular invasion (LVI), vascular invasion, BRAF status, microsatellite instability (MSI-H) status, and adjuvant systemic treatments were collected. To maintain consistency with previous studies, we defined primary tumors located in the cecum, ascending colon, and transverse colon as right-sided tumors, and tumors located in the splenic flexure, descending colon, sigmoid colon, and rectum as left-sided tumors	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is	Al ppacients complete the study (observational restrospective)	yes	

	adequate.			
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	Kras mutation status	no	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	no reported	no	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	117 patients on right sided, 227 patients on left sided	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			high
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	No clrear definition	no	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement	Observational retrospectiveWith a median follow-up of 73.6 months	yes	

	properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).			
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			moderate
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariate model. Right sided; age, primary tumor nodal metastases, lymphovascular invasion and vein invasion. Left sided: age, male sex, T category, primary tumor nodal metastase, vein invasion and kras status	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Variables that were found to have a statistically sig- nificant association with outcomes on the univariable analysis ($p < .05$) were included in the multivariable analysis.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	

Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	FS and OS were calculated from the date of surgery using the Kaplan–Meier method, and differences in RFS and OS were assessed with the Log-rank test. Cox proportional hazards regression models were used to identify potential predictors of survival. Variables that were found to have a statistically sig- nificant association with outcomes on the univariable analysis ($p < .05$) were included in the multivariable analysis.	yes	
	The selected statistical model is adequate for the design of the study.	Long rank tests and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Li li et al.** AÑO PUBICACIÓN: 2017			
Study identifier	ISSN 1479-6694			
Reviewer	Elena Chinchilla Ruiz			
	-			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	in stage II colorectal cancer patients without adjuvant chemotherapy after radical surgery	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	A total of 160 continuous stage II primary colo-rectal cancer patients who underwent radical resection from the Sixth Affiliated Hospital of Sun Yat-Sen University and Guangdong General Hospital from 1 October 2010 to 30 September	yes	

		2013 were included.		
Recruitment period	Period of recruitment is adequately described	from 1 October 2010 to 30 September 2013	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Sixth Affiliated Hospital of Sun Yat-Sen University and Guangdong General Hospital	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	The inclusion criteria were as follows: diagnosis of primary colorectal adeno-carcinomas by pathology; TNM stage II; follow- up time of at least 2 years (>24 months) and no adjuvant chemotherapy after radical surgery until further disease progression (recurrence, metastasis or death). Exclusion criteria were as follows: diagnosis of hereditary nonpolyposis colorectal cancer; familial adenomatous poly- posis that had developed into malignant colo- rectal cancer; no survival follow-up data and nopathological wax block for subsequent research.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	A total of 160 individuals	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1: clinical pathological data: genfer, age, tumor location, gross type, tissue typing, degree of differentation and TNM stage	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Al ppacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	

Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	exons 2 and 3 of KRAS	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Genomic DNA was extracted from paraffin wax using a DNA extraction kit (QIAamp DNA Tissue Kit, Qiagen, Germany). Sanger sequencing was used to detect the mutations inexons2and3ofKRAS,	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	45,6% mutation frecuency	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	no	no	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective.On follow-up of the 160 patients for 24–56 months	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			moderate
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by**			

	another factor that is related to PF and outcome).			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariate model: The correlations between prognosis and stage II colorectal cancer patients' gender, age, tumor location, TNM stage, pathological classification, histological type and differentiation degree were analyzed.	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	The Cox regression model was used to evaluate the effects of various factors onprognosis, estimate the risk ratio and calculate the 95% CI and p-value	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Survival was analyzed by the Kaplan–Meier method. Univariate analysis was conducted to analyze the relationship among different clini- cal and pathological features, gene mutations, dMMR status and progression-free survival (PFS) and OS, to compare the differences between groups. The Cox regression model was used to evaluate the effects of various factors onprognosis, estimate the risk ratio and	yes	

		calculate the 95% CI and p-value.		
	The selected statistical model is adequate for the design of the study.	multivariate regression model	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Oscar Murcia et al.** AÑO PUBICACIÓN: 2018			
Study identifier	https://doi.org/10.1371/journal.pone.0203051			
Reviewer	Elena Chinchilla Ruiz			
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Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	CRC, available tumour tissue and complete genotyping for BRAF, KRAS, CIMP and MSI status, from the nationwide and multicentre EPICOLON I and EPICOLON II projects	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	We enrolled a population-based cohort of 878 patients with CRC, available tumour tissue and complete genotyping for BRAF, KRAS, CIMP and MSI status, from the nationwide and multicentre EPICOLON I and EPICOLON II projects [13;14] in a retrospective observa-tional study (Fig 1)	yes	
Recruitment period	Period of recruitment is adequately described	between years 2000–2001 in EPICOLON I and 2006–2007 in EPICOLON II.	yes	
Place of recruitment	Place of recruitment (setting and	Hospital General	yes	

	geographic location) are adequately described	Universitario de Alicante		
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Fig 1: low diagram of patients included in the study. Patients excluded: missing values at CIMP, KRAS and BRAF. Multiple imputation at cases with one or two missing markers cohort of 878 patients Table2: Clinical characteristics: median of age, age at diagnosis, sex, TNM stage at disgnosis, tumor location, 1st line chemoterpahy	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals		yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).		yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Al ppacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS muta- tion at exon 1, including codons 12 and 13,	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information	KRAS muta- tion at exon 1, including codons 12 and 13, was identified by DNA	yes	

	on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	direct sequencing. We assessed both mutations by direct amplicon sequencing with BigDye v1.1 terminators and a 3500 Genetic Analyzer		
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	continuous variables are reported as mean ± standard deviation (SD)	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	324 cases had a somatic KRAS mutation (37%)	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	DFS time (interval of time between remission of disease and their reappearance)	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective.The median follow-up was 52 months (interquartile range 16–64)..	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	The multivariate analysis was performed by adjusting for potential confounder and interaction variables (age, sex, TNM stage, and chemotherapy) in a Cox regression model.	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also	Yes: obsrvational study, clinical variables collected	yes	

	characteristics, such as blind measurement and limited reliance on recall).			
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	The multivariate analysis was performed by adjusting for potential confounder and interaction variables (age, sex, TNM stage, and chemotherapy) in a Cox regression model.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	The imputation took into account BRAF and KRAS status, presence of CIMP, MMR status, sex, age, TNM stage, tumour location, treatment with chemotherapy, and DFS time. After imputation, we classified cases into subtypes 1 to 5.For overall prognosis, we compared differences in DFS time (interval of time between remission of disease and their reappearance) among the five subtypes by log rank test in a uni-variate analysis, expressing it graphically with Kaplan-Meier survival curves. The multivariate analysis was performed by adjusting for potential confounder and interaction variables (age, sex, TNM stage, and chemotherapy) in a Cox regression model. Subtype 4 was the subtype of reference.	yes	
	The selected statistical model is adequate for the design of the study.	long rank test and multivariate regresion models	yes	

Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Ryota Nakanishi et al.** AÑO PUBICACIÓN: 2013			
Study identifier	DOI 10.1007/s10147-012-0501-x			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Japanese patients with CRC,	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	We analyzed 254 consecutive patients with CRC who underwent surgical resection at the Department of Surgery and Science, Kyushu University Hospital, between 1994 and 2009. Histological diagnosis was based on the World Health Organization criteria [	yes	
Recruitment period	Period of recruitment is adequately described	between 1994 and 2009	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Department of Surgery and Science, Kyushu University Hospital,	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	no	no	
Adequate study participation	There is adequate participation in the study by eligible individuals	cohort of 878 patients	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table2: Clinical characteristics: median of age, age at diagnosis, sex, TNM stage at disgnosis, tumor location, 1st line chemoterpahy	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed**			moderate

	relationship between PF and outcome.			
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	Al ppacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS at codons 12 and 13	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	were determined by direct sequencing as previously described, Briefly, each region was amplified by PCR using the c-Ki-ras/12 primer set (forward, 50 - GACTGAATATAAAC TT GTGG-30 ;Purified PCR products were used as a template for cycle sequencing reactions using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). We confirmed all muta- tions by pyrosequencing using PyroMark KRAS v2.0 kit and BRAF Pyro kit according to the manufacturer's instructions (Qiagen, Hilden, Germany).	yes	
	Continuous variables are reported or	yes	yes	

	appropriate cut-points (i.e., not data-dependent) are used.			
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	33.5 % (85/254)	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	no	no	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective.The median fol- low-up time of these patients was 44.1 months (range, 1.0–189 months).	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			moderate
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariate analysis: tumor grade, infiltration, lympatic invasion and BRAF status	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Survival data were evaluated using the multivariate Cox proportional hazards model	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	

Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Survival curves were plotted using the Kaplan–Meier method, and the log-rank test was used to determine associations between individual variables and survival. Survival data were evaluated using the multivariate Cox proportional hazards model.	yes	
	The selected statistical model is adequate for the design of the study.	long rank test and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Ehsan Nazemalhosseini-Mojarad et al.** AÑO PUBICACIÓN: 2019			
Study identifier	https://doi.org/10.1007/s12029-019-00201-4			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Iranian CRC patients.	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	A total of 258 consecutive stages I–IV CRC patients, who underwent surgical resection of adenocarcinoma at	yes	

		gastroenter- ology and liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, from 2012 to 2016, were enrolled in this research		
Recruitment period	Period of recruitment is adequately described	from 2012 to 2016,	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran,	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Patients with the following conditions were excluded: Familial Adenomatous Polyposis coli (FAP) or hereditary non-polyposis CRC (HNPCC)	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	A total of 258	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Patients characteristicsDemographic and clinical information including age, sex, fam- ily history, tumor location, metastasis, tumor differentiation, and MSI status.	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor	**Goal: To judge the risk of measurement bias related to how PF was measured (differential**			

Measurement	**measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS codons 12, 13, and 61	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Pyrosequencing of KRAS codons 12, 13, and 61 was per- formed using the Therascreen KRAS Pyro Kit (QIAGEN), by manufacture's protocols. For pyrosequencing prepara- tion firstly, KRAS was amplified by primers in which one of them was biotinilated to immobilize with straptavidin beads (GE healthcare). PCR-Pyrosequencing reaction car- ried out on Thermocycler (eppendorf) contains 10 ng of genomic DNA. Two sets of seq primer (Therascreen KRAS Pyro Kit QIAGEN) were used for analysis of mu- tations in codons 12/13 and 61. Pyromark Q24 version2 software was applied to analyze Pyrosequencing results. Detection limit (LOD) for KRAS mutations was obtained as 3% by Pyro Kit (QIAGEN).	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	KRAS mutation was detected in 19 (15.4%) patients with MSS/MSI-L tumors at exon 2 (codons 12 and 13).	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Overall survival was computed since the date of cancer diagnosis up to the date of death or end of	yes	

		follow-up: May 2016.		
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective. The median follow-up time for overall survival (OS) was 5 years.	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariate analysis: gender, location of tumor, differentiation, tNM stage, family history, chemoteraphy, KRAS status and age od fisgnose	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Overall survival analyses were done through a Cox proportional hazard models that were used to estimate univariate and multivariate adjusted hazard ratio for OS according to mutation status.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	

Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Survival analyses were determined using variables as following: sex, age, tumor-node-metastasis stage, tumor location (colon versus rectum), and differentiation grade (well, moderate, and poor), family history, age of diagnosis, and MSI status. Prognosis of BRAF and KRAS mutations was evaluated according to overall survival (OS). Overall survival was computed since the date of cancer diagnosis up to the date of death or end of follow-up: May 2016. Overall survival analyses were done through a Cox proportional hazard models that were used to estimate univariate and multivariate adjusted hazard ratio for OS according to mutation status. Kaplan-Meier (log-rank test) curves were plotted. Statistical significance was recorded if P value was less than 0.05. .	yes	
	The selected statistical model is adequate for the design of the study.	long rank test and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Shuji Ogino et al.** AÑO PUBICACIÓN: 2019			
Study identifier	DOI: 10.1158/1078-0432.CCR-09-1570			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and**			

	***outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	stage III colon cancer patients enrolled in a National Cancer Institute (NCI)-sponsored clinical trial of postoperative adjuvant chemo-therapy (27	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	Patients in this study were participants in the NCI-sponsored Cancer and Leukemia Group B (CALGB) adjuvant ther- apy trial for stage III colon cancer comparing therapy with the weekly Roswell Park regimen of 5-fluorouracil (FU) and leucovorin (FU/LV) with the weekly bolus regimen of irinotecan, FU, and leucovorin (IFL; CALGB 89803; ref. 27). From April 1999 to May 2001, 1,264 patients were enrolled in the treatment trial.he current analysis was limited to 508 pa-tients for whom archived formalin-fixed paraffin-embedded tumor tis-sue was available and the KRAS gene was sequenced.	yes	
Recruitment period	Period of recruitment is adequately described	From April 1999 to May 2001	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	ALGB Statis- tical Center and Dana-Farber Cancer Institute	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Patients in the treatment trial (and thus this companion study) were eligible if they had undergone a com-plete surgical resection of the primary tumor within 56 d prior to study entry, and had regional lymph node metastases (stage III colon cancer) but no evidence of distant metastases. Moreover, patients were required to have a baseline Eastern Cooperative Oncology Group performance status of 0 to 2 (ambulatory; ref. 28) and have adequate bone marrow, renal, and hepatic function.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	A total of 508	yes	

Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Baseline characteristics: sec, age, mean age, body mass index, tumor location, T stage, N stafe, AJCC tumor dtage, performance status socore, clinical bowel perforation, clinical bowel obstruction, MSI status, treatment arm	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	We compared the baseline characteristics of the patients who were included in this study (with available KRAS data, n = 508) with those who were excluded from this study due to unavailability of tissue data (n = 756). We did not detect any significant or substantial difference between these two groups	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS codons 12, 13,	yes	

Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	DNA was extracted from paraffin-embedded tissue of colon cancer as previously described (29). We marked a tumor area on a H&E-stained slide, and dissected the tumor area from another tu-mor tissue section by a sterile needle for subsequent DNA extraction. PCR and pyrosequencing spanning KRAS codons 12 and 13 were done as previously described (29), and validated against Sanger sequencing method (29, 40). In our KRAS pyrosequencing assay, we routinely con- firmed the presence of a mutation by two different sequencing primers and by the creation of frameshifted reading of a mutant sequence rela- tive to a wild-type sequence in a pyrogram (	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	KRAS mutation in 178 (35%) patients.).	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	he primary end point was DFS, defined as time from the study enrollment to tumor recur- rence, occurrence of a new primary colon tumor, or death from any cause. In addition, we defined RFS as the time from the study enroll- ment to tumor recurrence or occurrence of a new primary colon tumor. For RFS, patients who died without known tumor recurrence were	yes	

		censored at last documented evaluation by treatment provider. Finally, OS was defined as the time from the study enrollment to death from any cause..		
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective. With median follow-up of 6.2 years a	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	We used stage-matched (or stratified) Cox proportional hazard models to calculate the HR of events according to tumoral KRAS status, adjusted for age at study entry (as a continuous variable), gen- der, baseline body mass index (≥30 versus <30 kg/m2), baseline perfor- mance status (0 versus 1-2), presence of bowel perforation or obstruction at time of surgery, treatment arm, tumor location (proximal versus distal), and MSI status (high versus low/MSS).	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable	We used stage-matched (or stratified) Cox proportional hazard models to	yes	

	groups).	calculate the HR of events according to tumoral KRAS status, .		
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	e Kaplan-Meier method was used to describe the distribu- tion of survival time according to KRAS status, and the log-rank test was carried out. We used stage-matched (or stratified) Cox proportional hazard models to calculate the HR of events according to tumoral KRAS status, adjusted for age at study entry (as a continuous variable), gen- der, baseline body mass index (≥30 versus <30 kg/m2), baseline perfor- mance status (0 versus 1-2), presence of bowel perforation or obstruction at time of surgery, treatment arm, tumor location (proximal versus distal), and MSI status (high versus low/MSS).	yes	
	The selected statistical model is adequate for the design of the study.	long rank test and multivariate regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**TOSHIRO OGURA et al.** AÑO PUBICACIÓN: 2014			
Study identifier	DOI: 10.3892/or.2014.3165			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	onsecutive primary CRC patients	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	he present study was conducted on 1,304 consecutive primary CRC patients at the Saitama Cancer Center from July 1999 to July 2008. Information on clinical data, including age at diagnosis, gender, tumor size, histological differentiation, tumor location, International Union against Cancer (UICC) stage and prognosis were collected from medical records. Tissue samples were surgi-cally excised after obtaining informed consent from each patient.	yes	
Recruitment period	Period of recruitment is adequately described	from July 1999 to July 2008.	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Saitama Cancer Center	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	NO	no	
Adequate study participation	There is adequate participation in the study by eligible individuals	A total of 1,304	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Clinicopathological and molecular features of all of the CRC samples: gender, age, location, tumor size, histological features, stage, KRAS, NRAS, BRAS and MSI	yes	

		status		
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			moderate
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS exon 2, 3 and 4	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	RAS mutations in exon 2 and 3 were detected by denaturing gradient gel electrophoresis (DGGE), and BRAF mutations in exon 15 by PCR-restriction fragment length poly-morphism (RFLP), as previously described. KRAS exon 4 using a Rotor-Gene Q (Qiagen, Hilden, Germany).	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	KRAS mutations were detected in	yes	

		42.4% (n=553		
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Overall survival (OS) time was calculated from the date of surgery to the date of death by any cause or censored at the last follow-up visi	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective. The median follow-up period was 5.6 years (interquartile range, 4.1-7.8 years)	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariate analysis: age, gender, tumor location, KRAS, NRAS, BRAF mutant, MSS, hystological subtype, mucionous components and extramural venous invasion	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	A multivariable model stratification by UICC stage was performed.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	

Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Overall survival (OS) time was calculated from the date of surgery to the date of death by any cause or censored at the last follow-up visit. Cox proportional hazards analysis was used to estimate clinicopathological- and biomarker-specific survival hazard ratios (HRs) and 95% confidence intervals (CIs). A multivariable model stratification by UICC stage was performed. All P-values were calculated from two-sided test, and P-values <0.05 were considered statistically signifi-cant.	yes	
	The selected statistical model is adequate for the design of the study.	cox proportional harzards analysis	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**A l Phipps et al.** AÑO PUBICACIÓN: 2013			
Study identifier	doi: 10.1038/bjc.2013.118			
Reviewer	Elena Chinchilla Ruiz			
	-			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues

1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	invasive CRC conducted in Western Washington State.	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	Details of the population-based study samples have been published elsewhere (Newcomb et al, 2007a, b). Briefly, eligible participants included men and women diagnosed with invasive CRC between January 1998 and June 2002 who, at the time of diagnosis, were aged 20–74 years and resided in King, Pierce, or Snohomish counties in Western Washington State. Women who resided in 10 additional Washington counties and were diagnosed during the same time period at ages 50–74 years were also eligible. During a second phase of study recruitment, we identified eligible participants as men and women with invasive CRC in this 13-county ascertainment area who were diagnosed at ages 18–49 years between April 2002 and July 2007At an average of 8.6 months after diagnosis, participants completed a structured telephone interview in which they were asked to provide detailed information on a number of potentialrisk factors, including smoking history, body mass index (BMI), family history of CRC, and use of selected medications. At the conclusion of the interview, participants were asked for consent to access diagnostic tumour specimens	yes	
Recruitment period	Period of recruitment is adequately described	etween January 1998 and June 2002	yes	

		andbetween April 2002 and July 2007.		
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Surveillance, Epidemiology, and End Results (SEER) cancer registry serving Western Washington State.	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Study eligibility was limited to English speakers with a publicly available telephone number. Of 3585 individuals contacted and identified as eligible, 463 (13%) were deceased, 351 (10%) refused participation, 128 (4%) could not be reached, and 24 (0.7%) completed only a partial interview.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	In total, 76% of eligible cases were enrolled in the study (N 1/4 2708).	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Study population characteristics: age at diagnosis. Sex, tumor site, stage at diagnosis, MSI status, BRAF mutation status and vital status	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low

3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS exon 2	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	DNA was extracted from paraffin-embedded formalin-fixed tumour tissue. In cases for whom tumour DNA was successfully extracted (N1⁄41989), the coding sequence of KRAS exon 2 was amplified (Oliner et al, 2010). Mutations in exon 2 were identified via forward and reverse sequencing of amplified tumour DNA (Alsop et al, 2006). Cases for whom KRAS testing failed (N 1⁄4 36) or produced equivocal results (N1⁄430) were classified as having unknown KRAS-mutation status. For quality control purposes, sequencing was also conducted on three cell-line controls (one containing the p.G12V mutation, one containing the p.G13D mutation, and one wild-type cell line).	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	Approximately 31% of cases had KRAS-mutated CRC.	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	The time axis for analysis was definedas days since diagnosis, with left censoring of participants until the date of study	yes	

		enrollment. We conducted separate survival analyses for disease-specific survival and overall survival. In all analyses, participants still alive at their last vital-status assessment were censored at that date. In analyses of disease-specific survival, we also censored persons who died due to causes other than CRC at the time of death		
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective. study follow-up period (mean ¼ 6.5 year	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Regression models included adjustment terms for age (5-year categories), sex, and study phase.Of these additional factors, only cigarette smoking and BMI were retained in our final analytic model as adjustment for other variables had minimal impact on effect estimates	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Regression models included adjustment terms for age (5-year categories), sex, and	yes	

		study phase.		
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	We evaluated associations between KRAS- mutation status and survival outcomes in the full cohort and within strata defined by patient characteristics (age at diagnosis, sex) and tumour characteristics (tumour site, stage, MSI status).Finally, we explored associations between different classes of KRAS mutations and survival outcomes, examining associations with specific mutations evident in X5% of cases, and, more generally, with codon 12 mutations and codon 13 mutations separately; differences in codon-specific associations were evaluated via tests for hetero-geneity.Regression models included adjustment terms for age (5-year categories), sex, and study phase. We also assessed potential confounding by several patient and tumour characteristics: cigarette smoking (never, former, current); BMI 2 years before diagnosis (o25.0, 25.0–29.9, X30.0kgm□2); race (white, non- white); regular use of non-steroidal anti-inflammatory drugs at baseline (no, yes); family history of CRC in first-degree relatives (no, yes); and tumour site (proximal colon, distal	yes	

		colon/rectum). Of these additional factors, only cigarette smoking and BMI were retained in our final analytic model as adjustment for other variables had minimal impact on effect estimates (o5% change).		
	The selected statistical model is adequate for the design of the study.	cox proportional harzards analysis	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**J. Smeby et al.** AÑO PUBICACIÓN: 2018			
Study identifier	doi/10.1093/annonc/mdy085/4922418			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	consecutive series (Oslo-series) of patients treated surgically for stage I-IV CRC	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	Totally 1197 primary tumor samples from a consecutive series (Oslo-ser- ies) of patients treated surgically for stages I–IV CRC at Oslo University Hospital, Norway between 1993	yes	

		and 2014 were analyzed (supplementary Table S1, available at Annals of Oncology online). Formalin-fixed paraf-fin-embedded tumor tissue was available from patients operated between 1993 and 2003 (n 1/4 761), while fresh frozen samples were available from patients operated between 2005 and 2014 (n1/4436).		
Recruitment period	Period of recruitment is adequately described	between 1993 and 2014	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Oslo University Hospital, Norway	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	no	no	
Adequate study participation	There is adequate participation in the study by eligible individuals	Totally 1197 primary tumor samples	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Distribution of mutations acording to clinicpathological and molecular characteristics: age, gender, MSI status, CMS, location, stage, pT, pN, differentiation, KRAS and BRAF	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			moderate
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor	**Goal: To judge the risk of measurement bias related to how PF was measured**			

Measurement	**(differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	exon 2: codons 12 and 13, exon 3: codon 61)	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	DNA extraction, determination of MSI status, and Sanger sequencing of mutation hotspots in KRAS	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	mutation rates of 31%	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Five-year OS and relapse-free survival were defined according to the guidelines by Punt et al. [26].	partial	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective 5 years follow up	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariable analysis: gender, age, MSI status, location, stage, diferentatiosn and KRAS, BRAF mutations	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing	Appropriate methods are used if imputation is	no missing confunder	no	

data	used for missing confounder data.	data		
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Multivariate regresion model	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	(supplementary Data, available at Annals of Oncology online)	partial	
	The selected statistical model is adequate for the design of the study.	long rank test and multivariate harzard ratios	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			moderate

Author and year of publication	**Xiang-Bin Wan et al.** AÑO PUBICACIÓN: 2019			
Study identifier	DOI: 10.3748/wjg.v25.i7.808			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	CRC patients receiving treatment at the Affiliated Tumor Hospital of Zhengzhou University	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	Tissue samples and clinical data (including gender, age at disease onset, tumor site, metastasis site, and tumor differentiation	yes	

		and stage) were collected from 220 CRC patients receiving treatment at the Affiliated Tumor Hospital of Zhengzhou University from January 2012 to December 2013		
Recruitment period	Period of recruitment is adequately described	from January 2012 to December 2013	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	Affiliated Tumor Hospital of Zhengzhou University	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	no	no	
Adequate study participation	There is adequate participation in the study by eligible individuals	220 CRC patients	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Clinicopathological features: including gender, age at disease onset, tumor site, metastasis site, and tumor differentiation and stage	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			moderate
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS codon 12 and codon 13	yes	

Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Formalin-fixed paraffin-embedded (FFPE) tissue samples were sectioned (3-5 μm thick) and deparaffinized through a series of xylene and ethanol solutions using standard procedures[18]. DNA was extracted from the sections using a QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was purified by ethanol precipitation, dissolved in distilled water, and analyzed for concentration and purity using a spectrophotometer (OD260/OD280 = 1.8 ± 0.2, OD260/OD230 ≥ 1.7). The total yield per sample was > 50 ng.The KRAS gene mutation status was analyzed by real-time qPCR using a Human KRAS Gene Mutation Detection Kit (Beijing ACCB Biotech Ltd., Beijing, China). Pre-denaturation was performed at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 60 s	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	62 (31.6%) carried a KRAS mutation i	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	overall survival and profresion free survival	partial	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of	Observational retrospective 4 years follow up	yes	

	outcome with valid and reliable test).			
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			moderate
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariable analysisMutación KRAS, MEK, ERK, BRAF, estadío T y N	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Multivariate regresion model	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	rogression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method and the log-rank test. A Cox proportional hazards model was applied to identify predictors of OS and disease-free survival.	yes	
	The selected statistical model is adequate for the design of the study.	long rank test and multivariate harzard ratios	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Abolfazl Yari et al.** AÑO PUBICACIÓN: 2020			
Study identifier	https://doi.org/10.1007/s12029-020-00426-8			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Southeast Iranian colorectal cancer (CRC) patients.	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	A hundred formalin-fixed, paraffin-embedded (FFPE) tumor blocks from patients diagnosed with colorectal cancer from February 2012 to August 2015 at the three different hospitals (Afzalipour, bahonar and mehregan hospitals) throughout Kerman province (southeast of Iran) were retrieved. Demographic, clinical, and clinicopathological data were obtained by reviewing the medical records	yes	
Recruitment period	Period of recruitment is adequately described	rom February 2012 to August 2015	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	three different hospitals (Afzalipour, bahonar and mehregan hospitals) throughout Kerman province (southeast of Iran)	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	The population study included patients with initial diagnosis of CRC and no patients had accepted adjuvant treatment at the time of sampling.no patients received anti-EGFR and/or anti- VEGF therapy during the study perio	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	100 CRC patients	yes	

Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Demographics and clinicopathological features: age of diagnosis, sex, smoking status, alcohol intake, family history, tumor location (right, left or rectum), differentiation grade (well, moderate or poor), TNM stage (I, II, III, or IV), lymph node metastasis, and distant metastasis.	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	KRAS (exon 2 and 3)	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	DNA was extracted from FFPE specimens using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol.The mutational analysis of KRAS (exon 2 and 3) was per-formed using PCR products and bidirectional sequencing from DNA samples. The primers used to evaluate exon 2 [14] and 3 [15] of KRAS were as	yes	

		previously described.		
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	KRAS mutation was identified in 29 (29%) of all the patient samples.	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Overall survival (OS) was defined since the date of diagnosis up to the date of death or last of follow-up visit.	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective 5 years follow up	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariaye analysis: sex, ahe, smoking status, alcohol intake, familiy history, tumor location, tumor size, differentiation, TNM stage, lymph node metastasis and distant.	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Multivariatelogistic regression analysis	yes	

	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	Logistic regression models were used to analyze the association based on the estimation of the odds ratios (ORs) and 95% confidence intervals (CIs). Overall survival (OS) was defined since the date of diagnosis up to the date of death or last of follow-up visit. The overall survival was plotted and analyzed by Kaplan–Meier (log-rank test). All statistical analyses were conducted by using SPSS 22.0 statistical package (SPSS Inc., Chicago, IL, USA). All p values were two-sided. The statistical significance was con- sidered if the p value < 0.05.	yes	
	The selected statistical model is adequate for the design of the study.	long rank test and logistic regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	Ye Yuan et al. AÑO PUBICACIÓN: 2021			
Study identifier	htOtpsl://1d0o.i1.o1r7g/71/01.17127476/1070284261 0018021711051721			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues

1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).	Chinese colorectal cancer patients	yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	A total of 7189 CRC patients (iCohort) were collected from January 2013 to December 2019.The following clini- cal characteristics were abstracted: age, sex, and tumor histology. Included in the study were 145 patients diagnosed with stage II–IV CRC at The Third Affiliated Hospital of Soochow University (sCohort) from January 2010 to December 2019. The clinical data of these patients were pooled retrospectively, and the factors included in the analysis were age, sex, pathology, clinical stage, and sur- vival. In addition, the KRAS state in the sCohort were detected by droplet digitalTM polymerase chain reaction (ddPCR)	yes	
Recruitment period	Period of recruitment is adequately described	from January 2010 to December 2019.	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	The Third Affiliated Hospital of Soochow University	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	Germline alterations were excluded.	partial	
Adequate study participation	There is adequate participation in the study by eligible individuals	total of 7189 CRC patients, only 145 survival information	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Clinicopathological characteritics of colorectal cancer patients of sCohort: age, sex, TNM stage, T stage, M stage, N stage, Tumor differentation and tumor location	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	

Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	whole exome	yes	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Genomic DNA was isolated from tissue samples using the ReliaPrepTM FFPE gDNA Miniprep System (Promega) and quantified using the QubitTM dsDNA HS Assay Kit (Thermo Fisher Scientific) following the manufacturers' instructions.KRAS mutations were detected by whole exome sequencing with 800× sequencing depth in a College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA) certified laboratory of 3D Medicines Inc. All pathologic or likely pathologic mutations of KRAS were considered. KRAS mutations, including single nucleotide variation, insertions/deletions, copy number variations, gene rearrangement, and fusions were assessed. Germline alterations were excluded.	yes	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	51 of 145 CRC patients were confirmed to have KRAS mutations (	yes	
Method used for missing	Appropriate methods of imputation are used for	No misisng data	yes	

data	missing 'PF' data.			
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			low
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	Progression-free survival was defined as the time from the date of first-line therapy administration to the progression of cancer, or death from any cause. OS was calculated from the date of first-line therapy administration to the date of death from any cause.	yes	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective 9 years follow up	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			low
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	Multivariaye analysis:agem tumor differentation and KRAS mutation	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.	Yes	yes	
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	multivariate analysis	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			

Reporting				
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	urvival description was illustrated by Kaplan–Meier curves, with the P-value determined by a log-rank test. HR was deter- mined through univariate and multivariate Cox regression. The associations between response and variables were examined by a univariate logistic regression. Variables with significant P-values or interest were included in the multivariate logistic regression.	yes	
	The selected statistical model is adequate for the design of the study.	long rank test and logistic regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low

Author and year of publication	**Meifang Zhang et al**. AÑO PUBICACIÓN: 2021			
Study identifier	htOtpsI://1d0o.i1.o1r7g/71/01.17127476/1070284261 0018021711051721			
Reviewer	Elena Chinchilla Ruiz			
Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Study Methods & Comments**	**Rating of reporting**	**Rating of "Risk of bias"**
Instructions to assess the risk of each potential bias:	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some 'issues' may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text exerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST).		yes	
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)	This retrospective cohort study included patients with incidental CRC diagnosed during 2010–2014 and recorded statuses of KRAS and tumor deposit	yes	

		in the National Cancer Database of the USA		
Recruitment period	Period of recruitment is adequately described	during 2010–2014	yes	
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described	National Cancer Database of the USA	yes	
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).	inclu- sion criteria were all incident CRC cases diagnosed during 2010–2014, with data of KRAS status, which became part of the NCDB (as Site-specific factor 9) for CRC in 2010.	yes	
Adequate study participation	There is adequate participation in the study by eligible individuals	total of 7189 CRC patients, only 145 survival information	yes	
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST).	Table 1:Baseline characteristics of resected incident, colorectal cancers with known KRAS status in National Cancer Database diagnosed during 2010–2014: age, sex, tumor location (colon versus rectum), microsatellite instability (MSI) status, KRAS status, pathologic tumor stage (the 7th AJCC staging manual, according to the data item TNM_EDITION_NUMBER), tumor grade (high ver- sus low), race, Charlson–Deyo score, chemotherapy status, and radiotherapy status.	yes	
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**			low
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.	All pacients complete the study (observational restrospective)	yes	
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.	no patients who dropped the study	no	
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.	no loss of follow-up	no	
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST).	no loss of follow-up	no	
	There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.	no loss of follow-up	yes	
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**			low

3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**			
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).	NO	no	
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	NO	no	
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.	yes	yes	
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.	Yes	yes	
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.	38%	yes	
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.	No misisng data	yes	
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**			moderate
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.	he end point was the OS	partial	
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).	Observational retrospective 4 years follow up	yes	
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.	Yes	yes	
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**			moderate
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.	P of multivariate Cox regression analyses adjusted for age, tumor grade, pathologic stage, Charlson–Deyo score, chemotherapy status, radiotherapy status, and race	yes	
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).	Yes: clinical variables collected	yes	
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).	Yes: obsrvational study, clinical variables collected	yes	
Method and Setting of Confounding	The method and setting of confounding measurement are the same for all study	Yes	yes	

Measurement	participants.			
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.	no missing confunder data	no	
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).	Multivariable logistic regression analyses were con- ducted to identify the factors independently linked to tumor deposit status and CRC OS.	yes	
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).	Cox multivariate regresion model	yes	
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**			low
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.	yes	yes	
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.	ogistic regresion models were used to assess potential associations. Multivariable Cox regression models with time-varying covariates were used for survival analyses, including the factors that had a p value less than 0.10 in univariate Cox regression models. Only the factors with significant time-variance were included as time-varying covariate. T.	yes	
	The selected statistical model is adequate for the design of the study.	logistic regresion models	yes	
Reporting of results	There is no selective reporting of results.	no selecitve reporting results	yes	
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**			low