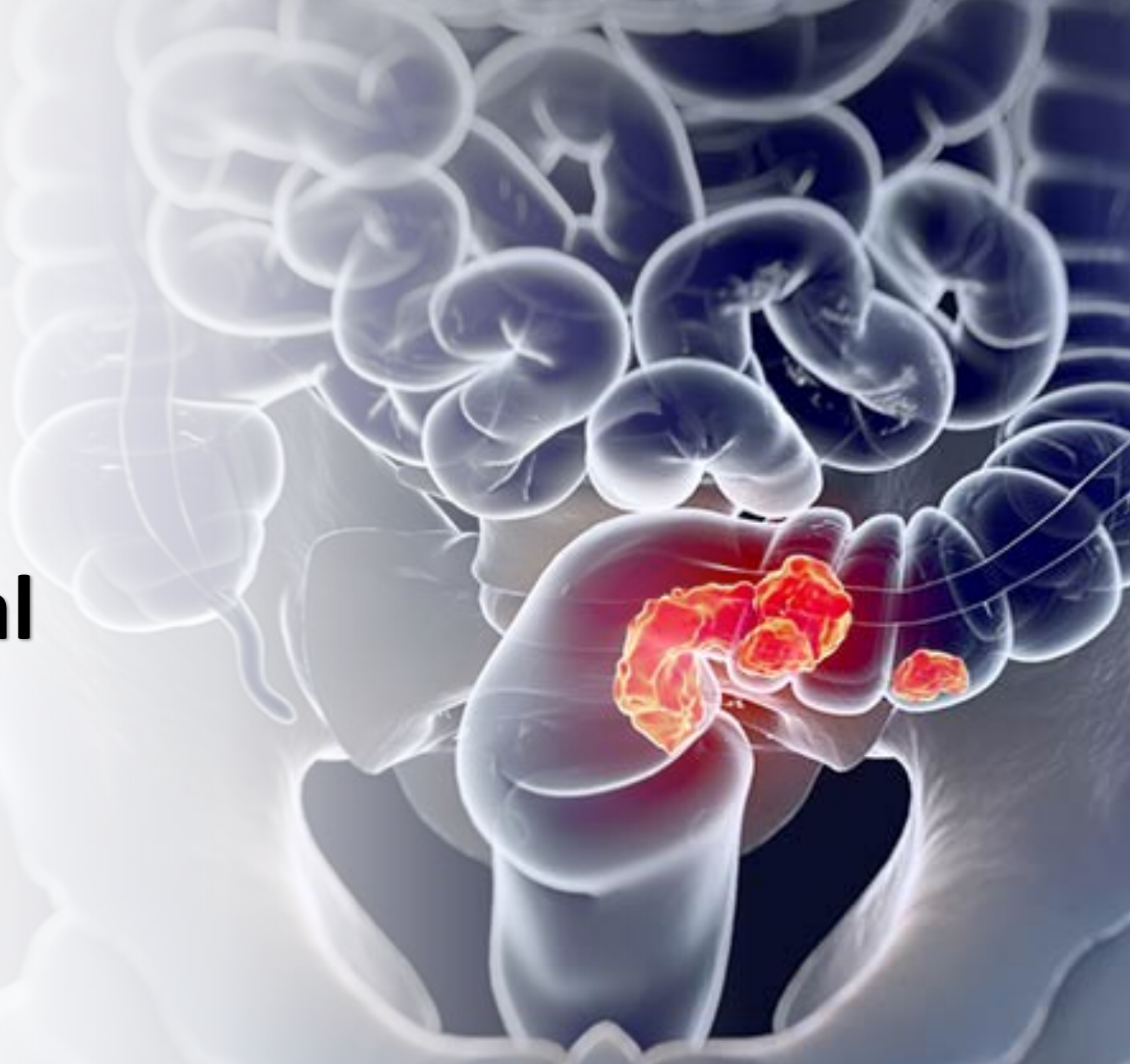
Asociación de los polimormos C677T y A1298C de la enzima MTHFR con la efectividad del tratamiento con quimioterapia en pacientes con cáncer colorrectal metastásico

Allan Ramos-Esquivel

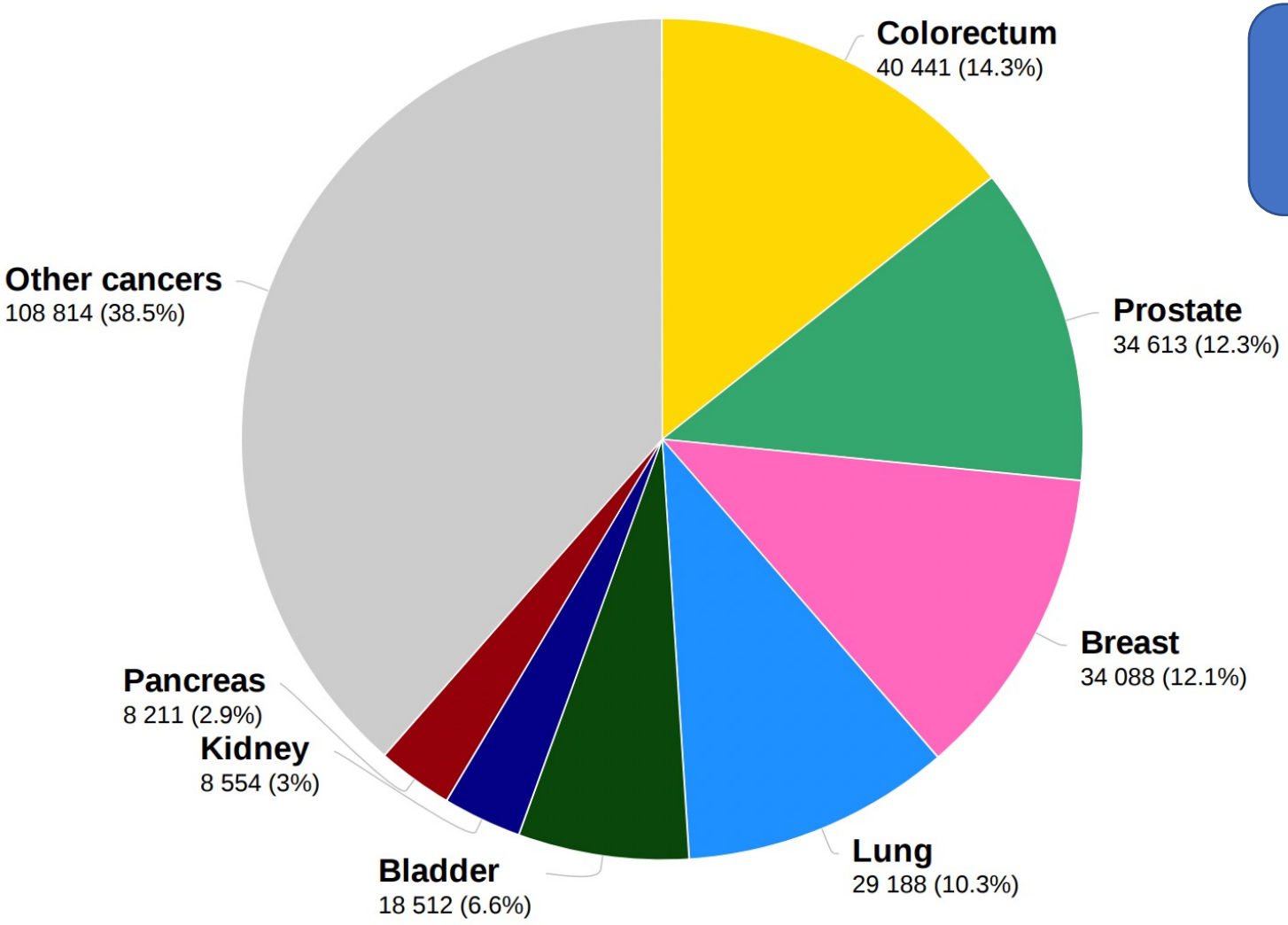
Jornades Doctorals en Farmacologia
2021

Universitat Autònoma de Barcelona

Cáncer colorrectal



Estimated number of new cases in 2020, Spain, both sexes, all ages



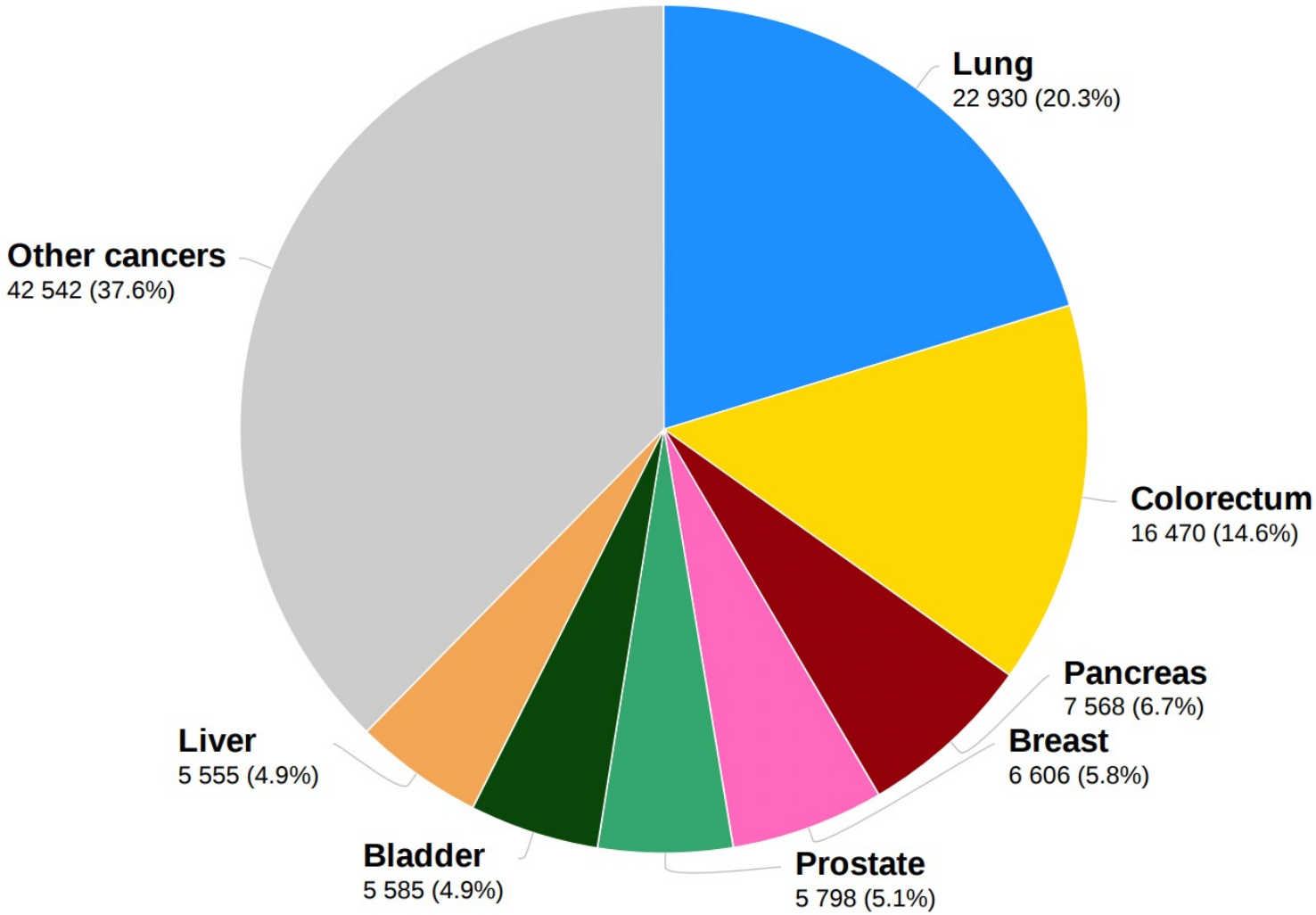
Malignidad más diagnosticada en hombres y mujeres

Total : 282 421

Data source: Globocan 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr)

International Agency for Research on Cancer
World Health Organization

Estimated number of deaths in 2020, Spain, both sexes, all ages



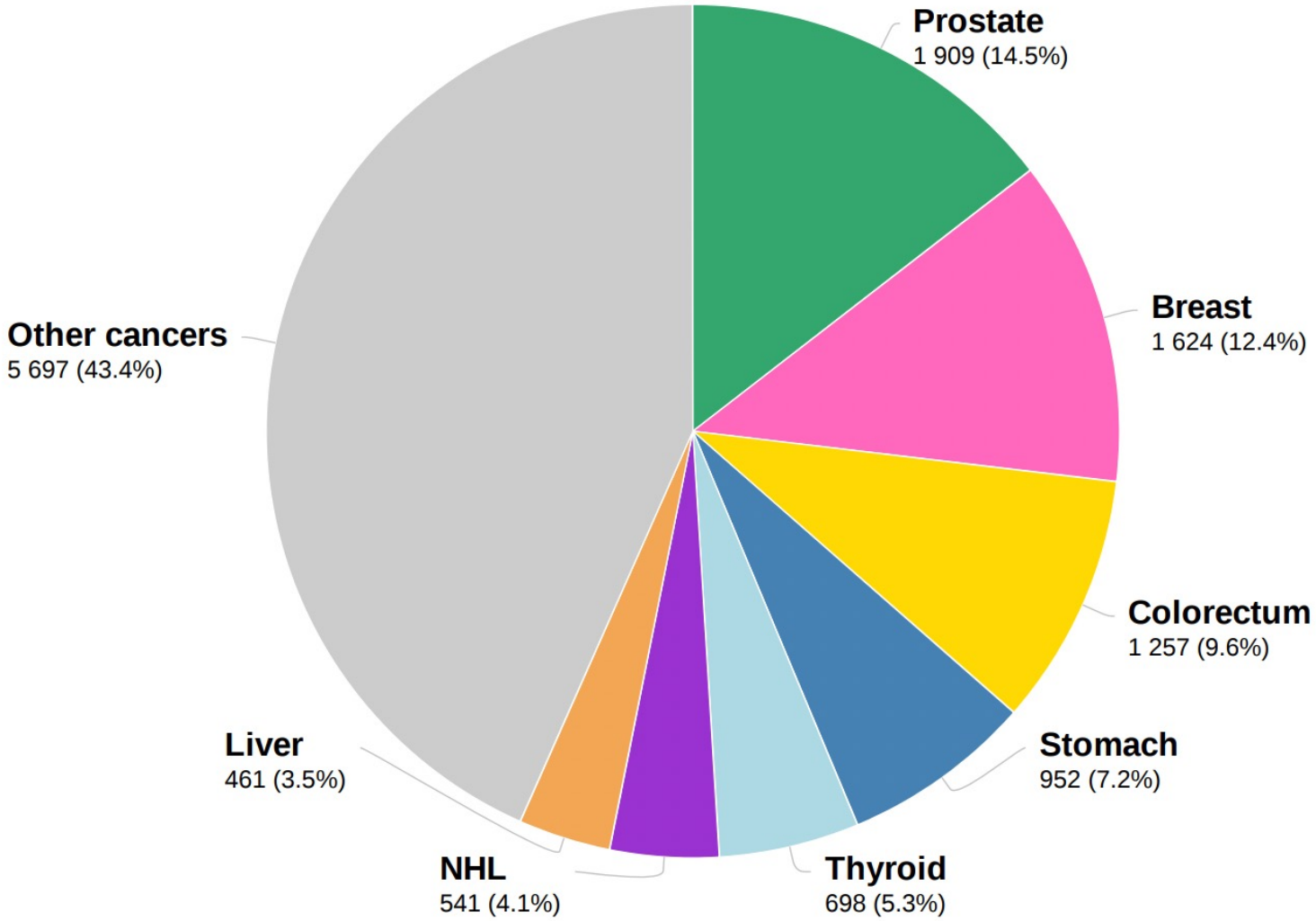
Total : 113 054

Segunda causa de mortalidad por cáncer

Data source: Globocan 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr)

International Agency for Research on Cancer
World Health Organization

Estimated number of new cases in 2020, Costa Rica, both sexes, all ages



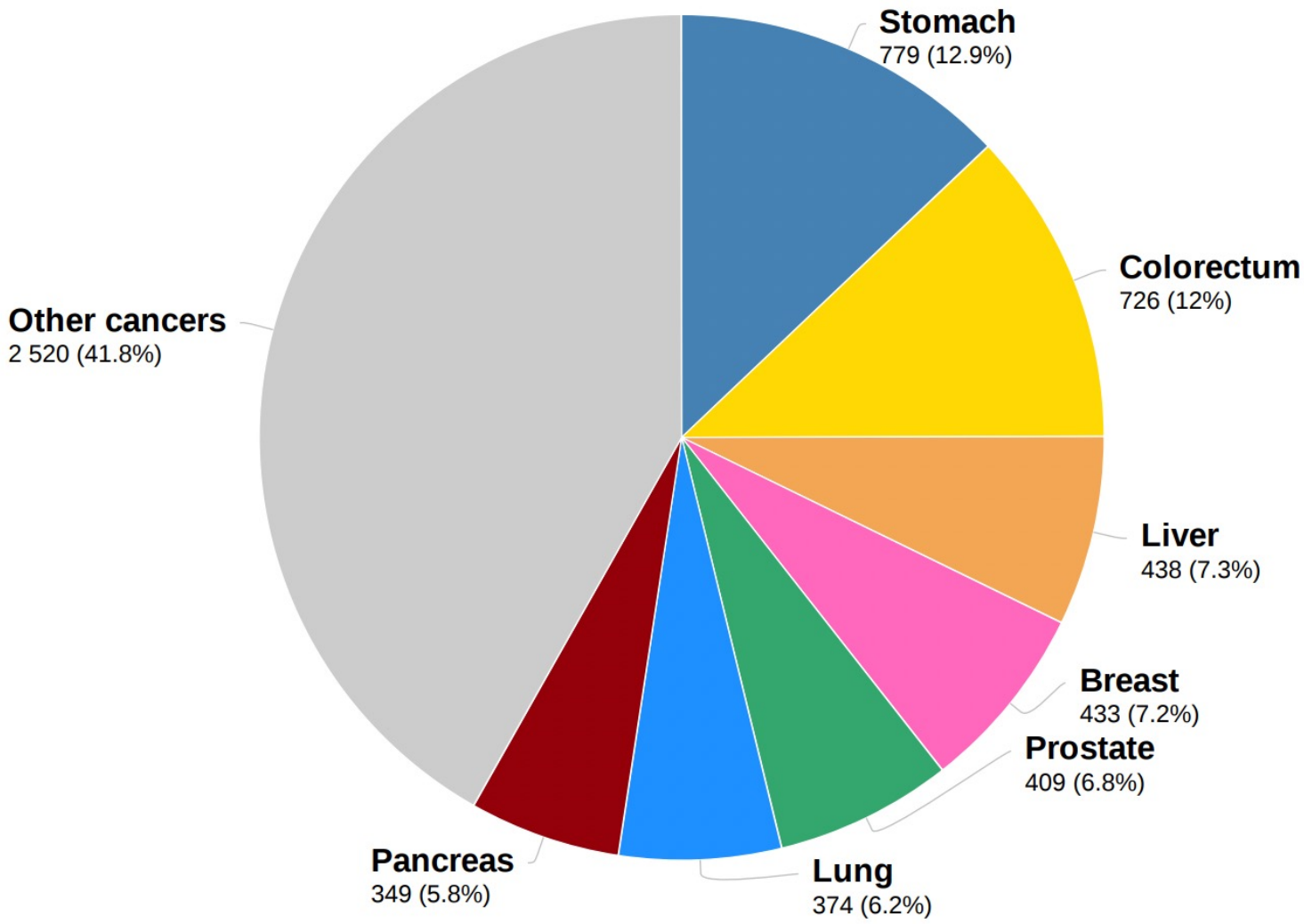
Total : 13 139

Segunda posición en incidencia de cáncer después del cancer de mama y próstata

Data source: Globocan 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr)

International Agency for Research on Cancer
World Health Organization

Estimated number of deaths in 2020, Costa Rica, both sexes, all ages



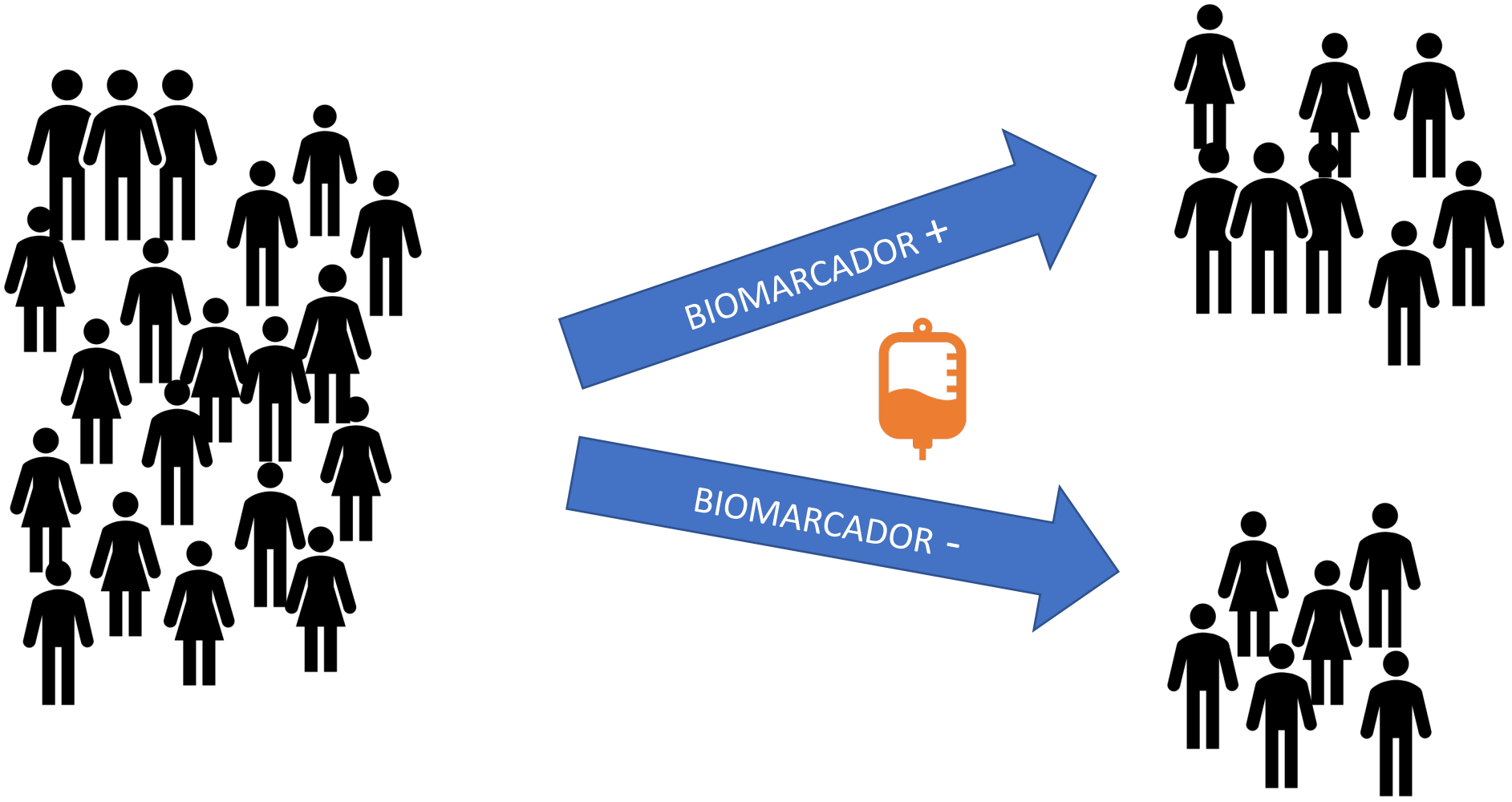
Total : 6 028

Segunda causa de mortalidad por cáncer

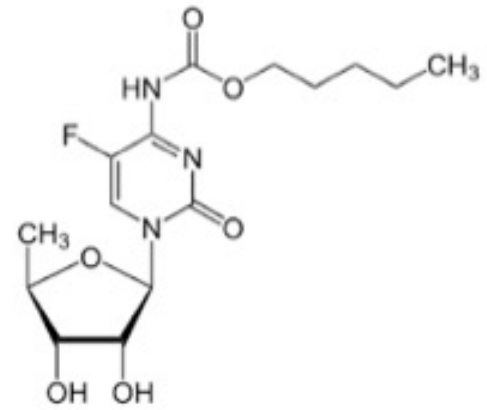
Data source: Globocan 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr)

International Agency for Research on Cancer
World Health Organization

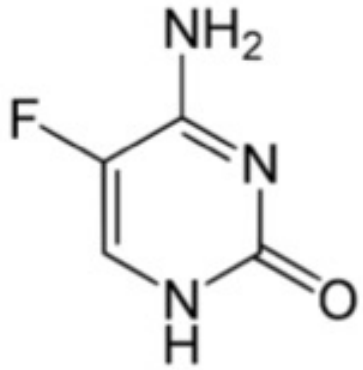
BIOMARCADORES FARMACOGENÉTICOS



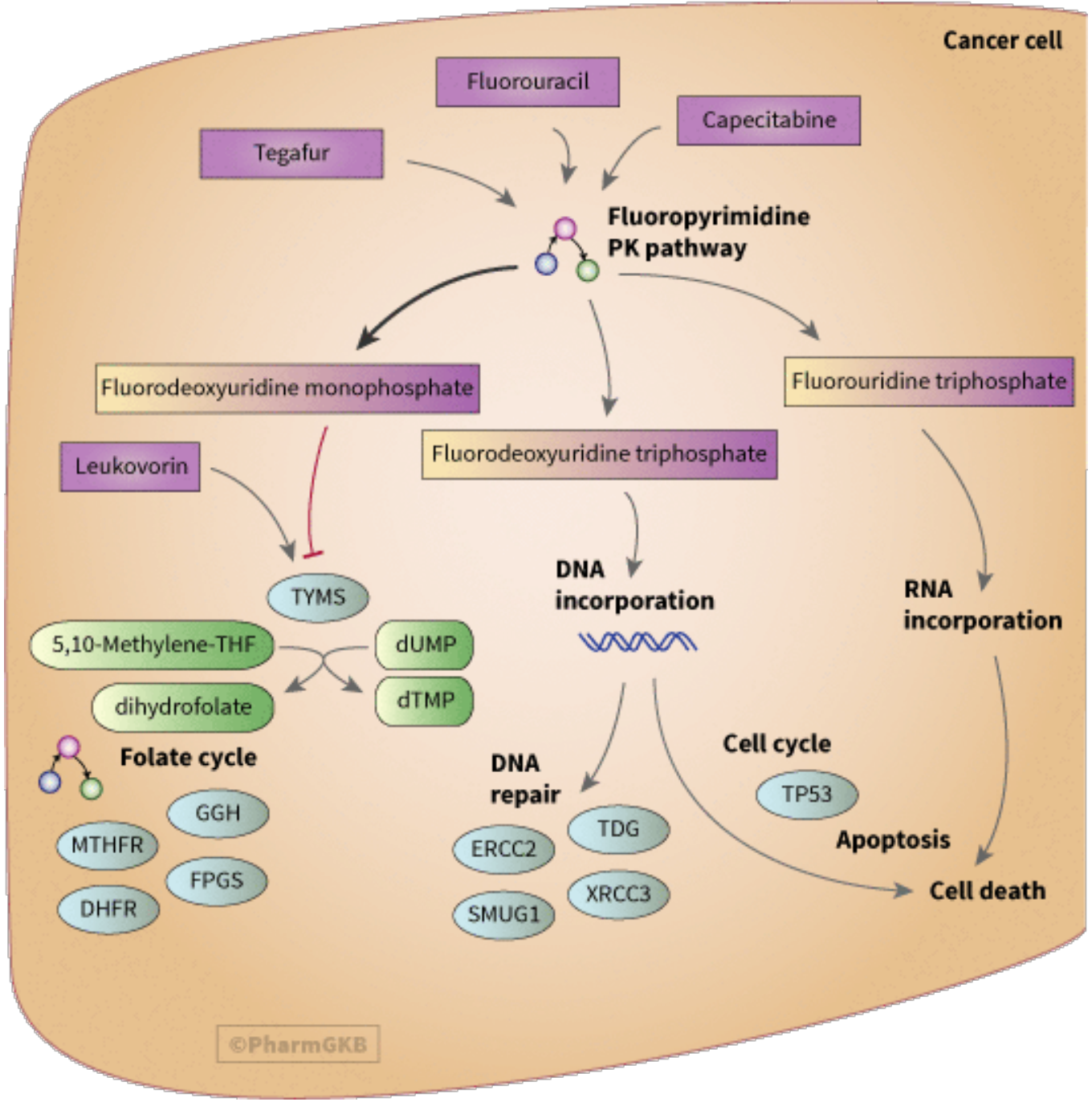
Fluoropirimidinas



Capecitabina



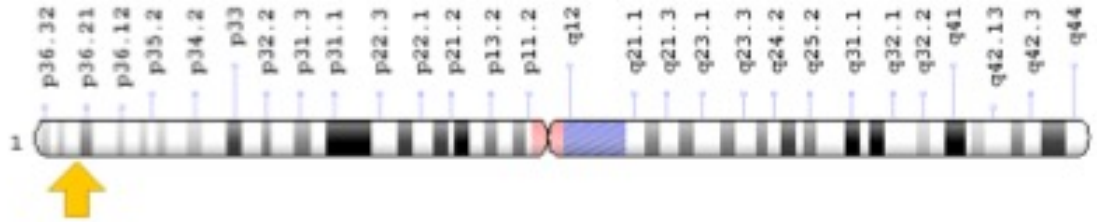
5-Fluoruracilo



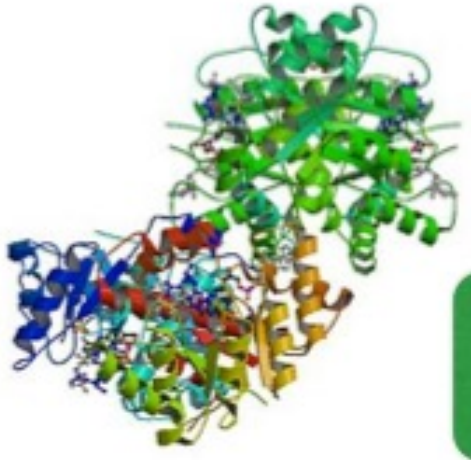
PharmGKB summary: fluoropyrimidine pathways. *Pharmacogenetics and genomics.* 2011

Metilentetrahidrofolato reductasa

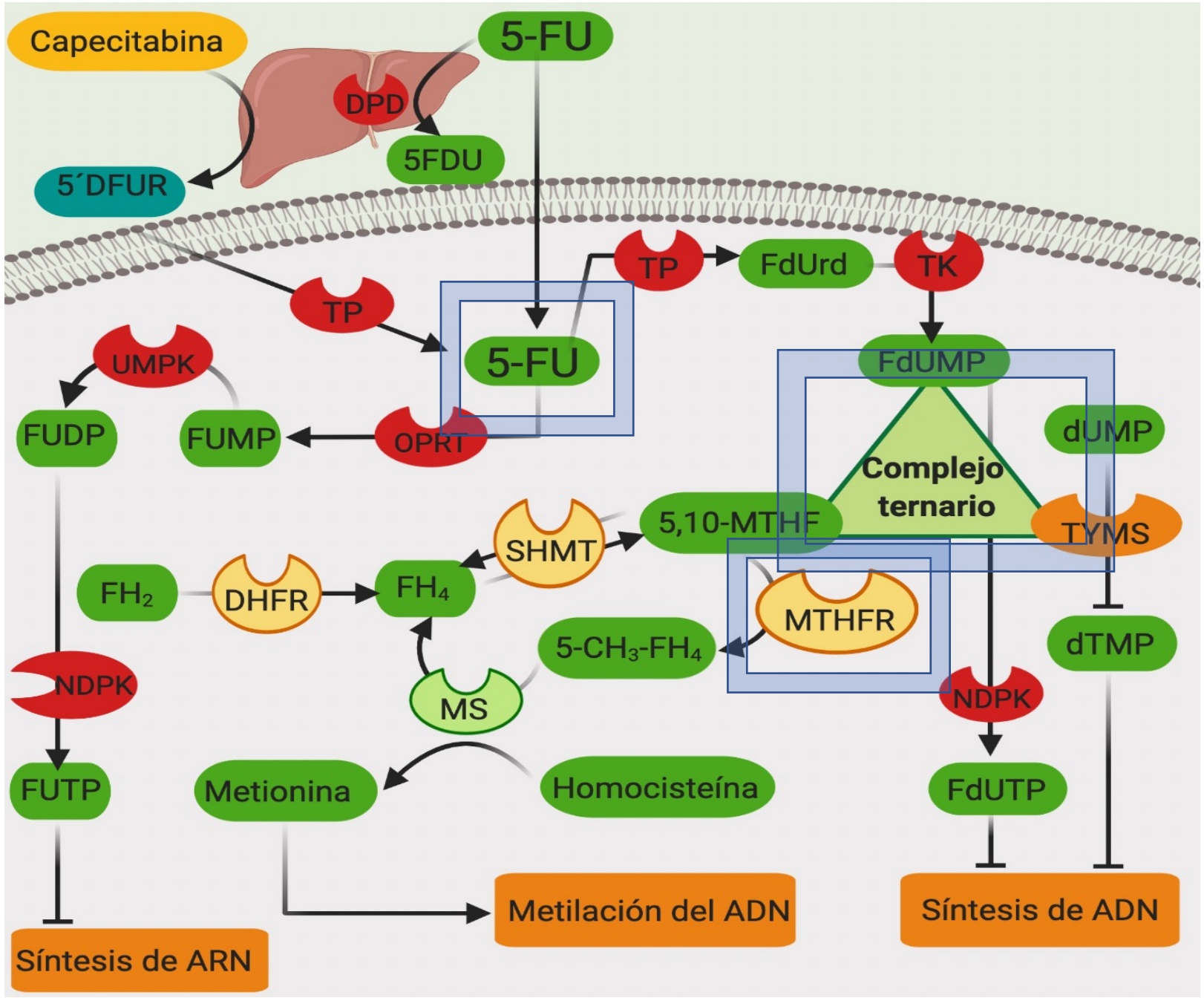
Metilentetrahidrofolato reductasa



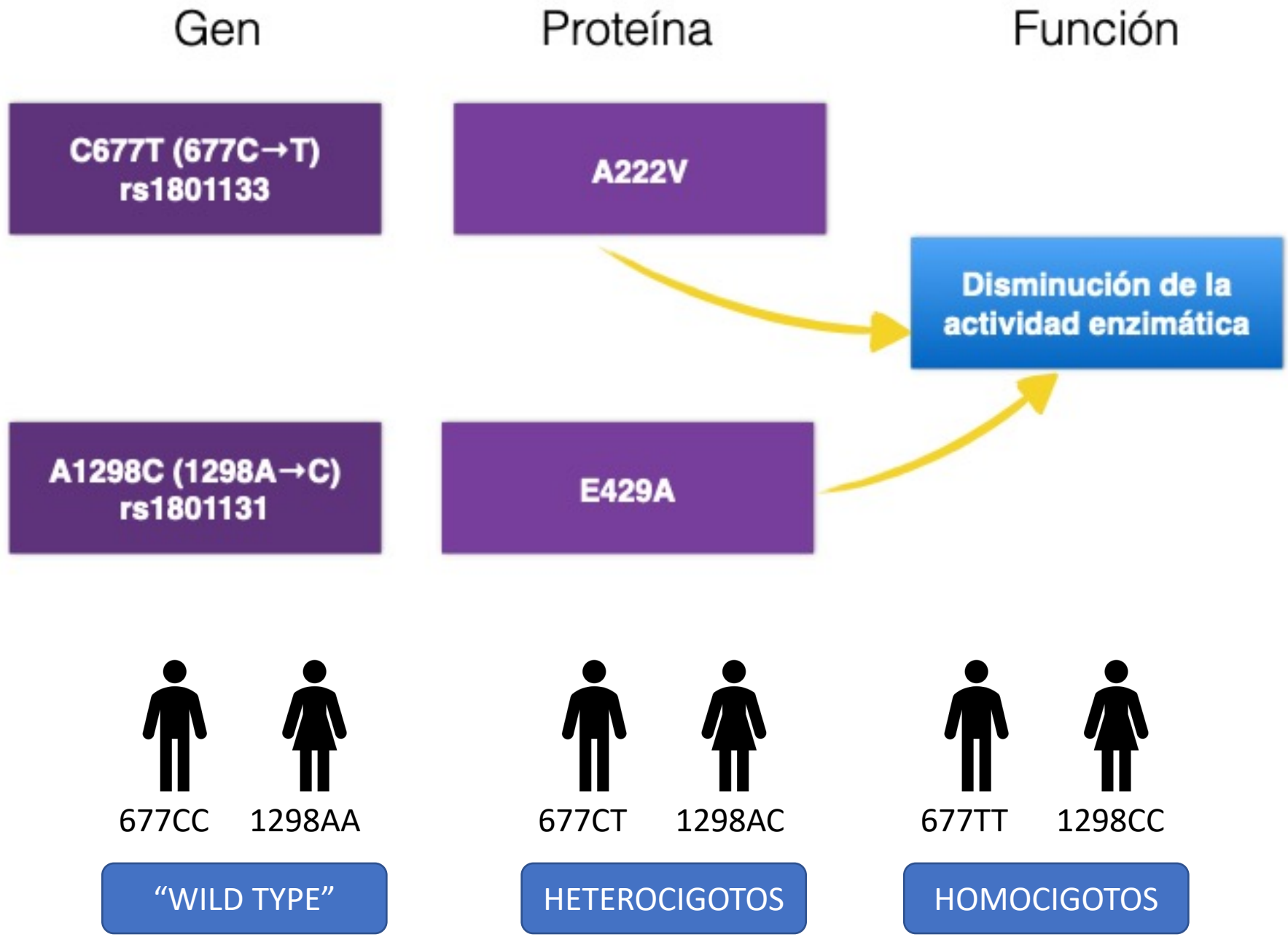
Gen compuesto de 12 exones
Localizado 1p36.3

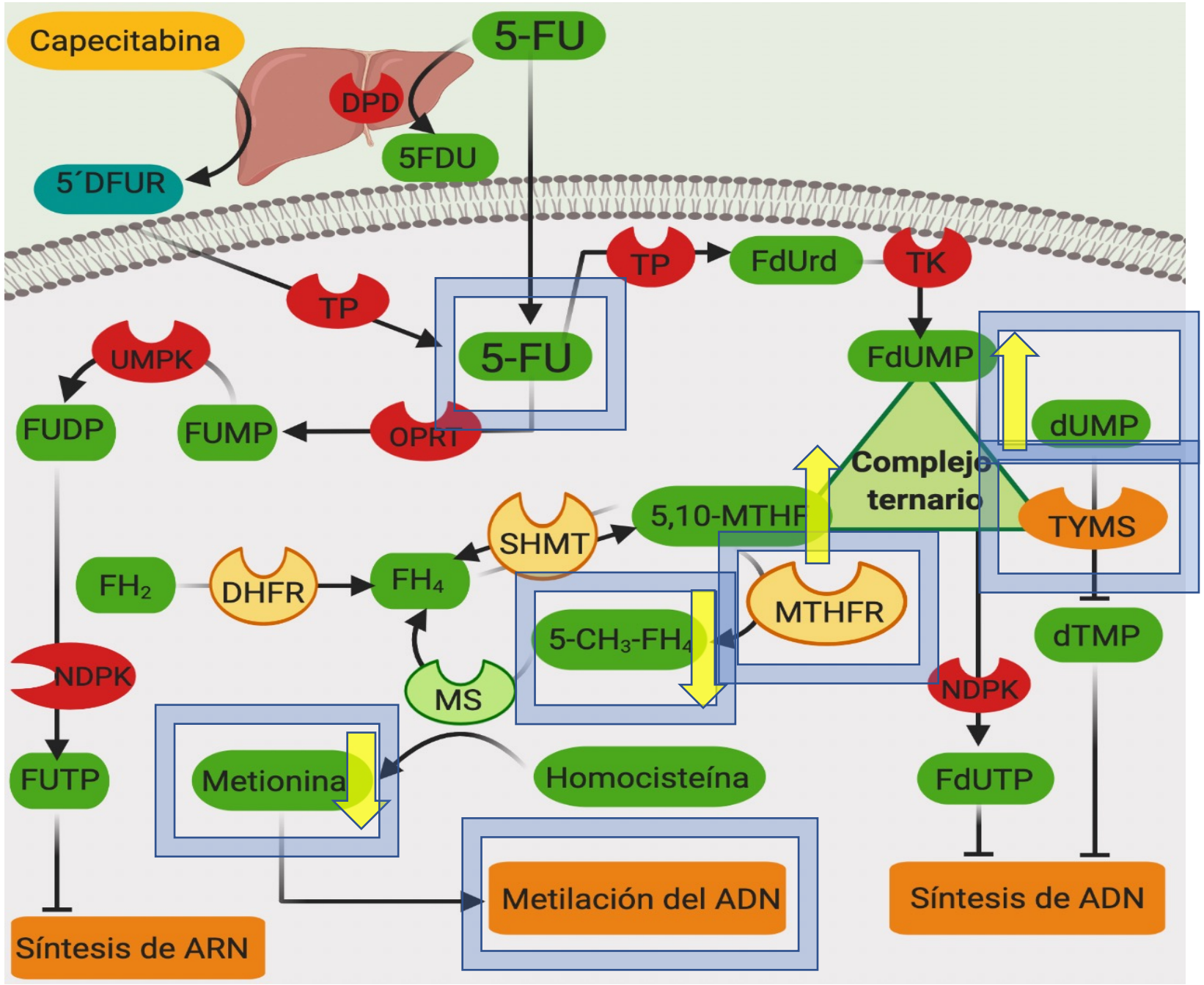


Proteína de 656 aminoácidos


Capecitabina
5-FU
DPD
5FDU
5´DFUR
TP
FdUrd
TK
TP
5-FU
FdUMP
UMPK
FUDP
FUMP
OPRT
dUMP
Complejo ternario
5,10-MTHF
SHMT
TYMS
FH2
DHFR
FH4
MTHFR
5-CH3-FH4
dTMP
NDPK
MS
NDPK
FUTP
Metionina
Homocisteína
FdUTP
Metilación del ADN
Síntesis de ADN
Síntesis de ARN

Polimorfismos del gen *MTHFR*


Gen
Proteína
Función
C677T (677C→T)
rs1801133
A222V
Disminución de la actividad enzimática
A1298C (1298A→C)
rs1801131
E429A
677CC
1298AA
677CT
1298AC
677TT
1298CC
“WILD TYPE”
HETEROCIGOTOS
HOMOCIGOTOS


Capecitabina
5-FU
DPD
5FDU
5´DFUR
TP
FdUrd
TK
TP
5-FU
FdUMP
UMPK
dUMP
FUDP
FUMP
OPRT
Complejo ternario
5,10-MTHF
SHMT
TYMS
FH2
DHFR
FH4
MTHFR
5-CH3-FH4
dTMP
NDPK
MS
NDPK
Metionina
Homocisteína
FUTP
FdUTP
Metilación del ADN
Síntesis de ADN
Síntesis de ARN

Hipótesis

LOS PACIENTES PORTADORES DE ALELOS MUTADOS TENDRÁN **MAYOR TOXICIDAD** PERO **MEJORES TASAS DE RESPUESTA** AL TRATAMIENTO BASADO EN FLUOROPIRIMIDINAS

Metodología

Metodología

- **Diseño**:
  - Estudio de cohorte prospectivo de pacientes con cáncer colorrectal metastásico tratados en primera línea
- **Determinación de polimorfismos:**
  - Extracción de DNA (línea germinal y somática), detección por PCR y análisis electroforético de fragmentos de restricción
- **Determinación medidas de eficiencia y toxicidad:**
  - Supervivencia libre de progresión, supervivencia global, tasa de respuesta (RECIST 1.1) y toxicidad (NCI-CTC 4.0)
- **Análisis estadístico:**
  - Equilibrio Hardy-Weinberg; comparación de tasas de respuesta con prueba chi-cuadrado, análisis de supervivencia Kaplan-Meier, análisis de regresión Cox para el cálculo del hazard ratio

Resultados

ANTICANCER RESEARCH *40*: 4263-4270 (2020)
doi:10.21873/anticanres.14428

Association of C677T and A1298C *MTHFR* Polymorphisms and Fluoropyrimidine-induced Toxicity in Mestizo Patients With Metastatic Colorectal Cancer

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[2]*Research Center in Hematology and Related Disorders CIHATA, University of Costa Rica, San Jose, Costa Rica*

Abstract. *Background/Aim: Enzymatic variants involved in fluoropyrimidine metabolism have been associated with adverse events (AEs). We assessed the association between C677T (rs1801133) and A1298 (rs1801131) methylenetetrahydrofolate reductase (MTHFR) polymorphisms and AEs in patients with first-line fluoropyrimidine-based chemotherapy. Patients and Methods: Fifty patients with metastatic colorectal cancer were prospectively followed-up during the first 4 cycles of fluoropyrimidine-based treatment to assess AEs. Germline DNA was analyzed to determine the C677T and A1298C MTHFR polymorphisms. The associations between MTHFR polymorphisms and toxicity were examined. Results: Individuals carrying at least one mutant allele of the MTHFR C677T polymorphism had increased risk to experience anemia (OR=1.69, 95% CI=1.13-2.53, p=0.005), neutropenia (OR=2.27, 95% CI=1.47-3.42, p<0.001) thrombocytopenia (OR=1.91, 95% CI=1.30-2.70, p<0.001), neuropathy (OR=1.77, 95% CI=1.16-2.70, p=0.02), diarrhea (OR=1.69, 95% CI=1.13-2.53, p=0.005), and hand-foot syndrome (OR=1.56, 95% CI=1.08-2.27, p=0.013), compared to patients carrying the wild type alleles. The presence of the mutant allele C of the MTHFR A1298C polymorphism was associated with increased risk of anemia (OR=2.75, 95% CI=1.01-7.48, p=0.02) and thrombocytopenia (OR=3.14, 95% CI=1.01-9.78, p=0.03); however, the prevalence of this allele in the sample was quite low (20%). Conclusion: MTHFR C677T and A1298C polymorphisms predicted toxicity in a subset of Mestizo patients with colorectal adenocarcinoma.*

The abstract of this paper was presented at the American Association for Cancer Research (AACR) Annual Meeting 2020.

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Key Words: Metastatic colorectal cancer, methylenetetrahydrofolate reductase, SNPs, fluoropyrimidine-based chemotherapy, side effects, Mestizo population.

Colorectal cancer (CRC) is the second most prevalent neoplastic disorder worldwide, and the second leading cause of cancer-related death in both sexes (1). Although during the last decades new therapies have become available for the treatment of this disease, fluoropyrimidine-based chemotherapy still remains the backbone of treatment for these patients (2). However, adverse drug reactions to fluoropyrimidine-based chemotherapy (*i.e.*, diarrhea, mucositis, vomiting, myelosuppression, neuropathy, and hand-foot syndrome) represent a clinical challenge due to the relatively high frequency of dose reductions or treatment discontinuations among affected patients (3).

Fluoropyrimidines, mainly capecitabine and its active metabolite 5-fluorouracil (5-FU), exert their cytotoxic effects in two different ways (Figure 1). First, 5-FU metabolites are extensively incorporated into RNA and DNA molecules, disrupting their normal functions. In addition, 5-FU inhibits the thymidylate synthase, in a reaction where the 5,10-methylenetetrahydrofolate (MTHF) acts as a methyl donor by forming a stable ternary complex with both the active 5-FU metabolite (5-fluoro-2-deoxyuridine-5-monophosphate), and the target enzyme (4). This inhibition precludes the *de novo* synthesis of thymidylate, which is required for DNA replication and repair. Intracellular concentrations of 5,10-MTHF are highly regulated by the MTHF reductase (MTHFR). This enzyme catalyzes the irreversible conversion of 5,10-MTHF to 5-methyltetrahydrofolate, and reduces the concentration of 5,10-MTHF available for binding to the ternary complex (5).

It has been previously shown that two common single-nucleotide polymorphisms (SNPs), C677T (rs1801133) and A1298C (rs1801131) reduce enzyme activity and lead to an altered distribution of intracellular folates (6). Individuals homozygous for the *MTFHR* C677T variant (TT) produce a

C677T and A1298C *MTHFR* gene polymorphisms and response to fluoropyrimidine-based chemotherapy in Mestizo patients with metastatic colorectal cancer

Allan Ramos-Esquivel[a,b], Ricardo Chinchilla-Monge[b], Jad Abbas[c] and Marta Valle[a]

Objective **To assess the association between C677T and A1298C methylenetetrahydrofolate reductase (*MTHFR*) single-nucleotide polymorphisms (SNPs) and response to first-line fluoropyrimidine-based chemotherapy for metastatic colorectal adenocarcinoma.**

Methods **A total of 68 patients were prospectively followed up in San Juan de Dios Hospital (San José, Costa Rica) from January 2019 to November 2020. Patients received first-line therapy with capecitabine or 5-fluorouracil in combination with oxaliplatin or irinotecan. Germline and somatic DNA was extracted from blood samples and paraffin-embedded tissue, respectively. Overall response rate (partial response + complete response) was assessed according to RECIST 1.1 criteria. Cox regression models were performed to identify the effect of *MTHFR* C677T and A1298C SNPs on progression-free survival (PFS) and overall survival (OS) (NCT registration number: 03852290).**

Results **Patients harboring one or both T alleles of the *MTHFR* C677T SNP had better overall response than homozygous wild-type individuals [odds ratio (OR): 3.21; 95% confidence interval (CI), 1.05–9.81; *P*=0.03]. No association was found between the *MTHFR* A1298C genotypes and overall response (OR: 0.75; 95% CI, 0.26–2.20; *P*=0.60). Patients with the *MTHFR* 677 TT and CT genotypes had longer PFS than CC individuals (hazard ratio: 0.53; 95% CI, 0.28–0.98; *P*=0.045), even after adjustment for confounders (hazard ratio: 0.50; 95% CI, 0.25–0.98; *P*=0.04). We found no association between the *MTHFR* A1298C SNP and PFS (hazard ratio: 1.35; 95% CI, 0.72–2.55; *P*=0.34). None of the SNPs was associated with OS.**

Conclusion **Patients carrying at least one mutant allele of the *MTHFR* C677T SNP had a better overall response and longer PFS than wild-type homozygous patients.**

Pharmacogenetics and Genomics XXX, **XXX**:000–000

Keywords: colorectal neoplasms, Costa Rica, methylenetetrahydrofolate reductase, single-nucleotide polymorphism.

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Received 24 January 2021 **Accepted** 4 April 2021

Características generales

Variable	Frecuencia n=68 (%)
Sexo femenino	35 (51.5)
Edad (años)	59.4 ± 13.7
Metástasis	
Sincrónica	16 (23.5)
Metacrónica	52 (76.5)
Sitio de metástasis	
Hígado	38 (55.9)
Pulmón	23 (33.8)
Ganglios linfáticos	18 (26.5)
Peritoneo	17 (25)
Metastasectomía	30 (44.1)
Esquema de quimioterapia	
FOLFOX	31 (45.6)
CAPEOX	25 (36.8)
FOLFIRI	12 (17.6)

MTHFR C677T
CC = 26.5%
CT = 50%
TT = 23.5%

MTHFR A1298C
CC = 70.5%
CT = 23.5%
TT = 6%

CAPEOX, capecitabine and oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin.

Polimorfismos *MTHFR* y toxicidad

Resultados

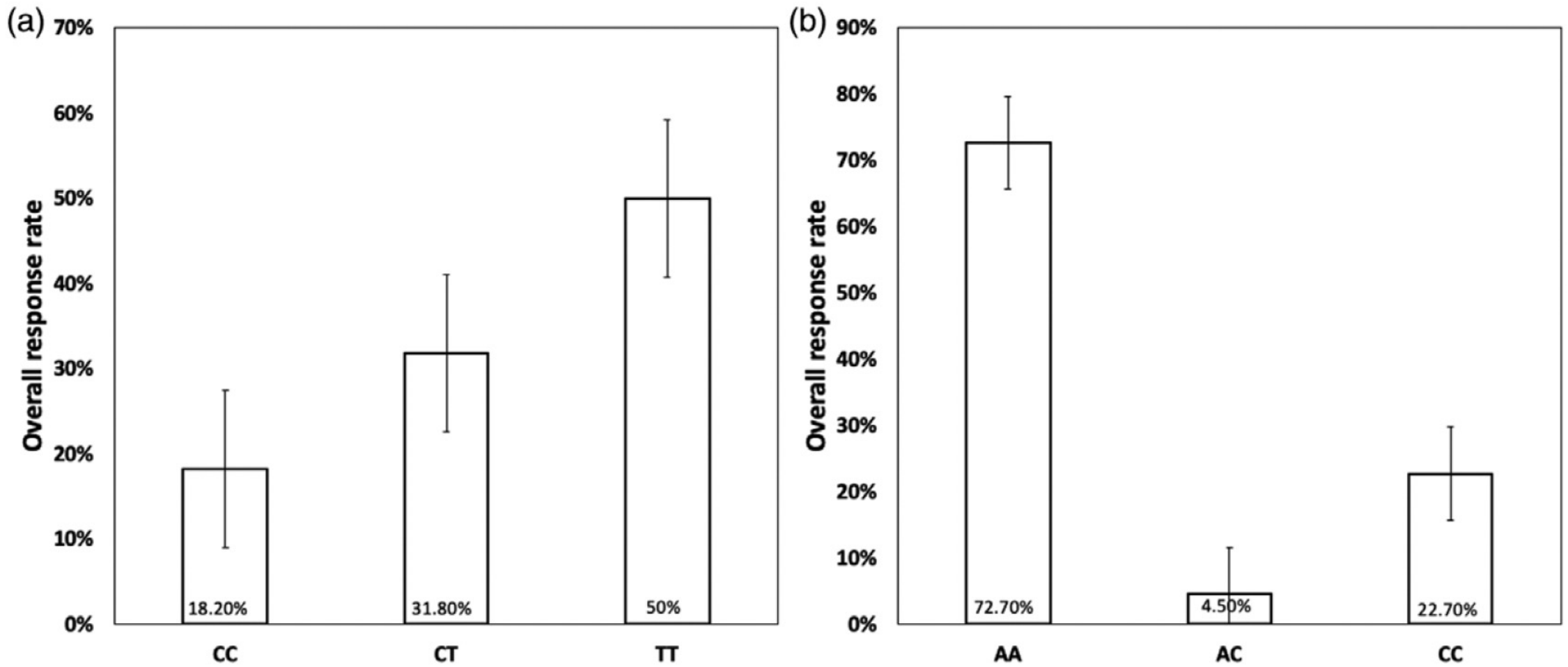
Table III. *Univariate analysis of mild/moderate or severe adverse events according to the presence of 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms.*

Toxicity	*MTHFR* C677T CT/TT *vs.* CC	*p*-Value	*MTHFR* A1298C AC/CC *vs.* AA	*p*-Value
	Odds ratio (95% CI)		Odds ratio (95% CI)	
Neutropenia	2.27 (1.47-3.42)	**<0.001**	2.34 (0.86-6.87)	0.12
Anemia	1.69 (1.13-2.53)	**0.005**	2.75 (1.01-7.48)	**0.02**
Thrombocytopenia	1.91 (1.30-2.70)	**<0.001**	3.14 (1.01-9.78)	**0.03**
Neuropathy	1.77 (1.16-2.70)	**0.02**	1.24 (0.53-2.89)	0.63
Fatigue	1.38 (0.98-1.92)	0.09	1.72 (0.56-5.20)	0.50
Diarrhea	1.69 (1.13-2.53)	**0.005**	2.00 (0.80-5.01)	0.12
Mucositis	1.30 (0.91-1.85)	0.14	1.45 (0.48-3.62)	0.61
Hand-foot syndrome	1.56 (1.08-2.27)	**0.013**	1.70 (0.68-4.27)	0.24
Nausea	1.55 (1.15-2.10)	**0.021**	2.78 (0.71-10.86)	0.17
Vomiting	1.48 (1.11-1.99)	**0.041**	4.92 (0.73-33.81)	0.08
Weight loss	1.18 (0.81-1.72)	0.70	3.50 (0.52-23.56)	0.24

Bold values indicate statistical significance.

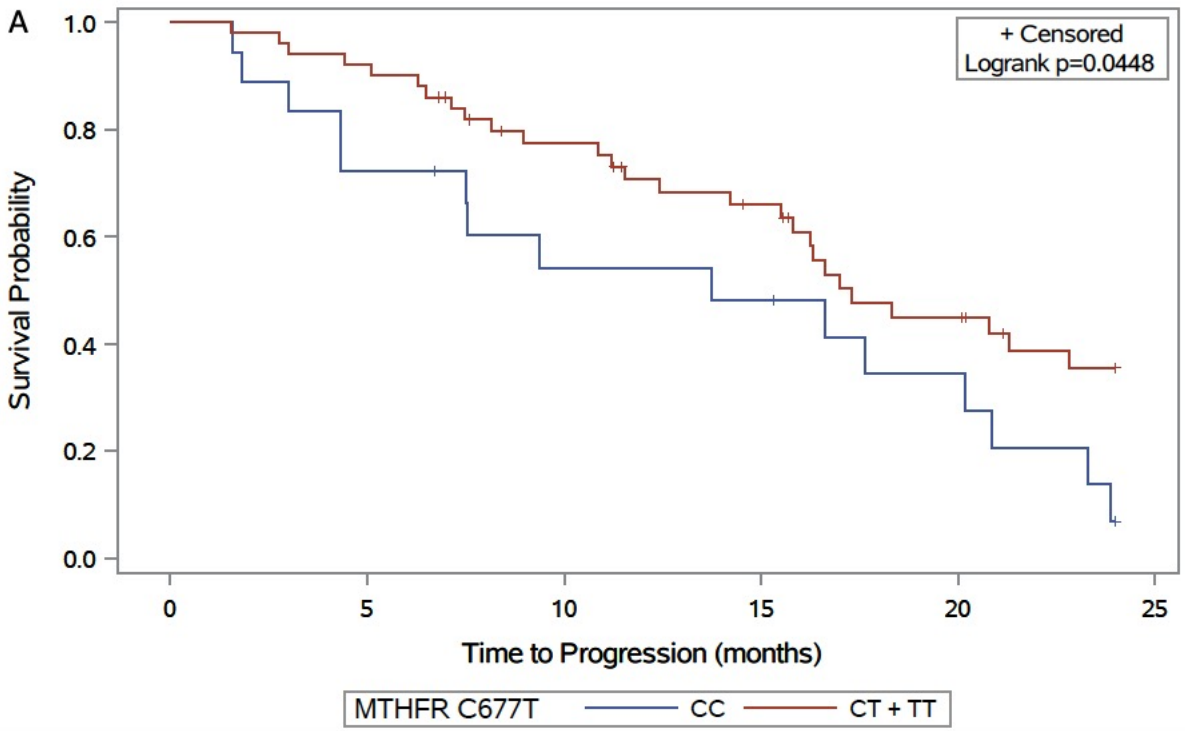
Anticancer Research 2020; 40:4263

Polimorfismos *MTHFR* y efectividad



Overall response rate according to the *5,10-methylenetetrahydrofolate reductase* (*MTHFR*) 6C77T (a) and A1298C (b) polymorphisms.

Pharmacogenetics Genomics 2021. In Press



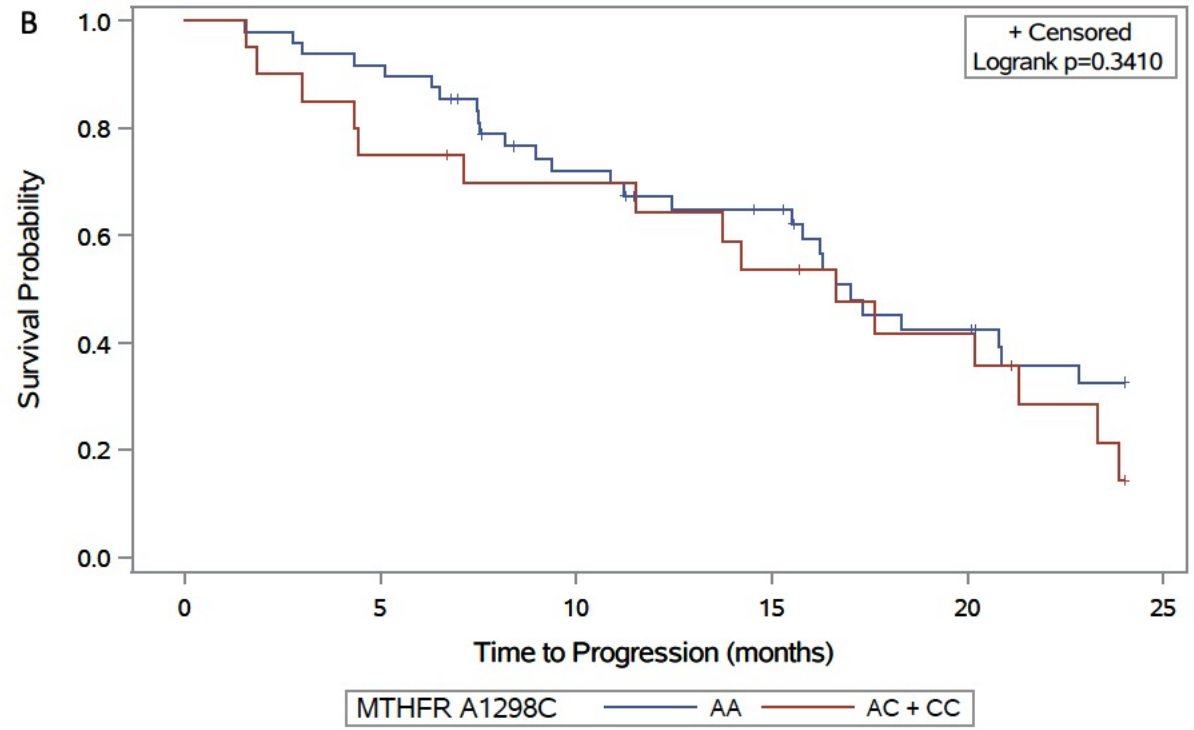


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Table 2 Univariate and multivariate models for progression-free and overall survival

Variable	Progression				Death			
	Univariate model		Multivariate model		Univariate model		Multivariate model	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Sex								
Female	1 (reference)				1 (reference)			
Male	0.98 (0.54–1.81)	0.96			1.03 (0.50–2.13)	0.93		
Age								
≥65 years	1 (reference)				1 (reference)			
<65	0.66 (0.45–1.67)	0.66			0.79 (0.34–1.85)	0.58		
ECOG Performance status								
1-2	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
0	0.44 (0.24–0.81)	0.009*	0.57 (0.30–1.08)	0.08	0.28 (0.13–0.58)	0.001*	0.34 (0.16–0.75)	0.007*
Primary tumor resection	0.43 (0.23–0.79)	0.006*	0.49 (0.25–0.97)	0.04*	0.31 (0.15–0.66)	0.003*	0.43 (0.19–0.98)	0.046*
Timing of metastastatic disease								
Synchronous	1 (reference)				1 (reference)			
Methacronous	0.74 (0.37–1.48)	0.39			0.69 (0.31–1.50)	0.34		
Metastatectomy	0.64 (0.35–1.19)	0.16	0.69 (0.36–1.33)	0.27	0.61 (0.29–1.28)	0.19	0.88 (0.40–1.95)	0.88
MTHFR C677T								
CC	1 (reference)		1 (reference)		1 (reference)			
CT + TT	0.53 (0.28–0.98)	0.045*	0.50 (0.25–0.98)	0.04*	0.86 (0.38–1.94)	0.71		
MTHFR A1298C								
AA	1 (reference)				1 (reference)			
AC + CC	1.35 (0.72–2.55)	0.34			1.24 (0.58–2.67)	0.58		

CI, confidence interval;*Statistically significant at $P<0.05$.

Pharmacogenetics Genomics 2021. In Press

Discusión

BJC British Journal of Canc

Br J Cancer. 2011 Nov 22; 105(11): 1654–1

Published online 2011 Nov 1. doi: 10.1038/b

Methylenetetrahydrofolate toxicity to 5-FU-based che

F Thomas,[1,2,*] A A Motsinger-Reif,[1,3,4] J M

J W Fleshman,[7] B R Tan,[5] and H L McLeod

Methylenetetrahydrofolate Colorectal Cancer: A Novel Genomic Predictor of Clinical Response to Fluoropyrimidine-based Chemotherapy[1]

Victor Cohen, Valerie Panet-Raymond, Nelly Sabbaghian, Isabelle Morin, Gerald Batist, and Rima Rozen[2]

Departments of Medicine [V. C., G. B.], Oncology [V. C., G. B.], Pediatrics [V. P-R., N. S., I. M., R. R.], and Human Genetics [R. R.], McGill University; Centre for Experimental Therapeutics in Cancer, Jewish General Hospital [V. C., G. B.]; and Montreal Children's Hospital Research Institute [V. P-R., N. S., I. M., R. R.], Montreal, Canada

tion was associated with an odds ratio of 2.86 (95% confidence interval 1.06–7.73) for a response in individuals with a valine allele.

Conclusions: **Our results show a link between the MTHFR polymorphism and tumor response to fluoropyrimidine-based chemotherapy and suggest that MTHFR genotyping may be of predictive benefit in selecting treatment regimens.**

ELSEVIER

Annals of Oncology

Volume 20, Issue 10, October 2009, Pages 1660-1666

original articles

gastrointestinal tumors

MTHFR polymorphisms and 5-FU-based adjuvant chemotherapy in colorectal cancer

S. Afzal [1] ✉, S.A. Jensen [2], B. Vainer [3], U. Vogel [4], J.P. Matsen [1], J.B. Sørensen [2], P.K. Andersen [5], H.E. Poulsen [1]

ıse (MTHFR) gene nse in colorectal

aindrault-Goebel,

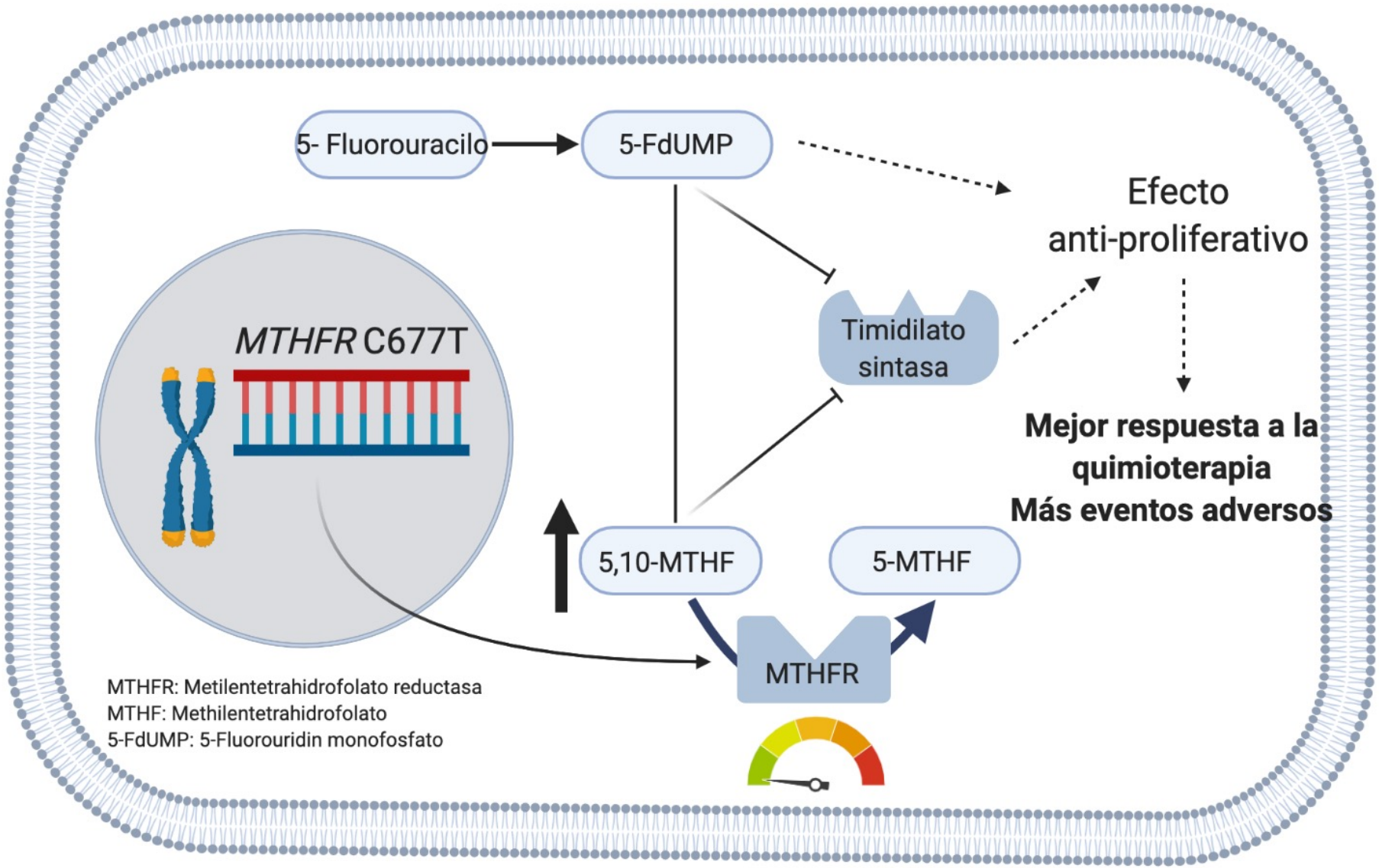
ard Lledo, Thierry André,

ery de Gramont

Posible causa de los resultados controvertidos

- **Frecuencia alélica *MTHFR* C677T y A1298C**
  - Alelo T (677) se encuentra en mayor porcentaje en población Mestiza vs. Caucásica
  - Alelo C (1298) poco representado en la población de studio
- **Método de análisis**
  - Modelos dominantes (CT + TT *vs* CC) tienden a reportar más asociaciones
- **Diseño del estudio**
  - Seguimiento prospectivo
- **Frecuencia de eventos adversos (EA)**
  - Mayor incidencia de EA en la cohorte estudiada
- **Tamaño muestral**
- **Efecto de otras mutaciones relevantes**

Conclusiones



MTHFR C677T es biomarcador farmacogenético de toxicidad y eficiencia (tasa de respuesta/SLP)

MTHFR A1298C es biomarcador farmacogenético de toxicidad

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