FARMACOGENOMICA EN ONCOLOGIA

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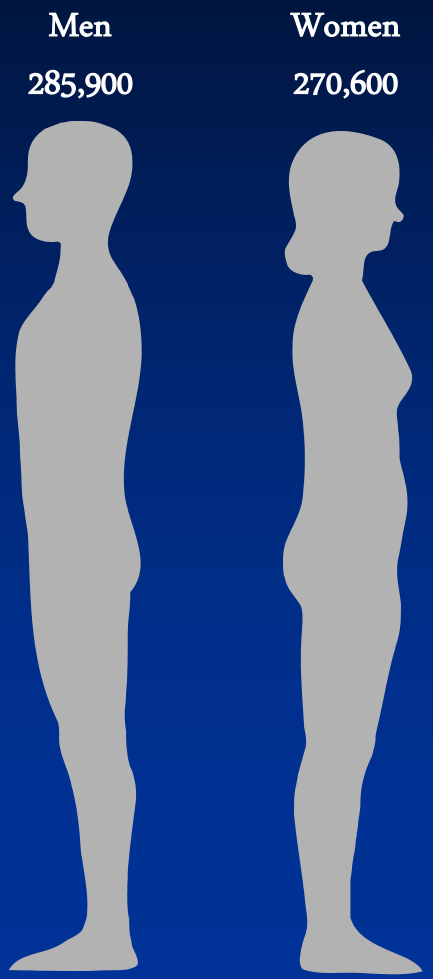
Can we optimize the selection of current therapies?

Is anatomy the best way to choose therapy?

Lung & bronchus	31%
Prostate	10%
Colon & rectum	10%
Pancreas	5%
Non-Hodgkin lymphoma	4%
Leukemia	4%
Esophagus	4%
Liver/intrahepatic bile duct	3%
Urinary bladder	3%
Kidney	3%
All other sites	22%

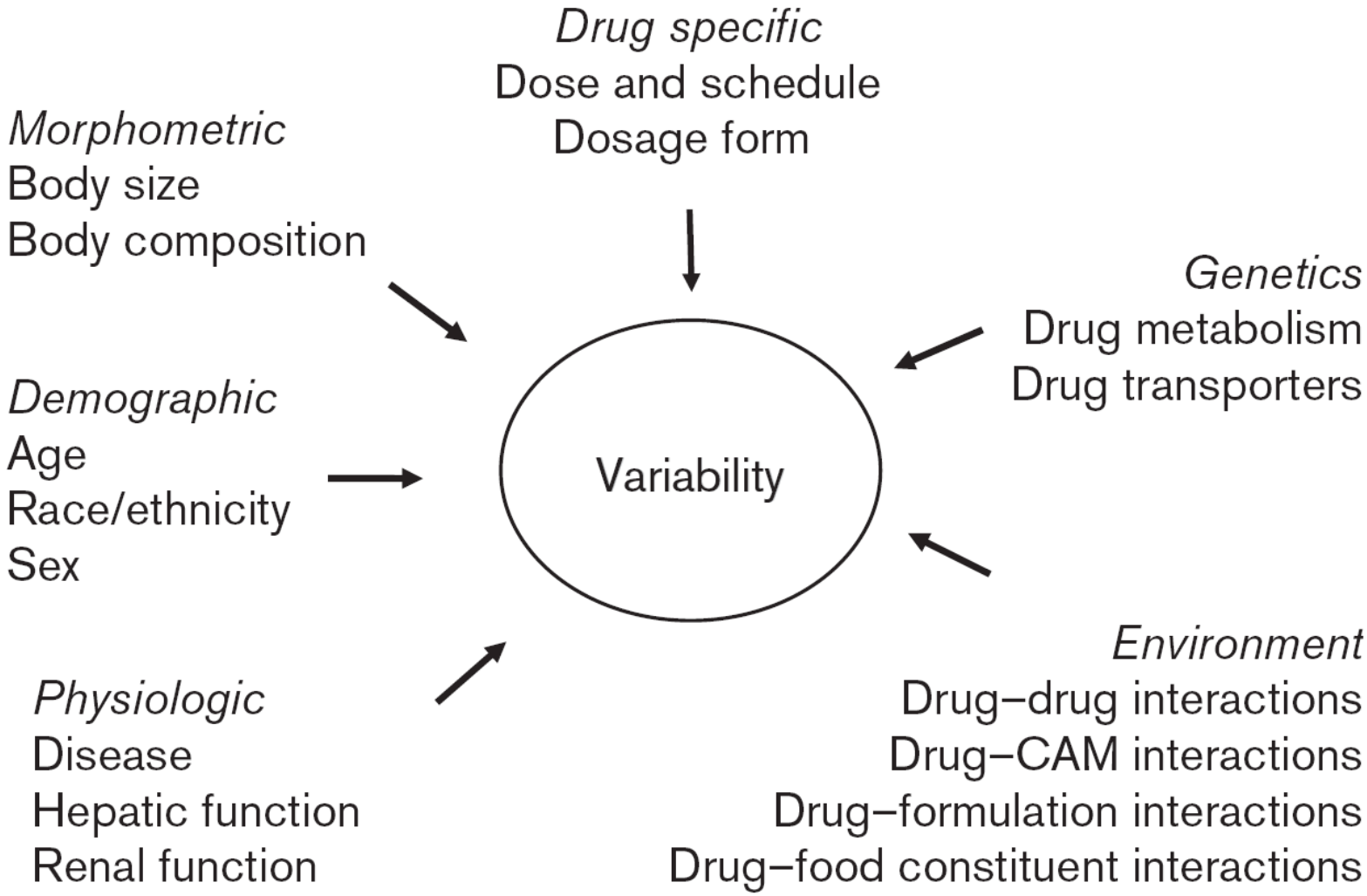


25%	Lung & bronchus
15%	Breast
11%	Colon & rectum
6%	Pancreas
5%	Ovary
4%	Non-Hodgkin lymphoma
4%	Leukemia
3%	Uterine corpus
2%	Brain/ONS
2%	Multiple myeloma
23%	All other sites

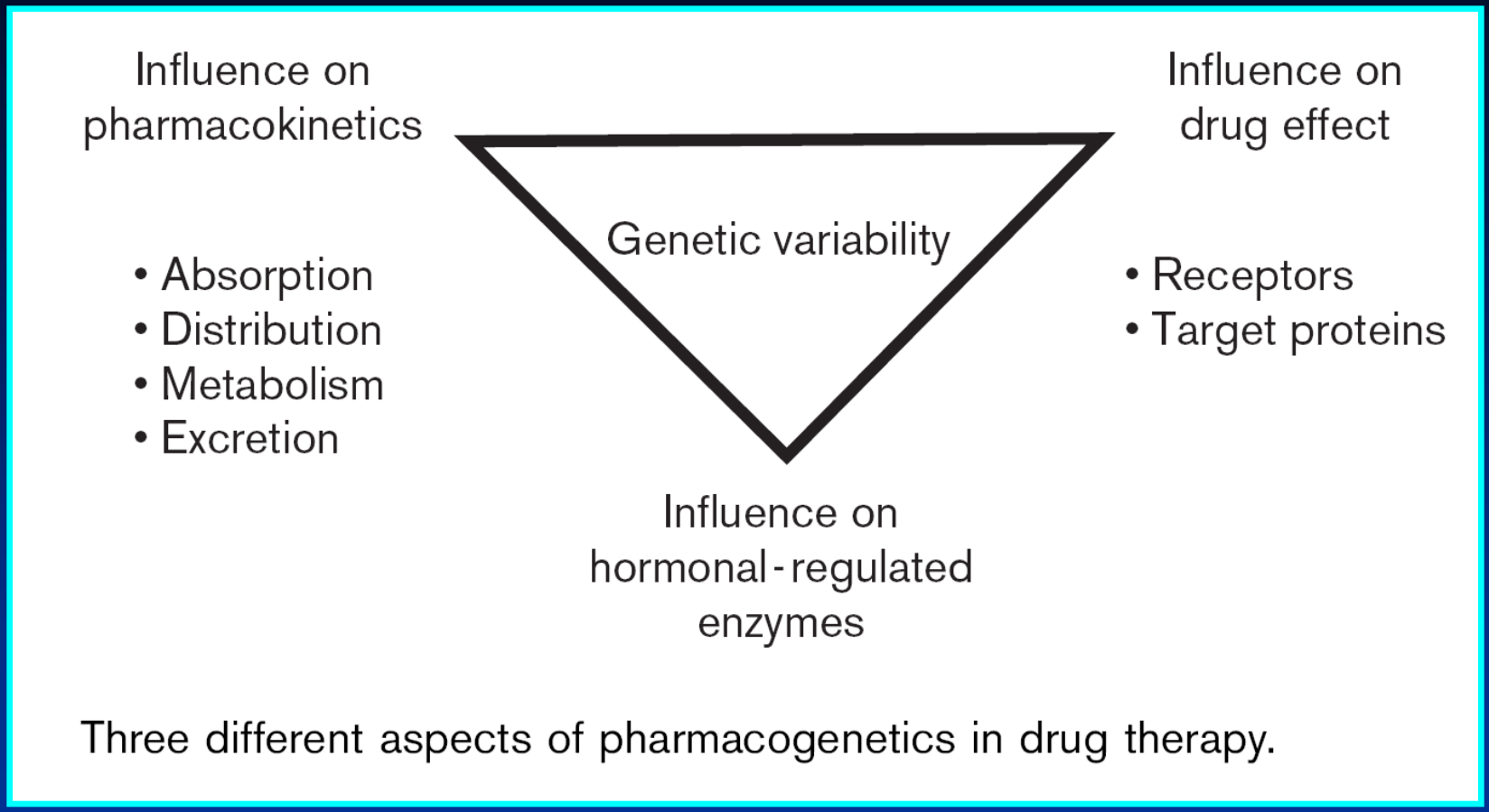
Hypotheses

- Tumor response to chemotherapy is influenced by the specific genes in a drug pathways
  - Subsets of tumors exist, based upon their gene expression

- Knowledge of a tumor's gene expression profile will aid in selecting drugs which are more likely to be active against the tumor (i.e. longer survival, decreased morbidity, increased resectability, etc.)

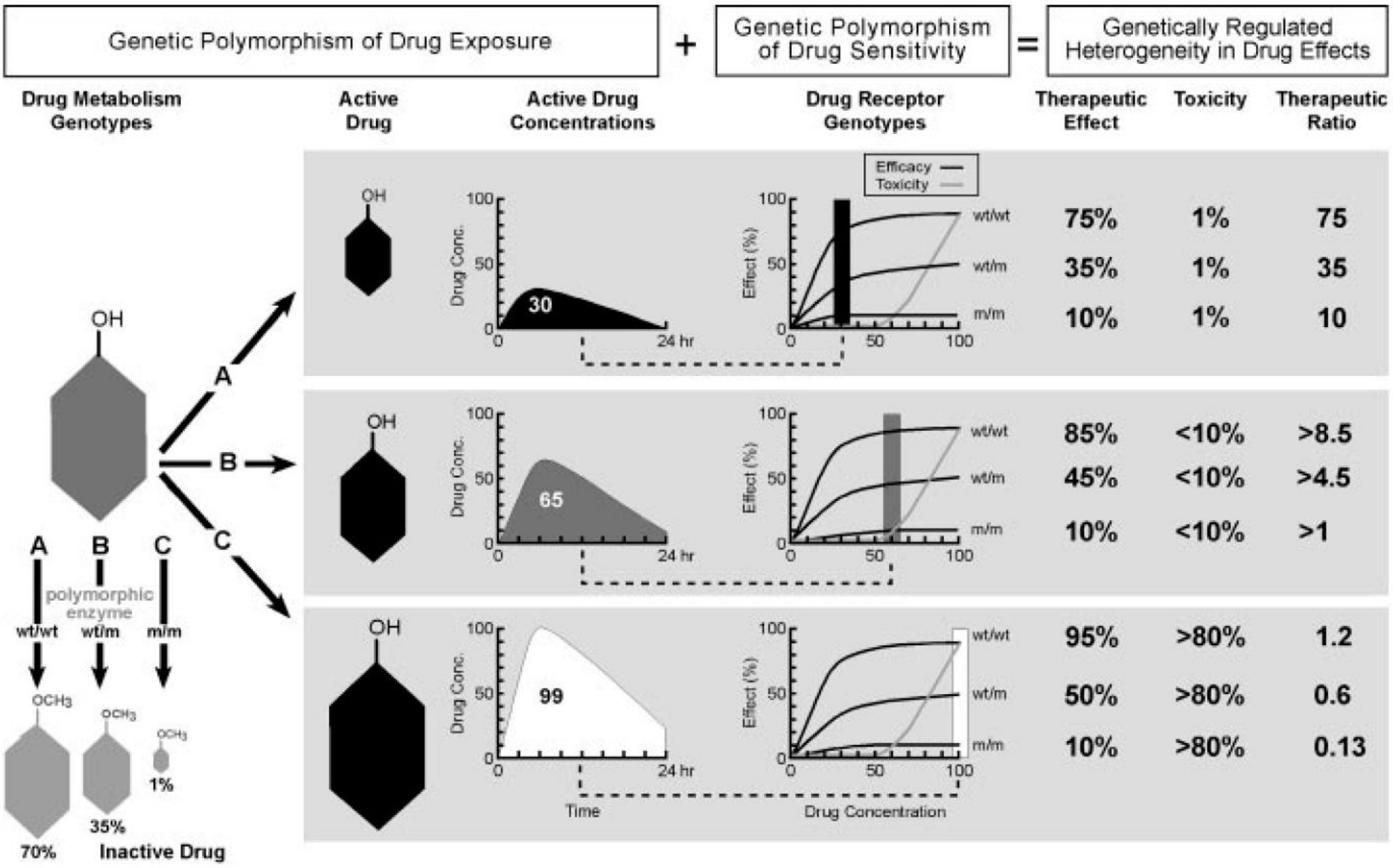


Sources of pharmacokinetic and pharmacodynamic variability. *CAM, complementary and alternative medicine.



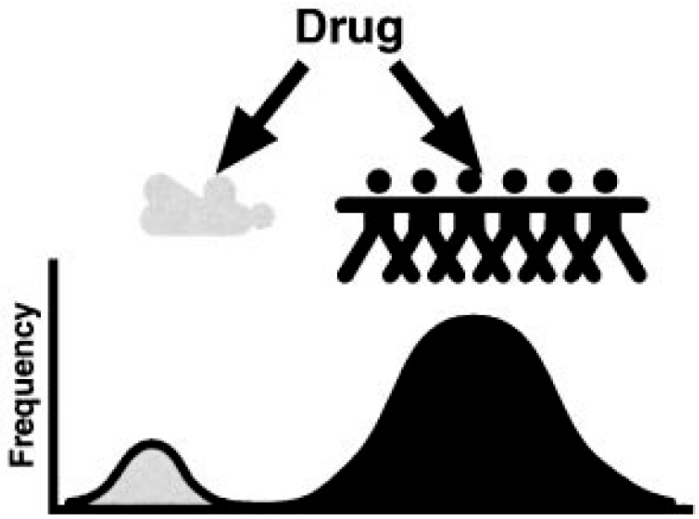
Three different aspects of pharmacogenetics in drug therapy.

- **Genetic variants within a specific candidate gene provide the mechanistic basis for many of the early examples in pharmacogenetics.**


Genetic Polymorphism of Drug Exposure
+
Genetic Polymorphism of Drug Sensitivity
=
Genetically Regulated Heterogeneity in Drug Effects
Drug Metabolism Genotypes
Active Drug
Active Drug Concentrations
Drug Receptor Genotypes
Therapeutic Effect
Toxicity
Therapeutic Ratio
OH
A
B
C
polymorphic enzyme
wt/wt
wt/m
m/m
OCH3
1%
35%
70%
Inactive Drug
Drug Conc.
30
24 hr
Efficacy
Toxicity
Effect (%)
wt/wt
wt/m
m/m
75%
35%
10%
1%
1%
1%
75
35
10
65
85%
45%
10%
<10%
<10%
<10%
>8.5
>4.5
>1
99
95%
50%
10%
>80%
>80%
>80%
1.2
0.6
0.13
Time
Drug Concentration

Pharmacogenetic Discovery

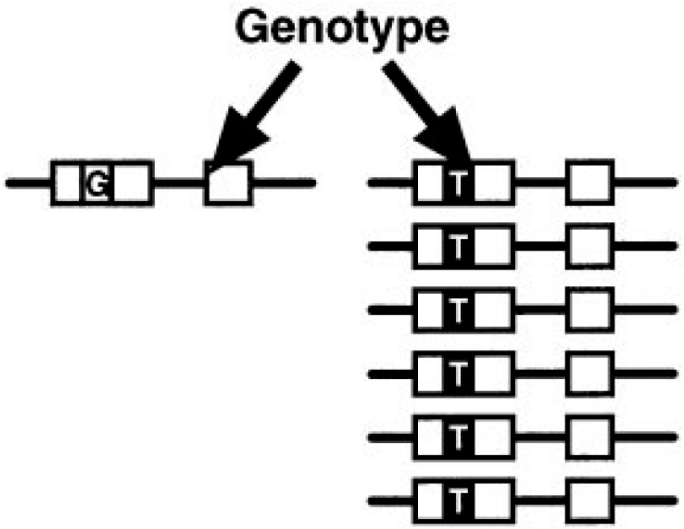
Pre-Genomics



Genotype

Compare genetic polymorphisms in *phenotypic* groups

Post-Genomics



Compare phenotypes in *genotypic* groups

Character of Population Phenotype Distributions Differs Based on Mutation Type

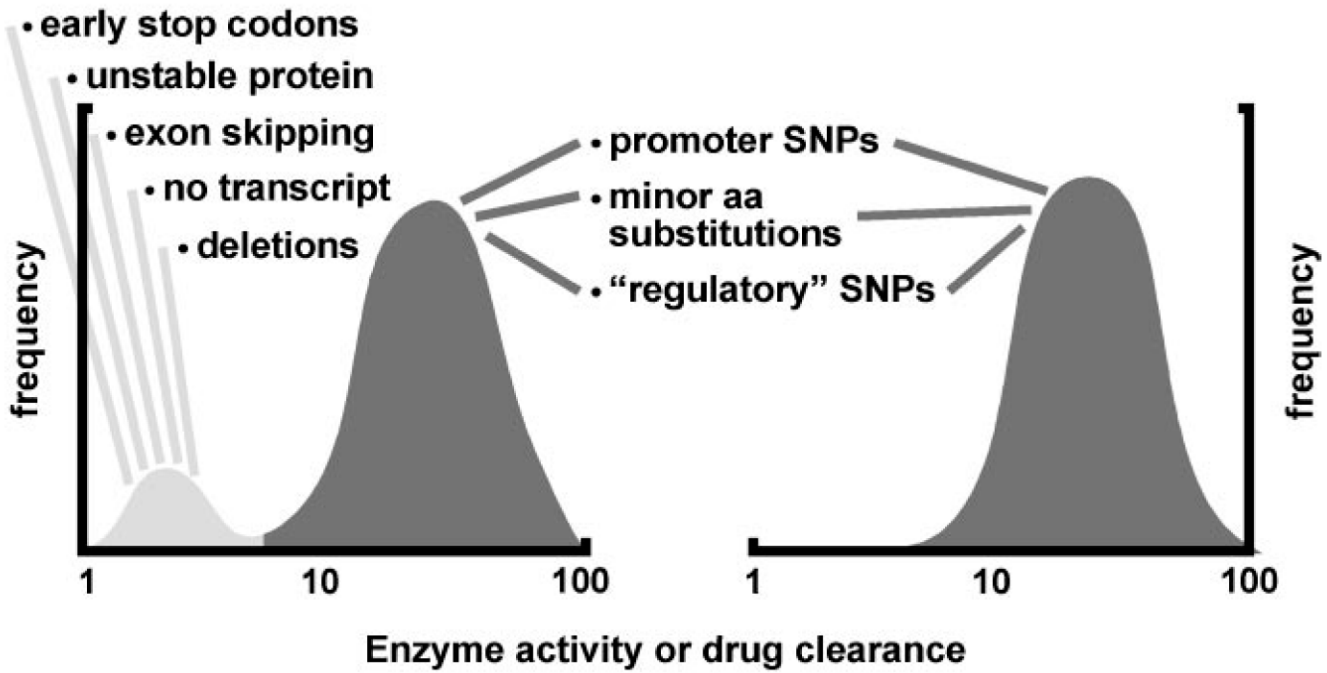
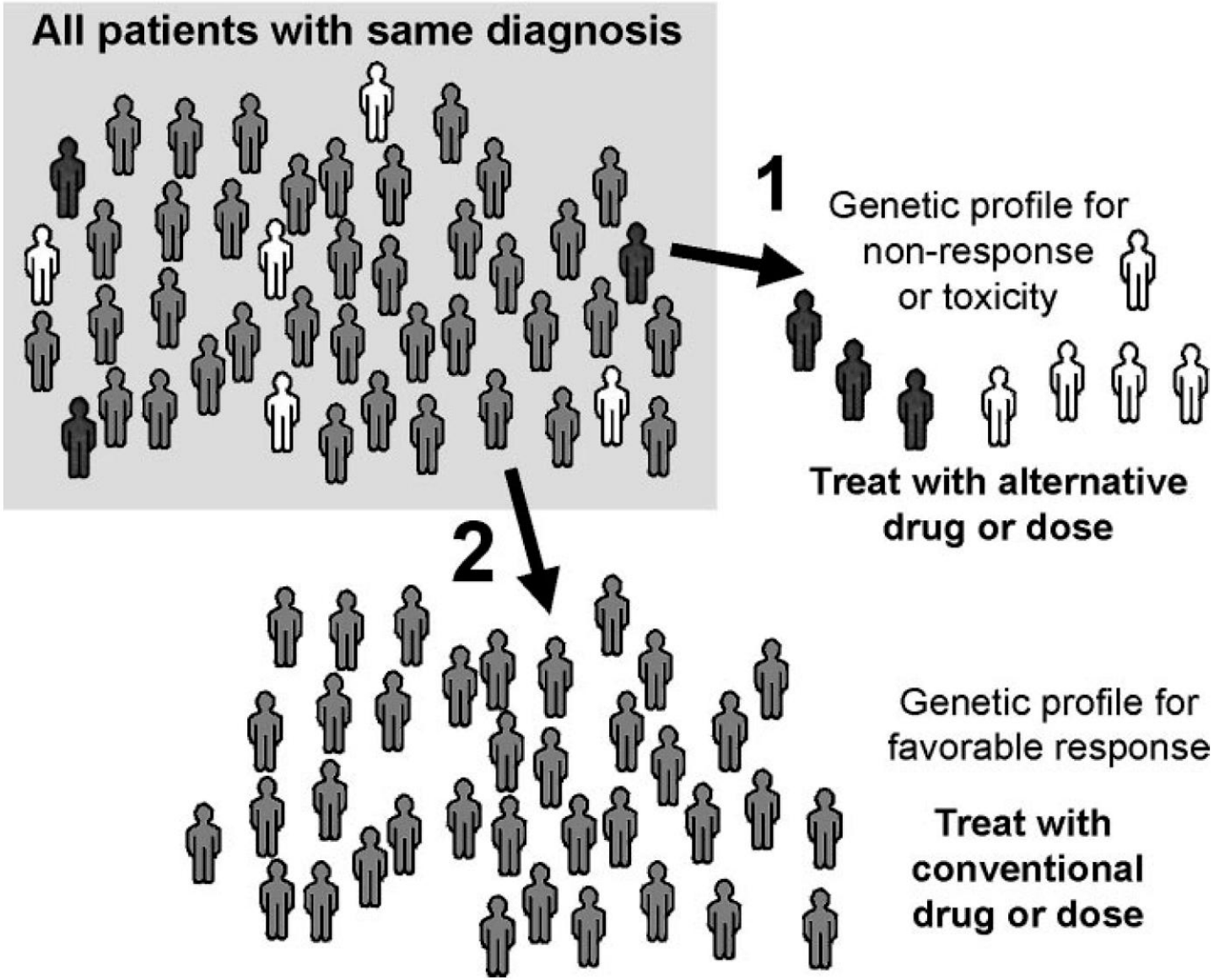


Figure 3 The left panel depicts the population distribution of drug metabolism phenotypes for most of the common genetic polymorphisms identified to date, which largely involve mutations that confer complete or near-complete loss of enzyme activity. The right panel depicts the population distribution of drug metabolism phenotypes for mutations associated with altered enzyme activity but not compete loss of function, reflecting the normal distribution of activity that typifies the metabolism and clearance of most medications. It is anticipated that at least some of the variability associated with the range of activity within a normal distribution will be inherited and due to mutations in the promoter regions of involved genes or amino acid changes that decrease or increase activity, but that do not eliminate activity

Potential of Pharmacogenomics



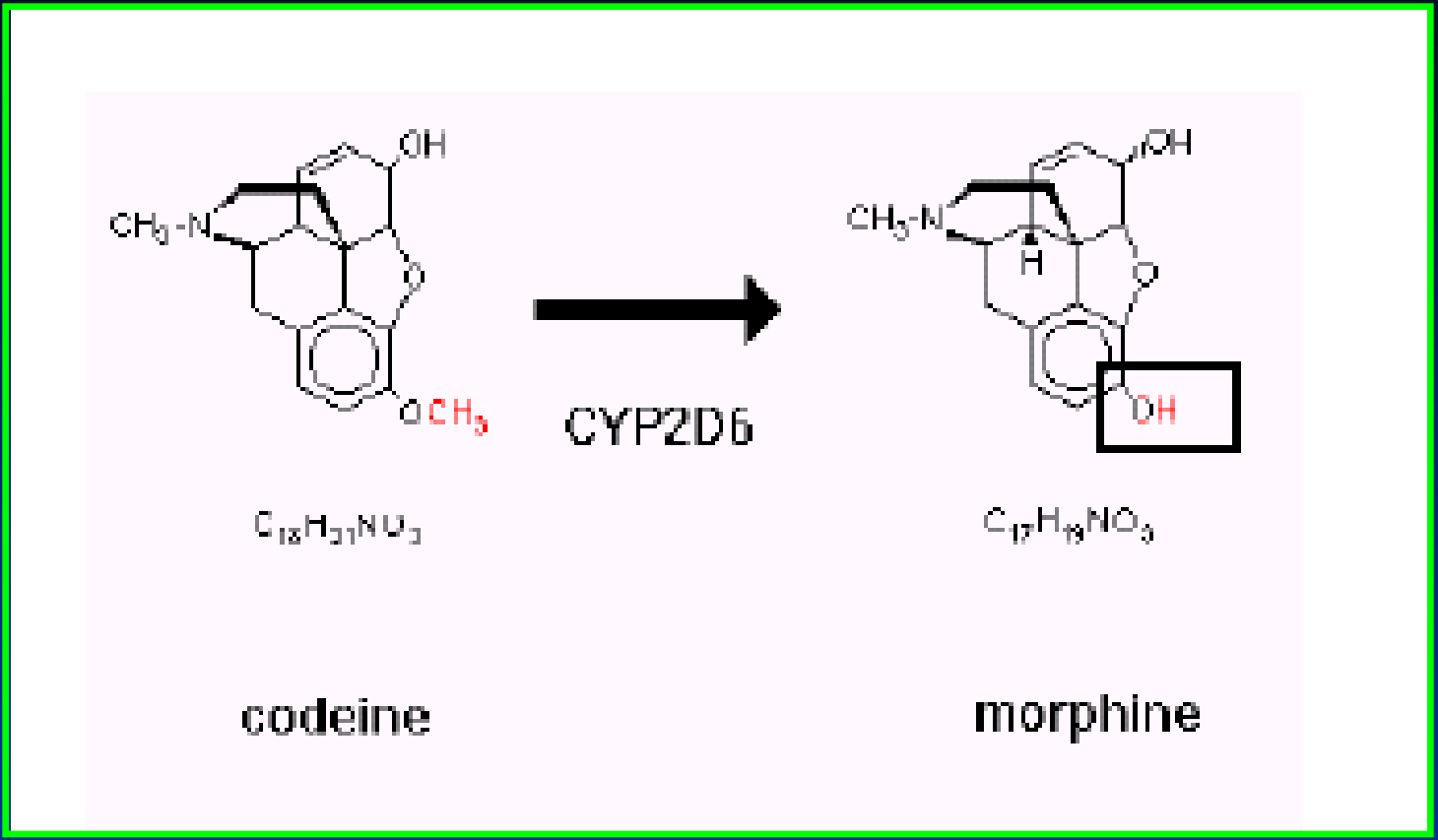
• El objetivo de la farmacogenómica es definir la contribución de las diferencias genéticas en la disponibilidad de las drogas o de los targets de las mismas en la respuesta a las drogas para mejorar la seguridad, y la eficacia de la terapia a través de la implementación de un tratamiento individualizado y guiado geneticamente.

• Si bien las diferencias heredables en cuanto a las enzimas metabolizantes son rasgos monogenéticos, el efecto farmacológico global de una droga es generalmente poligénico, determinado por numerosos genes que codifican para proteínas involucradas en múltiples vías del metabolismo de la droga, efecto terapéutico y disponibilidad de la misma

❖**POLYMORPHISMS** in both the individual's genome, as well as the tumour genome, will affect drug response—tumours are expected to be of the same genetic make up with respect to specific polymorphic sites as somatic tissue, unless new mutations have occurred or the site is subject to chromosomal loss. More than 1% frecuency in population.

❖Drug-related toxicity almost exclusively depends on the genotype of non tumour tissue. Inherited polymorphisms will have a key role with respect to toxicity, a crucial dose-limiting factor in most cancer chemotherapy regimens.

O-Dealkylation of Codeine by CYP2D6



-2-10% población homocigota para alelos CYP2D6 no funcionales → resistentes al analgésico.

-Existen grandes diferencias inter-individuales en los niveles de control del dolor con dosis standard de codeína.

Enzimas metabolizantes polimórficas y drogas anti-cancer

Table 1. Possible effects of genetic polymorphism on protein structure and function

Type of polymorphism	Effect on protein expression and function	Example		Affected anticancer drug(s)
Nonsynonymous SNP in coding region	Altered amino acid or early stop codon resulting in a variant protein	*GSTP1*B* (313A>G, Ile105Val)	Altered substrate affinity [51]	Platinum agents
Synonymous (silent) SNP in coding region	Similar protein, but mRNA translation capacity may be altered, resulting in lower or greater protein expression	*ERCC 1*(19007C>T, Asn118Asn)	Lower mRNA levels and possibly lower enzyme activity [6, 7]	Platinum agents
Deletion or insertion in coding region	Frameshift or stop codon at another position, resulting in different protein	*CYP2D6*6* (1707delT, 118 frameshift)	No enzyme activity left [52]	Tamoxifen
SNP in noncoding region	May induce alternative protein splice variants; may affect protein transcription or stability	*DPYD*2A* (IVS14+1G>A, exon 14 skipping)	No enzyme activity left [53]	5-fluorouracil, capecitabine, tegafur
Deletion or insertion in noncoding region	May induce alternative protein splice variants; may affect protein transcription or stability	Thymidylate synthase 3′ UTR 6 bp deletion	Lower mRNA level → lower enzyme activity [54, 55]	5-fluorouracil, capecitabine, tegafur
SNP in promoter region	Similar protein, but expression may be altered	*CYP2C19*17* (−806C>T and −3402C>T)	Greater enzyme activity [56]	Cyclophosphamide, ifosfamide
Microsatellites (variable number of tandem repeats)	Similar protein, but expression may be altered	*UGT1A1*28* $(TA)_6TAA \rightarrow (TA)_7TAA$	Lower enzyme activity [57, 58]	Irinotecan
Gene copy number variants	Similar protein, but expression may be altered	*CYP2D6*1XN*	Multiple copies lead to greater activity [13]	Tamoxifen
Gene deletion	No protein transcribed	*GSTM1*0* (null allele)	No enzyme activity left [16, 59]	Platinum agents, melphalan

Abbreviations: SNP, single nucleotide polymorphism; UTR, untranslated region.

Farmacogenómica de enzimas metabolizantes

Tiopurina-S-metiltransferasa (TPMT)

- Enzima citosólica que inactiva 6-MP, Azatioprina y 6-TG en tejidos hematopoyéticos (drogas para LLA)
- Crom 6p22 10 exones, 9 intrones, 34Kb
- Distribución trimodal de actividad TPMT:
  - ~ 90% alta actividad
  - ~ 10% actividad intermedia
  - ~ 0.3% actividad nula

-A < actividad TPMT se acumulan nucleótidos TG intracelulares y ↑ toxicidad hematopoiética severa (mielosupresión, fiebre)

-Existen 23 variantes polimórficas pero solo tres se traducen en > 95% de las deficiencias reportadas

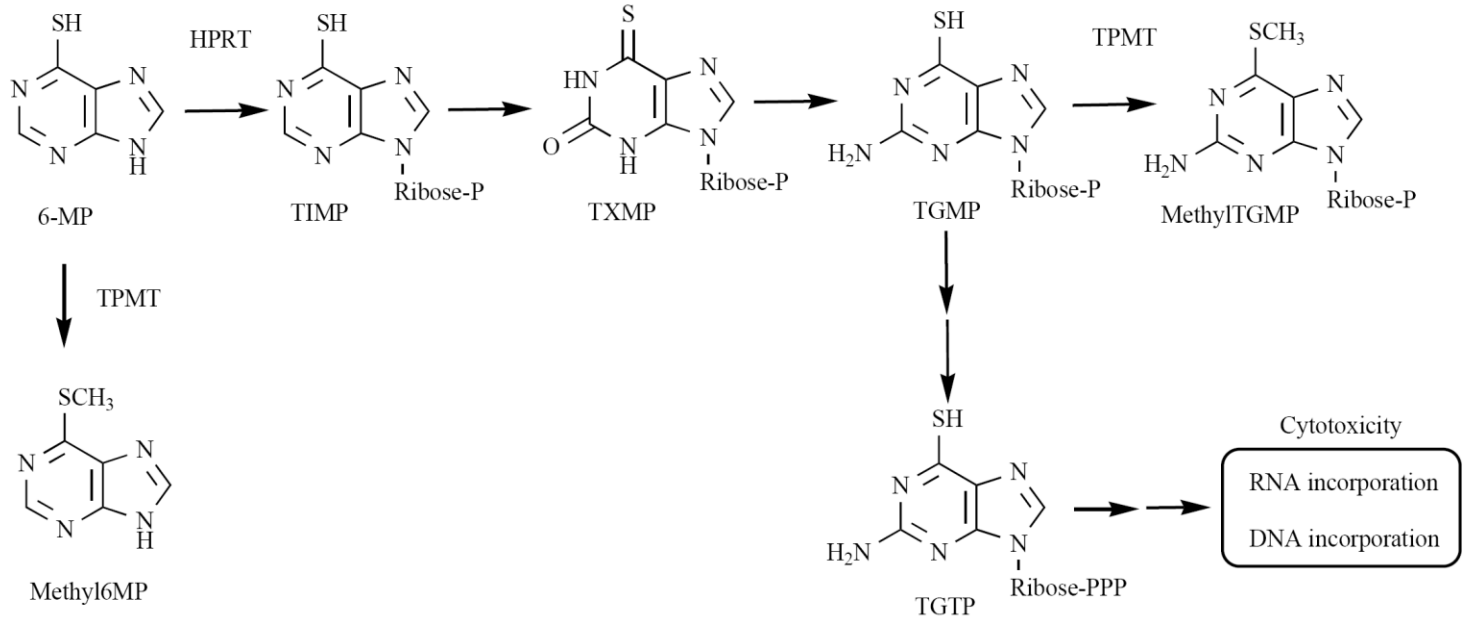


Fig. (1). Putative biotransformation pathway of 6-mercaptopurine. From references [18, 19].
6-Mercaptopurine, 6-MP; Thioinosine monophosphate, TIMP; hypoxanthine guanine phosphoribosyl transferase, HPRT; thioxanthosine monophosphate, TXMP; thioguanosine monophosphate, TGMP; thioguanosine triphosphate, TGTP.

Table 1 Ethnic frequency (%) of allelic variants in the *TPMT* gene

Allelic variant	SNPs	Caucasian	African-Americans	Asians	Hispanics	Africans
*TPMT*2*	G238C	0.17–0.5	0.4	0	0.7–2.2	0
*TPMT*3A*	G460A A719G	3.2–5.7	0.8	0–0.3	1.5–3.1	0
*TPMT*3C*	A719G	0.17–4.8	2.4	2.9–3.7	1.0	7.6

SNP, single nucleotide polymorphism.

TPMT

- Se comprobó un 98% concordancia entre fenotipo/ genotipo
- Heterocigotas niveles intermedios de actividad TPMT, homocigotas pobre metabolizantes o nulos.
- Con reducción de dosis al 90% en homocigotas obtienen igual sobre- Vida que wt con dosis habitual. Reducción de dosis 50% en heterocigo

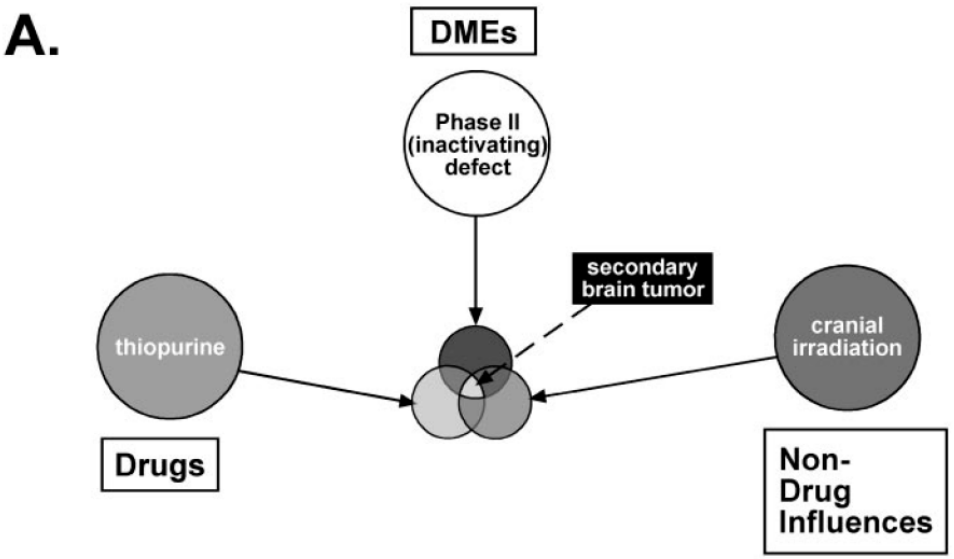
- 2004 FDA cambió el prospecto y RECOMIENDA Test de actividad y/o genotipo para TPMT para ajustar la dosis de las drogas.

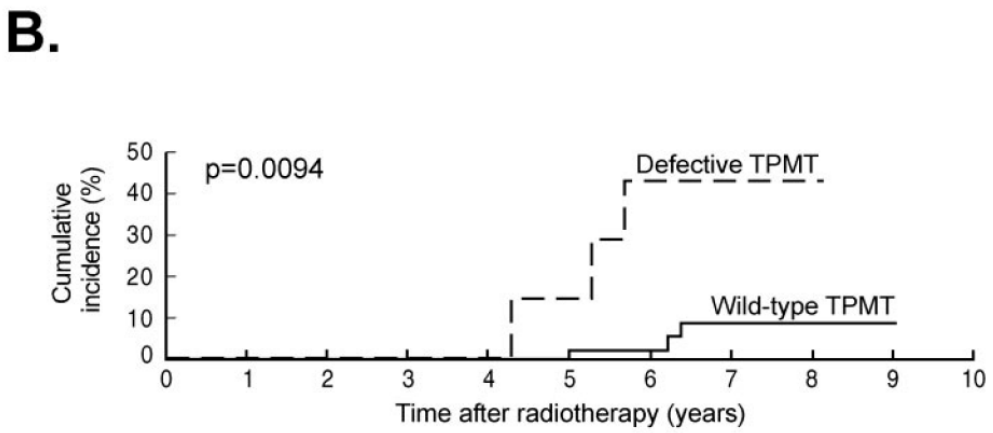
- Los pacientes con mayor actividad TPMT (wt) 2.9 veces >riesgo de recaída que los deficientes (> actividad TPMT > exposición a la droga efectiva que los pobres metabolizantes (↑ dosis en wt?)

- Los pacientes con ↓ actividad tienen > riesgo de cànceres secundarios

Compromiso entre toxicidad y eficacia

Interactions of Genetic Polymorphisms and Treatment May Result in Adverse Effects





Estimated cumulative incidence of radiation-associated secondary malignant brain tumor in patients treated with concomitant mercaptopurine and irradiation.

Inherited difference in drug metabolizing enzymes (DME) can alter the systemic exposure to affected substrates and thereby predispose to adverse interactions with other components of therapy. The top panel depicts such an interaction among the genetic polymorphism of TPMT, the affected thiopurine substrate mercaptopurine), and concomitant treatment with cranial irradiation. Only those patients with ALL who were treated with cranial irradiation for CNS leukemia developed a subsequent malignant brain tumor 4–8 years after being cured of their ALL. However, as depicted in the bottom panel, the cumulative incidence of a brain tumor was significantly higher among patients who inherited a deficiency of TPMT (homozygous wild type or heterozygous) (40 vs 8.3%) and were treated with mercaptopurine concomitantly with their radiation therapy

METABOLISMO DEL FOLATO Y ANTIFOLATOS

- Agentes anti-folato: son drogas cuyos blanco de acción son las enzimas del metabolismo del folato (ej: dihidrofolato reductasa) → usadas para el tratamiento de varios cánceres

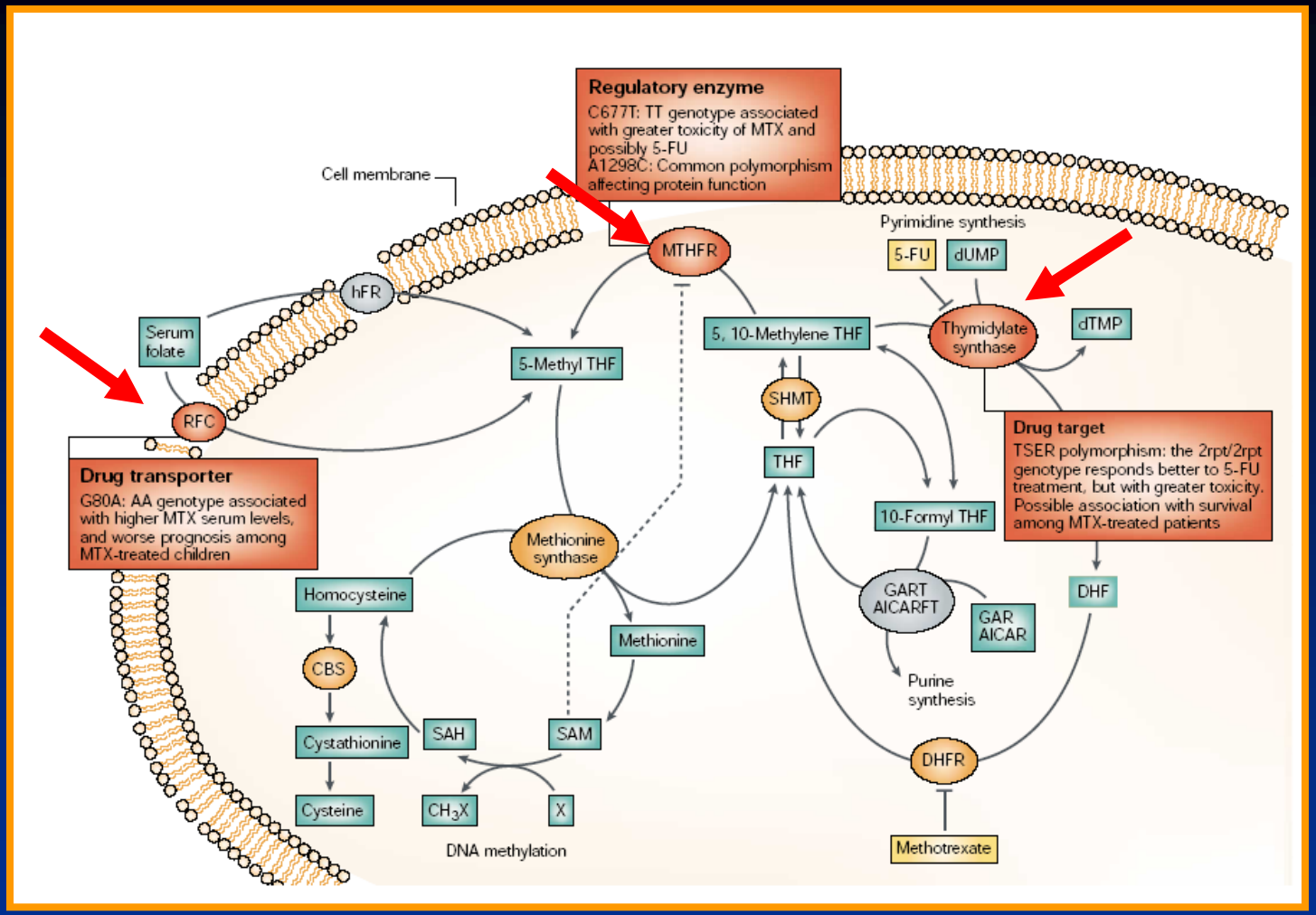
-Metotrexate (DHFR) y 5-fluorouracilo (Capecitabine) → inhibidores de la vía del folato (TS)

- Reducen la proliferación de células neoplásicas por disminuir la síntesis de nucleótidos.

-Las fallas en la síntesis de nucleótidos llevan a la incorporación de uracilo al DNA durante la replicación, resultando en rupturas de la doble cadena. Metabolitos activos de fluoropirimidinas también se incorporan al DNA y RNA, causando daños en la estructura e interfiriendo con la síntesis de RNA y las funciones celulares.

-Como el folato es requerido para la síntesis de DNA y la reparación, las células normales en rápida división del tracto digestivo y la médula ósea son las más afectadas causando la toxicidad (mucositis y mielosupresión).

Folate metabolism and related pathways



Polymorphisms have been found that are associated with pharmacogenetic outcomes in three key proteins in these pathways: the drug transporter protein reduced folate carrier (RFC); the regulatory enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR); and the drug target thymidylate synthase. Key enzymes are denoted as ovals, substrates as rectangles. Red ovals denote enzymes with genetic polymorphisms that have been investigated in pharmacogenetic studies. Orange ovals denote enzymes for which functional genetic polymorphisms have been described. 5-FU, 5-fluorouracil; AICAR, 5-aminoimidazole-4-carboxamine ribonucleotide; AICARFT, AICAR formyltransferase; CBS, cystathionine-β-synthase; DHF, dihydrofolate; DHFR, DHF reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; GAR, glycinamide ribonucleotide; GART, Phosphoribosylglycinamide formyltransferase; hFR, human folate receptor; MTX, methotrexate; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; X, various substrates for methylation

5-FU

TEJIDO TARGET

1- Anabolismo – nucleotidos tóxicos incorporados al DNA y RNA
2- Inhibe a la Timidilato Sintasa en la vìa de replicaciòn del DNA

HIGADO
DPD metaboliza 5-FU a Dihidrofluorouracilo inactivo

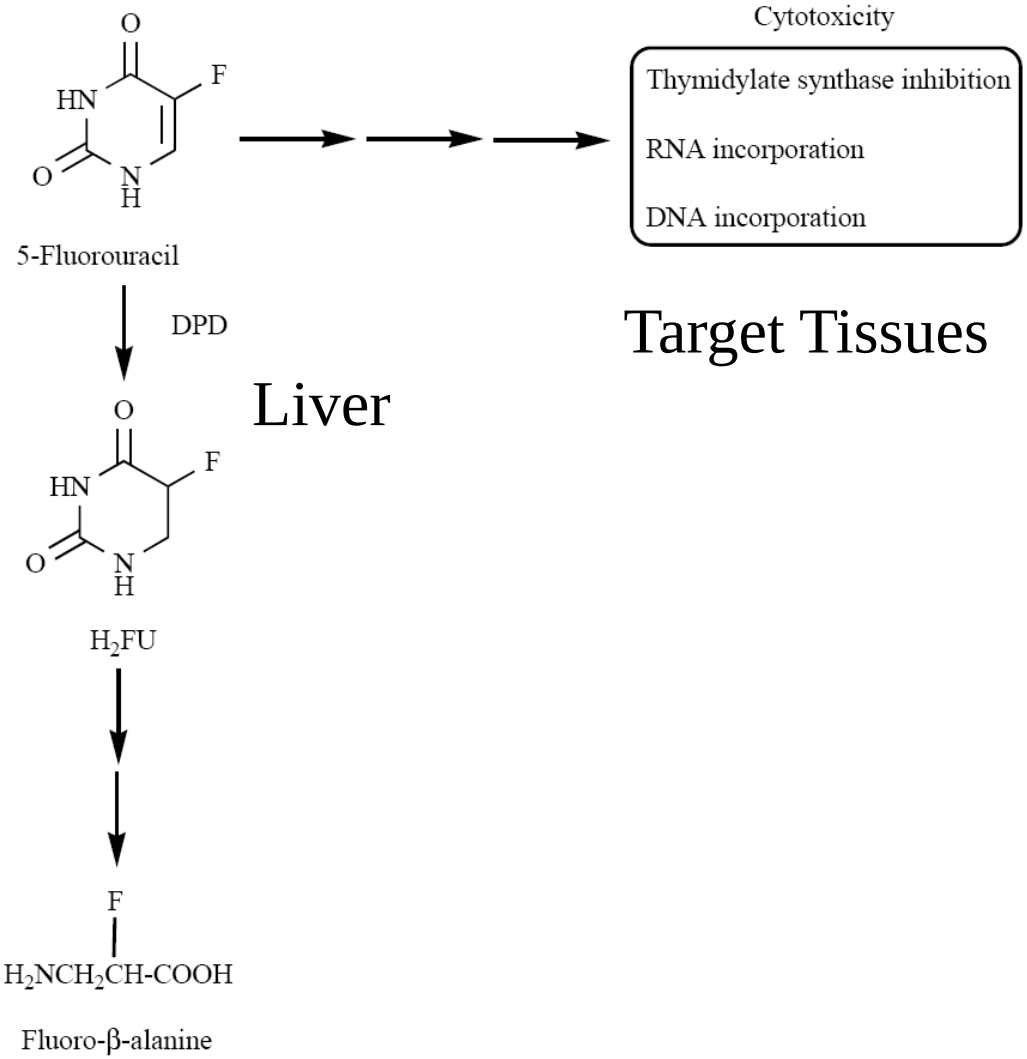
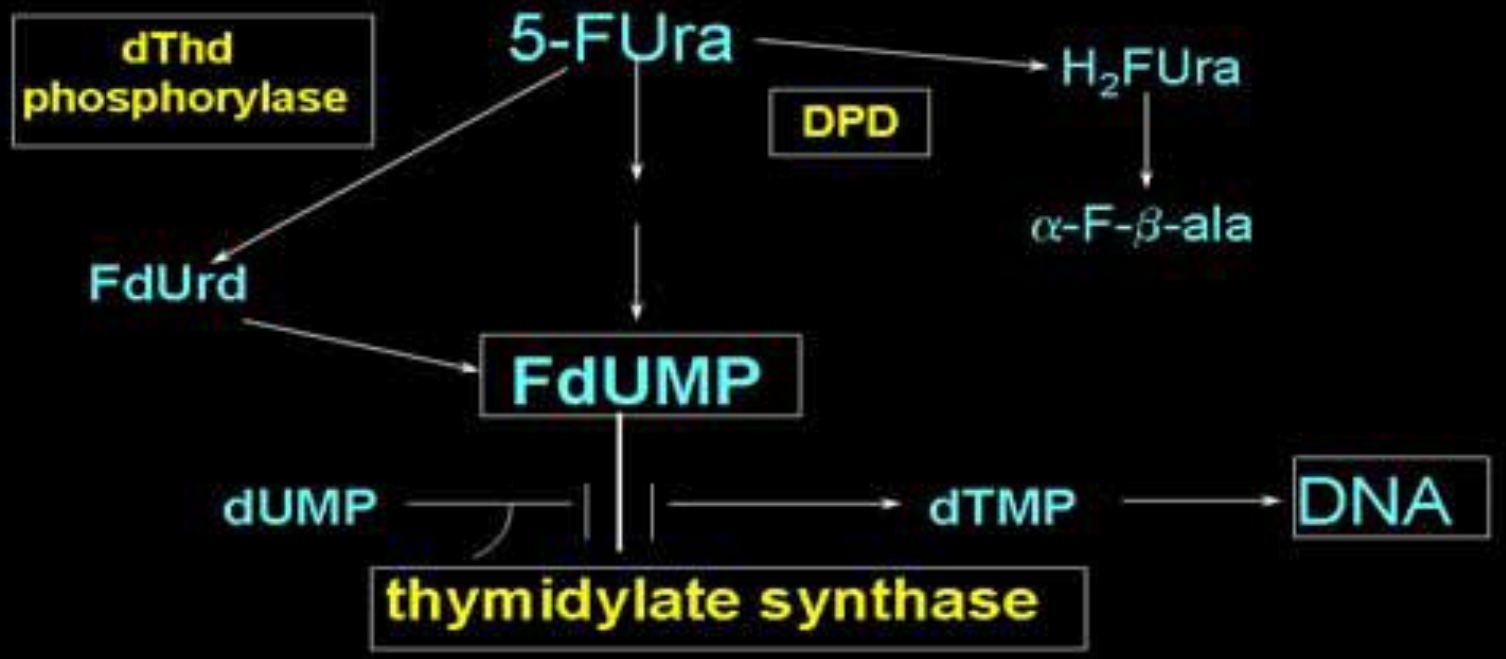


Fig. (3). Metabolic pathway of 5-fluorouracil. From reference 81. Dihydropyrimidine dehydrogenase, DPD; Fluorodihydrouracil, H_2FU.

Dihydroxypiridine dehydrogenase (DYPD variants)

- Expressed in the liver catabolizes uracyl and thymidine (pyrimidines)
- Responsible for catabolism of 5-fluorouracil
- more than 85% administered 5-FU is inactivated by DPD
- Absent in ~ 3% of Caucasians
- Deficient patients treated with conventional doses of 5-FU experience diarrhea, stomatitis, mucositis, myelosuppression, neurotoxicity and death.

Metabolism and mechanism of action of 5-Fluorouracil (5-FUra)



-Mutaciones y SNPs pueden causar deficiencias en la actividad de DPD

-Deficiencia en DPD puede producir severa toxicidad para 5-FU (3-5%)

-La mutación más frecuente afecta el sitio donor de splicing IVS14+1G>A (1/4 pts con toxicidad lo tiene).
-Actividad DPD es dificil de testear y el test por genotipo es poco sensible.
- Aùn con DPD normal hay pts que experimentan toxicidad.

-Se está desarrollando un método para testear rapidamente la actividad DPD a priori

- Faltan datos que apoyen el valor predictivo de DPD para toxicidad para 5-FU

Table 2 Ethnic frequency (%) of the *IVS14+1G>A* variant in the *DYPD* gene

Ethnic/nationality	Allele frequency (%)
European	0.47–2.2
Turkish	0.75
Taiwanese	0.0–2.7
Japanese	0
African-Americans	0

Thymidylate Synthase Polymorphism (TS)

TSER (5´enhancer region)

- Tandemly repeated sequence at the 5' end is polymorphic and contains double or triple repeats (230 vs 250bp)
- These sequences modulate the expression of the hTS gene in *in vitro* studies, trible repeats are associated with higher TS expression
- Identifying the genetic polymorphism of TS may be predict toxicity and response to therapy.

Frequency of TS 5' polymorphism

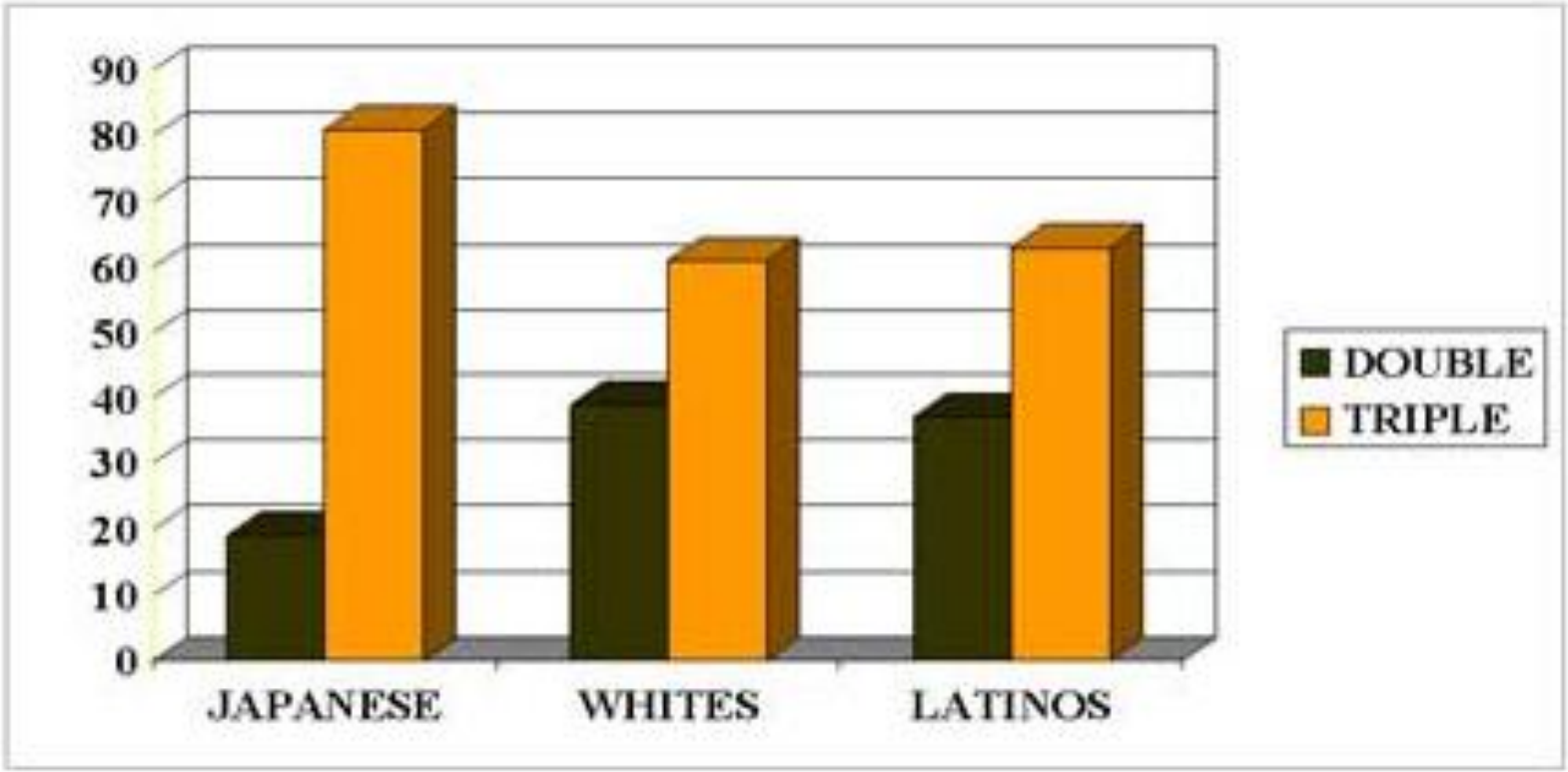


Table 1 | **Polymorphisms in folate metabolism and response to cancer drugs**

Polymorphism	Study population	Findings	References
TSER 28-base-pair repeats			
	Tumour tissue from 50 patients with metastatic colorectal cancer receiving 5-fluorouracil	2rpt/2rpt individuals (*n* = 11) had significantly greater toxicity	36
	65 patients with rectal cancer receiving pre-operative 5-fluorouracil	3rpt/3rpt individuals (*n* = 27) had a lower probability of tumour downstaging compared with 2rpt/2rpt individuals (*n* = 13)	77
	Tumour tissue from 221 patients with Dukes' C colorectal cancer receiving either surgery alone or surgery and 5-fluorouracil	No association between *TSER* polymorphism and survival. Similar effects of 5-fluorouracil treatment seen among each of the *TSER* genotypes	59
	24 patients with metastatic colorectal cancer receiving 5-fluorouracil	3rpt/3rpt homozygous individuals had decreased median survival	58
	205 children with acute lymphoblastic leukaemia treated with methotrexate	3rpt/3rpt homozygous children (*n* = 50) had significantly shorter event-free survival times compared with those with the other *TSER* genotypes	68
	24 patients with metastatic colorectal cancer receiving capecitabine	Individuals with the 2rpt/2rpt genotype (*n* = 4) had a greater response rate compared with individuals with the 2rpt/3rpt (*n* = 12) or homozygous 3rpt/3rpt genotypes (*n* = 8)	78

❖ **Patients who were homozygous for the *TSER*-double-repeat genotype (2rpt/2rpt; associated with lower thymidylate synthase expression) were more likely to respond to 5-fluorouracil therapy, but were also more likely to experience significant toxicity**

TS 5' polymorphism predict gene expression in normal liver

TS Genotype	Normal liver tissue		
	n	TS mean	95% CI*
L/L	7	8.21	4.79, 14.06
S/L	14	4.56	3.12, 6.68
S/S	5	3.19	1.69, 6.03

* CI denotes confidence interval.

The Pharmacogenomics Journal, 2001,1,65-70

TS gene polymorphism predicts response to chemotherapy

	L/L	L/S	S/S
Response	2	3	4
No Response	20	17	4

p=0.041 (Fisher exact test)

The Pharmacogenomics Journal, 2001,1,65-70

TS gene polymorphism predicts toxicity to 5-FU chemotherapy

	L/L	L/S	S/S
Grade 1	9	1	0
Grade 2	7	12	3
Grade 3	6	6	5

p=0.008 (Fisher exact test)

The Pharmacogenomics Journal, 2001,1,65-70

Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA levels in tumor tissues and the efficacy of 5-fluorouracil in patients with non-small-cell lung cancer.

Lung Cancer. 2004 Aug;45(2):189-96

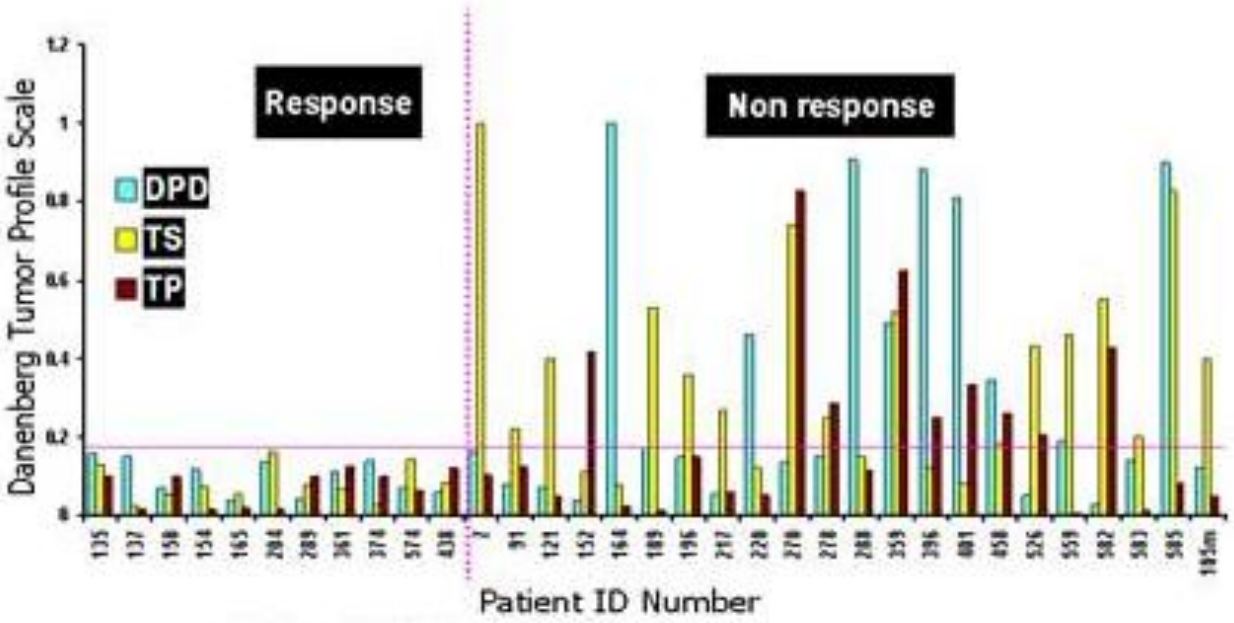
❖The authors examined 116 stage I-IIIA NSCLC patients for intra-tumoral expression of thymidylate synthase (TS) and (DPD) using TaqMan transcription RT-PCR assay to clarify the correlation between gene expression and the efficacy of 5-FU in patients with NSCLC.

Patients who were administered 5-FU alone after surgery comprised the 5-FU group (n = 30), and those who underwent only surgery comprised the control group (n = 86).

When dichotomized at the mean TS and DPD mRNA level, patients with low-DPD tumors who were administered 5-FU had a significantly better prognosis than those who did not receive adjuvant treatment (p = 0.041). In addition, in the 5-FU group, 10 patients with both low-TS and low-DPD tumors have not had any relapse, whereas 8 of the 20 patients with either high-TS or high-DPD tumors developed distant metastasis after surgery.

Based on these results, the quantitation of TS and DPD mRNA levels may predict the efficacy of 5-FU after surgery for patient with NSCLC.

DPD, TS and TP Gene Expression vs Response to 5-FU/LV in Colorectal Cancer



Actual Clinical Trial Data

Salonga et al. Clin Cancer Res 2000; 6: 1322-1327.

The patients with **low expression of all three of the genes** had significantly **longer survival** than patients with a high value of any one of the gene expressions. The results of this study show that intratumoral gene expression level of DPD is associated with tumor response to 5-FU and that the use of more than one independent determinant of response permits the identification of a high percentage of responding patients.

MTHFR

-Enzima polimórfica central de la vía del Folato que desvía metabolitos del folato hacia la síntesis de metionina.

-Polimorfismo funcional C677T (alanina222valina) se asocia con 30% de la actividad del wt (677CC) en el 677TT.

Varias enfermedades se asocian a este polimorfismo: ALL, polipos colorectales y cáncer de colon, defectos del tubo neural y posiblemente enfermedad cardiovascular.

-La presencia de 677TT se ha asociado a mayor toxicidad hematológica y en mucosas al metotrexate

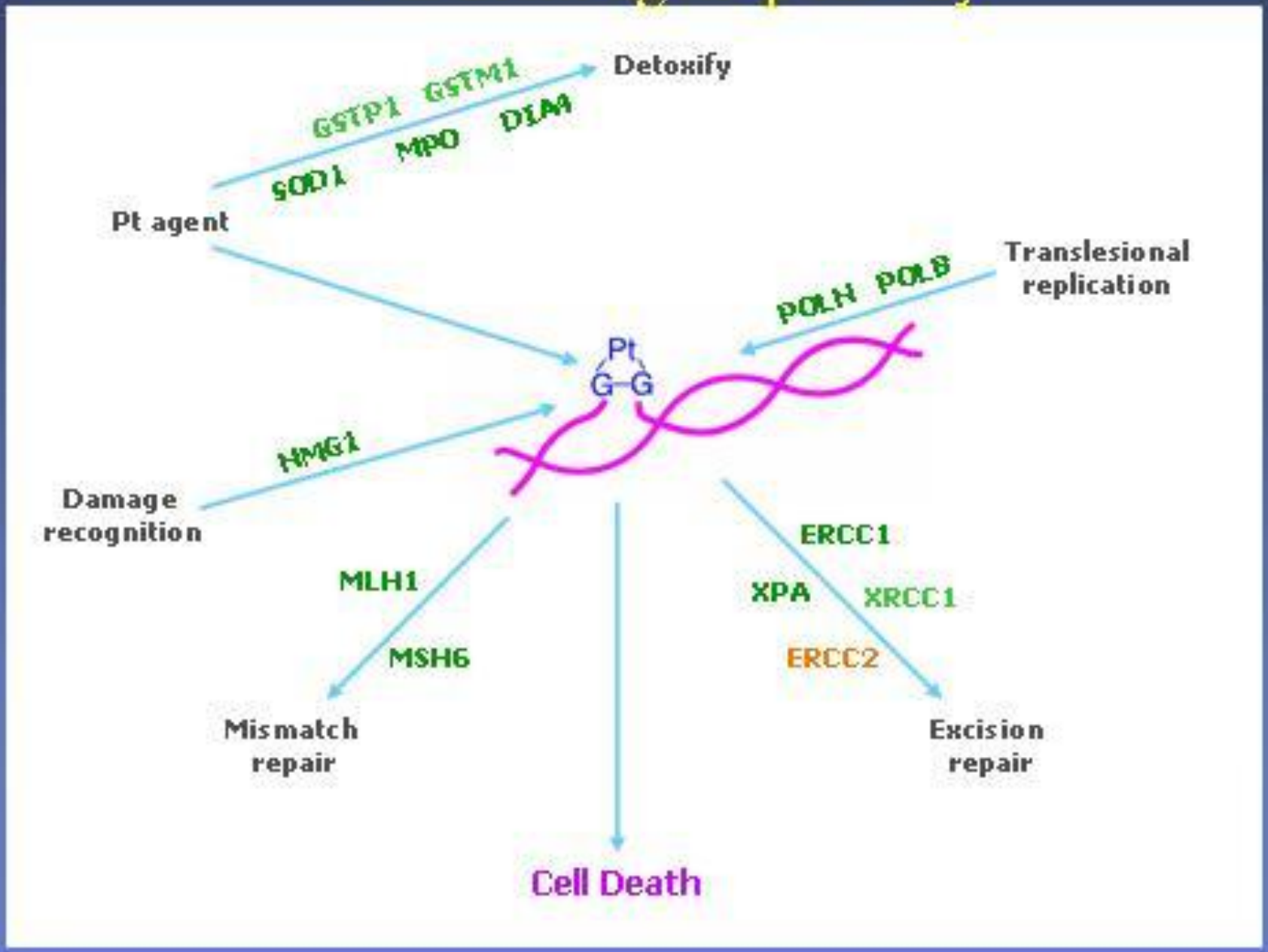
Genes involved in 5-Fu/oxaliplatin pathways

5-FU pathway:	5‘-TS; 3‘-TS
Platinum pathway:	XPD*751*; XPD*156*; XPA; ERCC1-*19007*; GSTT1, GSTM1,GSTP1*105* XRCC1-*399*

Polimorfisms in folate and response to cancer drugs

Polimorfism	Study population	Findings
MTHFR C677T		
	51 patients with stage III colon cancer receiving 5-fluorouracil and leucovorin	No differences in survival based on genotype 677TT (n = 4)
	6 patients with early breast cancer receiving cyclophosphamide, methotrexate and 5-fluorouracil	5 of the 6 patients who experienced grade IV toxicity (NCI-CTC) had the 677TT genotype
	220 patients with CML following haematopoietic-cell transplant	Patients with 677TT genotype (n = 36) receiving methotrexate experienced increased oral mucositis and somewhat slower platelet recovery
	61 patients with acute leukaemia receiving maintenance chemotherapy including methotrexate	Increased methotrexate toxicity among patients with the 677TT genotype (n = 15)
	6 children with acute lymphoblastic leukemia who had had methotrexate-related neurotoxicity	Lymphocytes from the two children with the 677TT genotype (n = 2) exhibited greater *in vitro* methotrexate sensitivity compared with those with the 677CC (n = 2) or 677CT genotypes (n = 2)
	43 patients with ovarian cancer receiving either methotrexate alone or in combination with carboplatin	677TT individuals (n = 13) had a significantly higher risk of experiencing grades III–IV toxicity (WHO criteria), and had significantly higher plasma homocysteine levels after methotrexate treatment
	43 patients with metastatic colorectal cancer receiving fluoropyrimidine chemotherapy	Individuals with the 677TT genotype (n = 17) were significantly more likely to respond to treatment than with those with the 677CT (n = 21) or 677CC genotypes (n = 5)
Reduced folate carrier G80A		
	204 children with acute lymphoblastic leukaemia treated with methotrexate	Patients with the 80AA genotype (n = 45) had significantly shorter event-free survival compared with those with the 80GG genotype (n = 61); patients with the 80AA genotype had higher plasma methotrexate levels than those with other genotypes, suggesting decreased cellular uptake of methotrexate

Platinum agent pathway



GSTP1 Glutathione S-Transferase P1

- Enzima detoxificante que protege a las células de la acción de agentes alquilantes y especies reactivas de oxígeno

-Detoxifica metabolitos reactivos de la ciclofosfamida usada contra varios tumores sólidos

-Detoxifica compuestos de platino (oxiplatino)

-Polimorfismo I105V (isoleucina a valina, 33% en caucásicos), actividad reducida de GSTP1

- Homocigotas Val poseen mayor respuesta a ciclofosfamida + 5FU que los homocigotas Ile.

GST-P1 polymorphism

- Codon 105 Val-Ile (G-A) GST-P1 directly associated with enzyme activity
- associated with survival in breast and head/neck cancer
- associated with cancer risk testicular, bladder and esophageal

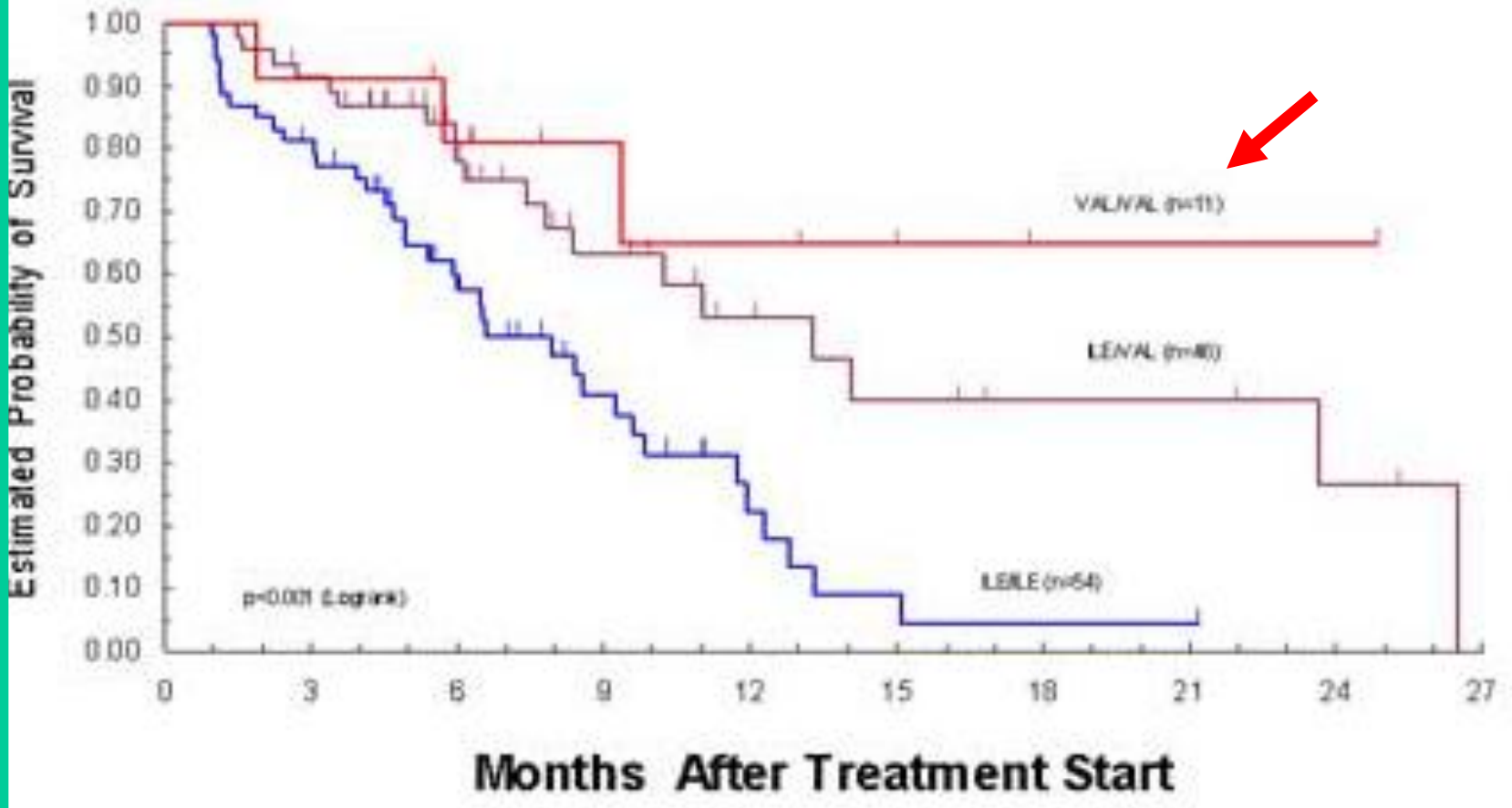
GSTP1-polymorphism and Survival

GSTP1 Genotype	No. of Patients	Median Survival Time (95% CI[1])	Comparison of Survival Genotype	p-value[2]
			Overall	<0.001
ILE/ILE	54	7.9 months (5.4, 9.6)	ILE/ILE vs. VAL/VAL	0.009
ILE/VAL	46	13.3 months (8.4,26.5)	ILE/VAL vs. VAL/VAL	0.35
VAL/VAL	11	24+ months (9.4,)	ILE/VAL vs. ILE/ILE	0.002

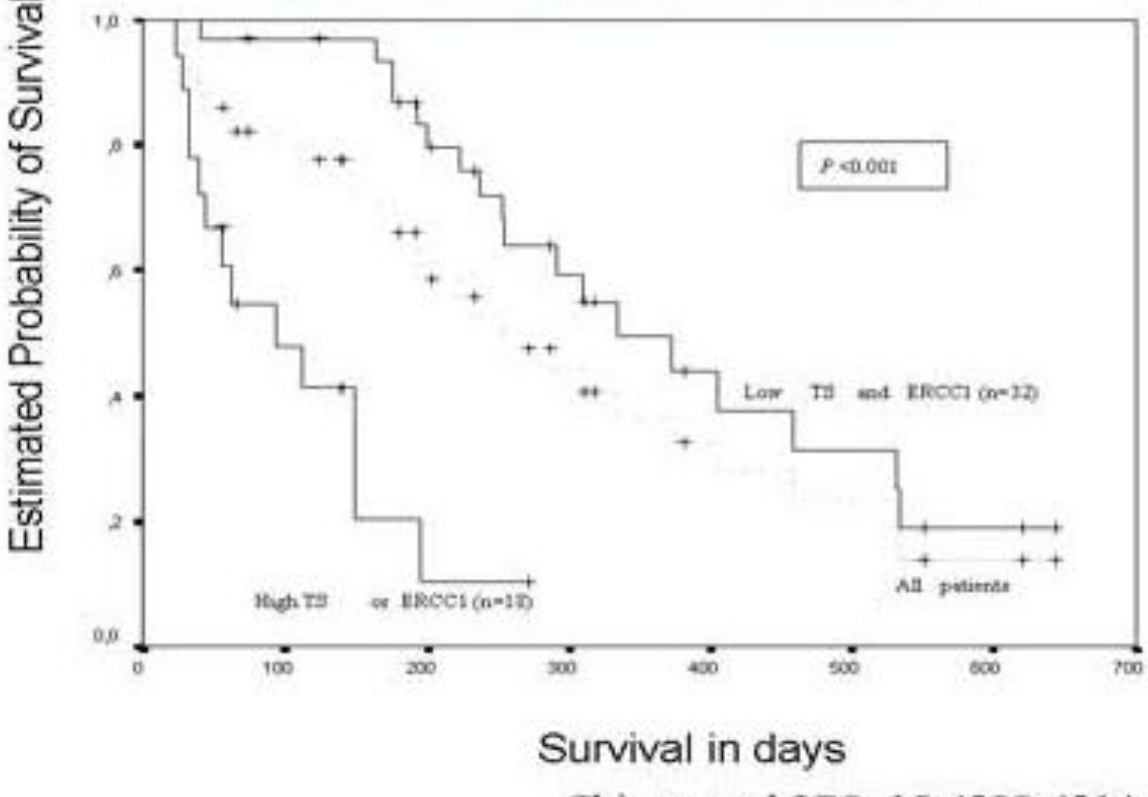
1. 95% confidence interval
2. Based on Logrank test. Since the overall p-value was significant at the 0.05 level, pairwise comparisons were made.

Colorectal cancer pts

GSTP1-polymorphism



TS and ERCC1 predicts survival to 5FU/Oxaliplatin Therapy



Shirota et al JCO, 19:4298-4304, 2001

These data suggest that intratumoral ERCC1 (excision cross-complementing gene) mRNA and TS mRNA expression levels are independent predictive markers of survival for 5-FU and oxaliplatin combination chemotherapy in 5-FU-resistant metastatic colorectal cancer.

Patients Characteristics

- 106 patients with metastatic colorectal cancer who failed 5-FU/LV and CPT-11 treated with 5-FU/Oxaliplatin

- Median Age (Range): 60 (24 - 83)
- Median Follow-Up: 11.4 (3.3-30.9) months
- Median TTP: 4.7 (4.2, 5.9) months
- Median Survival: 9.4 (6.6, 13.2) months

Br J Cancer. 2004 Jul 19;91(2):344-54.

ERCC-1 polymorphism

- ERCC1 a highly conserved enzyme, is specific to the nucleotide excision repair (NER) pathway
- ERCC1 gene contains a very common polymorphism at codon 118
- Ovarian cancer cell lines showed a 50% reduction in DNA adduct repair in a cell line containing the polymorphism compared to the "wild-type."

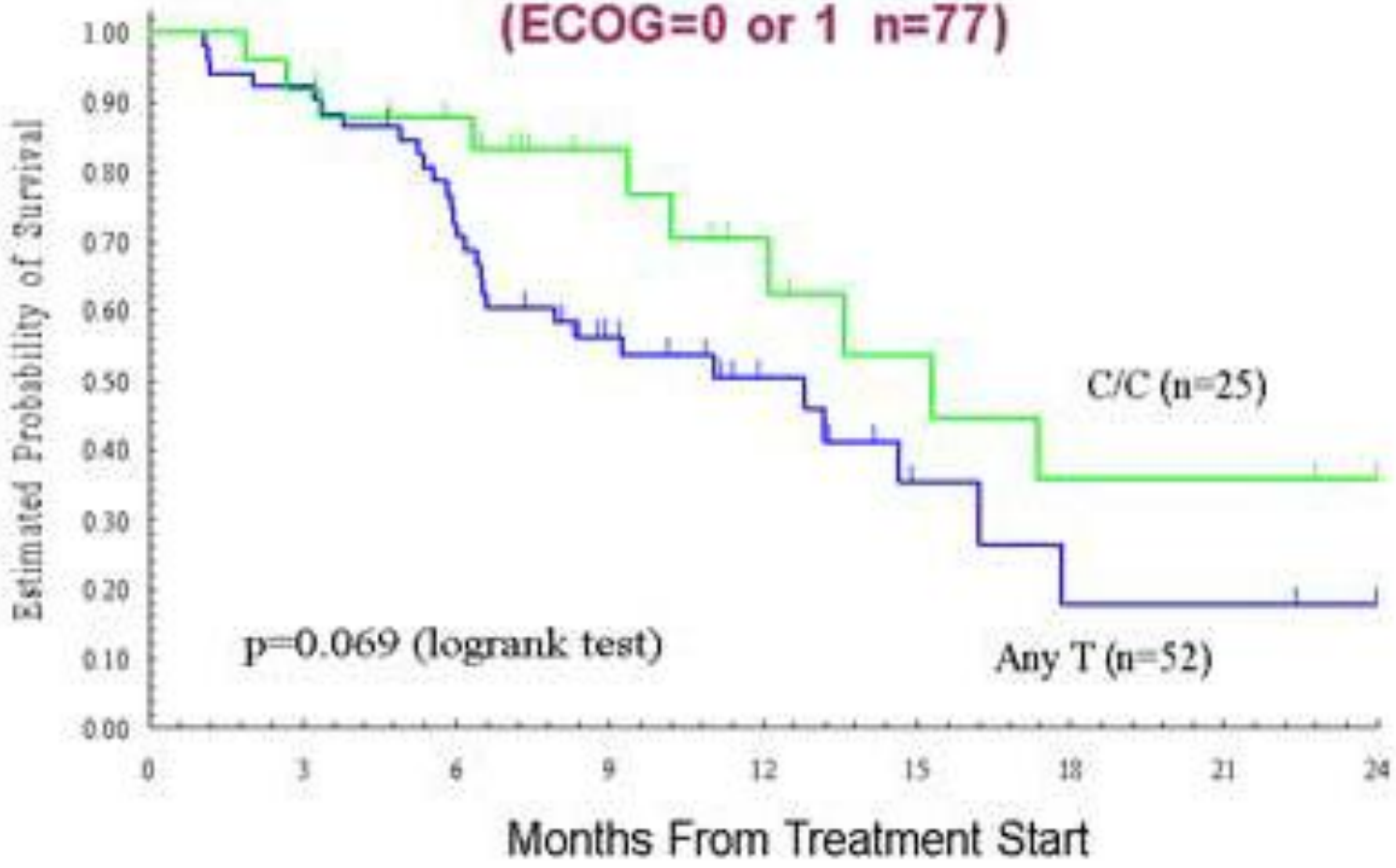
ERCC1 polymorphism and mRNA level in 31 patients

	No. patients	Geometry Mean of mRNA	95% Confidence Interval	p-value*
Genotype				0.096
C/C	11	2.31	(1.47, 3.61)	
C/T	12	2.91	(1.89, 4.48)	
T/T	8	4.91	(2.90, 8.32)	
Genotype				0.041
Any C	23	2.60	(1.91, 3.54)	
T/T	8	4.91	(2.91, 8.28)	

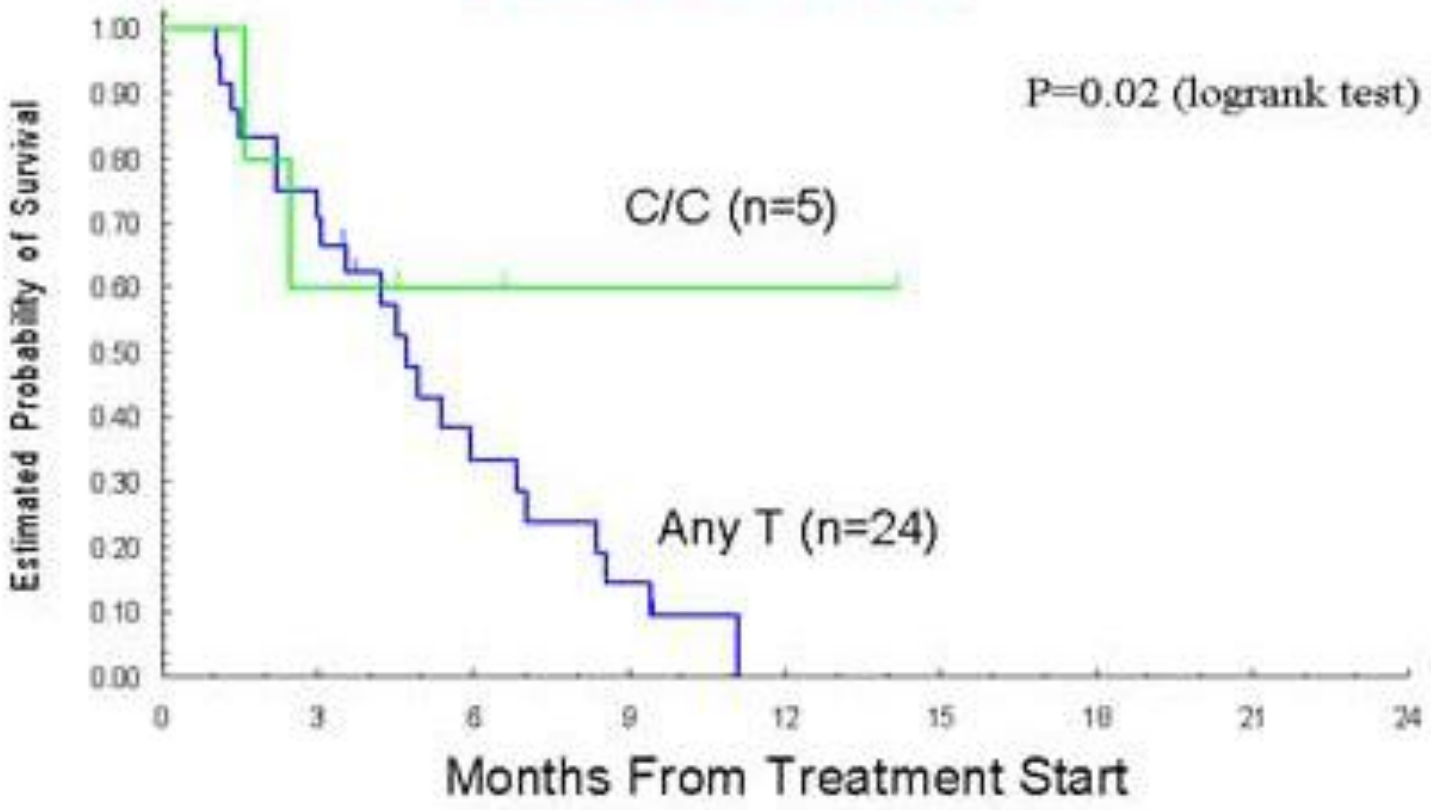
* based on least significant difference test

Association of ERCC19007 with Colorectal Cancer

(ECOG=0 or 1 n=77)



Association of ERCC19007 with Colorectal Cancer (ECOG=2 n=29)



Polimorfismos CYP (Citocromo P450)

-Familia de >60 enzimas expresadas en varios tejidos y principalmente en hígado.

- Metabolismo oxidativo de compuestos endógenos y aproximadamente 90% de todas las drogas.

-Las enzimas de mayor relevancia para la oncologìa clìnica son : CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 y CYP3A5.

-Polimorfismos en enzimas CYP correlacionan con distinta toxicidad y eficacia para ciertas drogas antineoplásicas.

CYP2D6

-Metaboliza del 20-25% de todas las drogas de uso clínico.

-22q23, existen 101 variantes incluyendo 26 alelos nulos.

-26 % alelos funcionales en caucàsicos y solo 50 % en asiáticos.

- Además de los polimorfismos, la duplicación del gen produce metabolizantes ultra-rápidos (2% suecos, 3.6% alemanes, 7-10% españole 10% italianos, 20% árabes y 29% etíopes)

Table 7 **Ethnic frequency (%) of allelic variants in the *CYP2D6* gene**

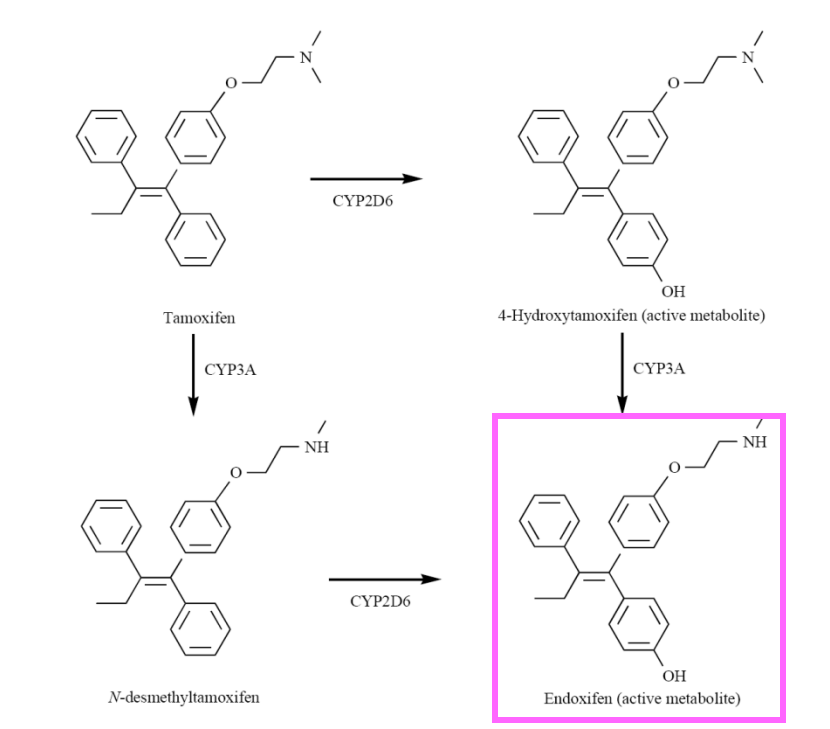
Allelic variant	SNPs	Caucasians	African-Americans	Asians	Hispanics	Africans	Middle Easterns
*CYP2D6*2*	C2850T	22–34	17.5–26.9	9.2–20	18.5–22.8	10.9–78	10–16
*CYP2D6*3*	2549A del	1.0–3.9	0.3–0.6	0–0.8	0	0–0.5	0–0.8
*CYP2D6*4*	G1846A	11.6–23.0	5.8–8.5	0.5–1.2	3.6–10.3	1.2–7.0	3.5–11.3
*CYP2D6*5*	2D6 deleted	1.6–7.3	6.0–6.9	4.5–6.2	2.3–4.2	33–6.1	0.1–1.5
*CYP2D6*6*	1797T del	0.7–1.4	0.5			0	0.7
*CYP2D6*10*	C100T	1.4–8.0	2.5–7.5	38.1–70	1.8–7.4	3.1–8.6	0.3–6.1
*CYP2D6*17*	C1023T	0.1–0.3	14.6–26	0–0.5	0	9.0–34	0.1–0.3

SNP, single nucleotide polymorphism.

Polimorfismos CYP2D6 y Tamoxifeno

- Modulador selectivo del receptor de estrógenos usado en pacientes con cáncer de mama en todos los estadíos con receptores hormonales +.

-≈35% de las pts con ca. A vanzado ER+ no responden a tamoxifeno y todos los tumores desarrollan resistencia aunque respondan
Tamoxifeno requiere del metabolismo hepàtico para incrementar su eficacia:



- Endoxifeno es 100 veces màs potente que el tamoxifeno como antagonista del estrógeno

- CYP2D6 es la enzima limitante en la formación de endoxifeno

-Variantes polimorficas de CYP2D6 se asocian a menores niveles de endoxifeno plasmático, mayor riesgo de recaída y menor indicencia de reacciones adversas en post-menopáusicas tratadas con tamoxifeno por 5 años .

AMPLICHIP P450

Table 1: Clinically Relevant Drug Substrates for Metabolism[66]

CYP2D6			
Beta Blockers	**Antidepressants**	**Antipsychotics**	**Others**
Carvedilol	Amitriptyline	Haloperidol	Atomoxetine
Metoprolol	Clomipramine	Risperidone	Codeine
Propafenone	Desipramine	Thioridazine	Dextromethorphan
Timolo	Imipramine		Flecainide
	Paroxetine		Mexiletine
	Venlafaxine		Ondansetron
			Tamoxifen
			Tramadol
CYP2C19			
Proton Pump Inhibitors	**Anti-epileptics**	**Antidepressants**	**Others**
Omeprazole	Diazepam	Amitriptyline	Cyclophosphamide
Lansoprazole	Phenytoin	Clomipramine	Progesterone
Pantoprazole	Phenobarbitone		

Impact of phenotype on drug metabolism

Roche

Diagnostics

Ultrarapid metabolizers[1]	Extensive metabolizers[2]	Intermediate metabolizers[2]	Poor metabolizers[1]
•Too rapid drug metabolism •No drug response at ordinary dosage (non-responders)	•Expected response to standard dose	•May experience some or a lesser degree of the consequences of poor metabolizers	•Too slow or no drug metabolism •Too high drug levels at ordinary dosage •High risk for side effects

In the case of pro-drugs the opposite phenomenon occurs: Ultrarapid metabolizers may suffer adverse events, and poor metabolizers may not respond [3]

1. Ingelman-Sundberg. *Trends in Pharmacological Sciences.* April 2004. Vol. 25, No. 4.
2. David Mrazek. *Current Psychiatry Online.* September 2004. Vol. 3, No. 9.
3. AmpliChip® CYP450 Test package insert.

AMPLICHIP

Population Differences for CYP2D6

Roche

Diagnostics

Allele	Predicted Enzymatic Activity	Japan	China	Caucasian EU	Caucasian US	Black American	Black African
*1	Normal	42-43%	23%	33-37%	37-40%	29-34%	28-56%
*2	Normal	9-13%	20%	22-33%	26-34%	20-27%	11-45%
*4	None	<1%	0-1%	12-23%	18-23%	7-9%	1-7%
*5	None	5-6%	6%	2-7%	2-4%	6-7%	1-6%
*10	Reduced	39-41%	50-70%	1-2%	4-8%	3-8%	3-9%
*17	Reduced	*	*	<1%	*	15-26%	9-34%
*41	Reduced	*	*	20%	*	*	*

Percentages represent ranges of allelic frequencies reported in published studies

AmpliChip® CYP450 Test package insert; from Bradford, L.D. 2002. Pharmacogenomics. 3:229-243

AMPLICHIP

UDP-GLUCURONOSILTRANSFERASA 1A1 (UGT1A1)

❖ For drugs that mimic natural molecules, such as the antifolates, antipyrimidines, anti-oestrogens and others, there are pre-existing biological pathways that define the disposition and activity of the agents.

❖However, many anticancer agents do not follow an existing biological pathway, but, rather, interact with a pharmacological pathway that consists of a diverse array of genes that would not have direct interplay in normal cell biology.
❖The construction of these pharmacological pathways entails the use of knowledge regarding drug absorption, excretion, activation and other metabolic functions.

❖Example: TOPOISOMERASE INHIBITOR **IRINOTECAN.**

A great complexity of factors that influence an individual drug, with a need for activation of irinotecan to SN-38; transporters causing efflux of both parent and metabolite; Citocromo P450-mediated inactivation of parent drug; glucuronidation of the active metabolite; variation in the cellular therapeutic target; and then a series of death genes that mediate the ultimate fate of the cell.

IRINOTECAN

❖The drug has demonstrated potent activity against many types of human cancer (gastrointestinal and pulmonary malignancies).

❖Also the addition of irinotecan to first-line therapy with fluorouracil and leucovorin has led to improved survival in patients with advanced colorectal cancer.

❖However, irinotecan does have significant side effects, including both acute and delayed diarrhea, neutropenia, and a vascular syndrome.

❖Can we identify patients who will receive antitumor benefit from irinotecan without experiencing severe, life-threatening, or fatal toxicity?

Possible pathways of irinotecan metabolism.

❖ Irinotecan (CPT-11) can be converted into the active metabolite SN-38 by carboxylesterases (CES) outside or inside the cell.

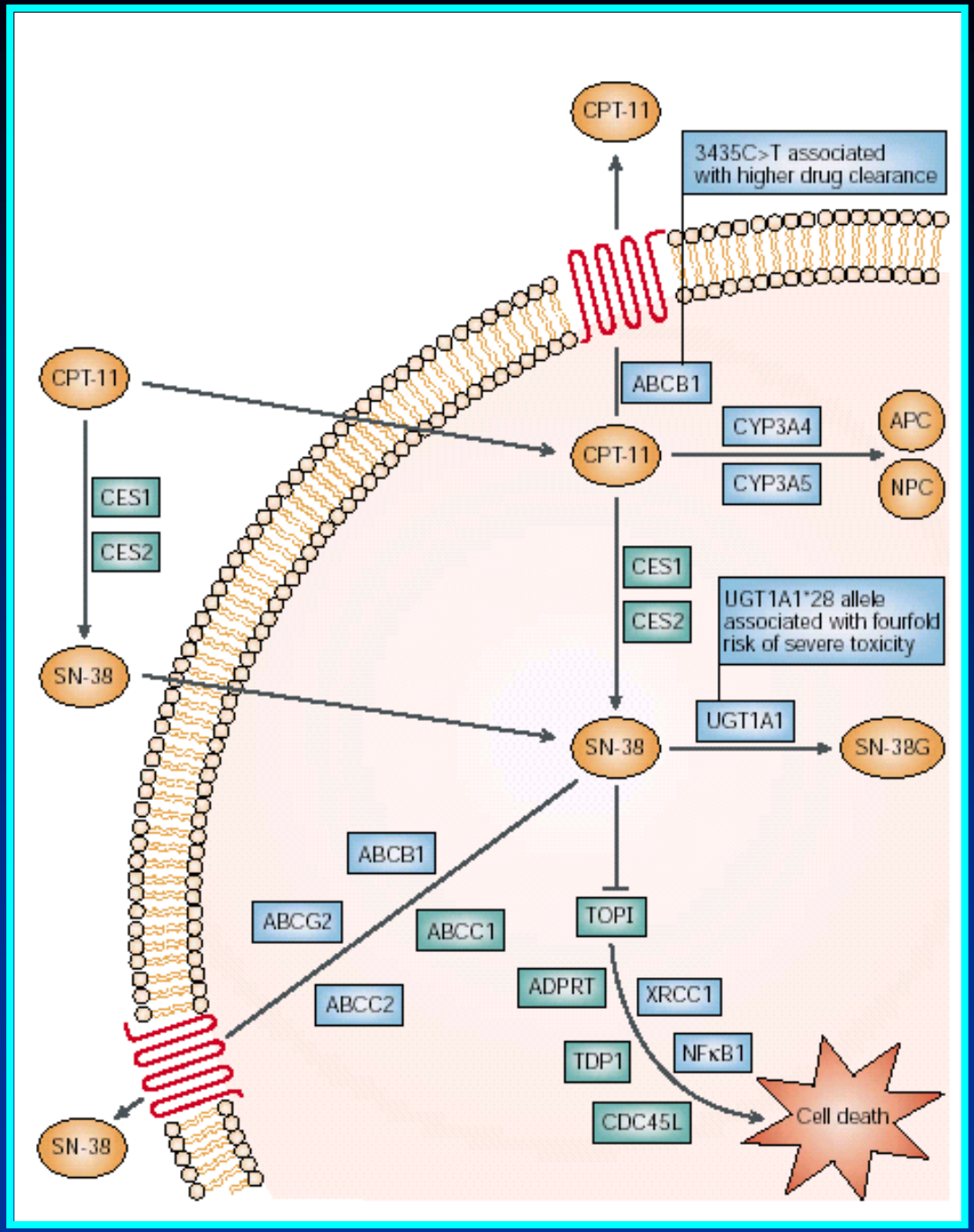
❖ CPT-11 and SN-38 are both substrates for the ATP-binding cassette (ABC) transport proteins —P-glycoprotein (ABCB), ABCC and ABCG — which transport the drug out of the cell.

❖ Alternatively, CPT-11 and SN-38 can be inactivated by cytochrome P450 enzymes (CYP) or by adition of glucuronic acid by the uridine diphosphate glucuronosyltransferases **(UGTs).**

❖ If SN-38 persists, it binds to its target topoisomerase I (TOPI), interfering with DNA synthesis and repair processes, culminating in cell death.

While each of these steps has the potential to substantially regulate irinotecan activity, it is glucuronidation by the protein UGT1A1 that has the clearest potential impact on patient care.

Possible pathways of irinotecan metabolism



ADPRT, ADP-ribosyltransferase; APC, inactive metabolite of SN-38; CDC45L, cell-division cycle 45L; NPC, inactive metabolite of SN-38; SN-38G, SN-38 glucuronide; TDP, tyrosyl-DNA phosphodiesterase; XRCC1, X-ray-repair cross-complementing defective-1.

UGTs

Represent major phase II drug metabolizing enzymes

Utilizes UDP-glucuronic acid as a cosubstrate from various substrates, such as bilirubin,hormones, drugs, and other xenobiotics

Formation of hydrophylic glucuronides from lipophylic substrates, facilitating the elimination through the bile and urine (glucuronidation is a “detoxification” reaction)

>16 human UGTs divided into two families—UGT1 and UGT2.

UGT1 family: (2q37) One large locus with 13 first exons, each with its own promoter and enhancer regions, spliced to identical exons 2 to 5. Only nine functional transcripts (UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7,1A8, 1A9, 1A10).

UGT2 locus: 7 separate genes clustered on chromosome 4q13 (*UGT2A1, 2B4, 2B7, 2B10, 2B11, 2B15, 2B17*).

- **UGT1A1 is also capable of forming bilirubin glucuronides**
  (Inheritable unconjugated hyperbilirubinemias Crigler-Najjar syndrome type 1 and type 2 and Gilbert's syndrome result of low activity *UGT1A1* gene or promoter alleles.)

- **More than 30 variant *UGT1A1* alleles have been identified.**

- **Innocenti et al reported an association between high pretreatment bilirubin levels and risk of developing grade 4 neutropenia after irinotecan therapy**
  (high steady-state bilirubin is indicative of low *UGT1A1* activity)

- **A dinucleotide repeat in the promoter region associated with Gilbert's – syndrome is known to influence gene expression**. The presence of 7 TA repeats, rather than the wild-type number of 6, results in the variant allele *UGT1A1*28*. (associated with ↓ gene expression and ↓ glucuronidation in human liver microsomes)

- ***UGT1A1*28* has also been shown to be associated with a higher chance of developing severe diarrhea and leukopenia during irinotecan therapy.**

Table 10 Ethnic frequency (%) of TA repeat variants in *UGT1A1*

No. of TA repeats	Caucasians	African-Americans	Asians	Hispanics
5	0	4.5	0	0
6	65.7–71.1	63.6	85–100	50–62.5
7	29.0–34.3	18.2	0–15	37.5–50
8	0	13.7	0	0

Table 1. Literature Assessment of *UGT1A1* 7/7 Promoter Genotype (*UGT1A1* *28) and Irinotecan Toxicity

Reference	Irinotecan Dosage	Study Design	Sample Size	Pharmacokinetic Relationship	Toxicity Relationship
Ando et al[27]	Variety of doses and schedules	Retrospective	118	Not evaluated	7/7 genotype had 5.2-fold risk of grade 4 leukopenia and/or grade 3/4 diarrhea ($P < .001$)
Iyer et al[26]	300 mg/m^2 over 90 minutes	Prospective	20	7/7 genotype SN-38 glucuronidation ratio is 3.9-fold lower than 6/6 patients ($P = .001$)	7/7 genotype had 2.5-fold lower ANC nadir than 6/6 patients ($P = .04$)
Innocenti et al[22]	350 mg/m^2 over 90 minutes	Prospective	66	7/7 genotype SN-38 glucuronidation ratio is 1.8-fold lower than 6/6 patients ($P = .03$)	7/7 genotype had 9.3-fold risk of grade 4 leukopenia ($P = .001$)

Abbreviation: ANC, absolute neutrophil count.

- These studies provided the first clear demonstration that determination of *UGT1A1* genotypes may be clinically important for the prediction of irinotecan toxicity

- We are faced with a molecular tool that can predict approximately 50% of all cases of grade 4 neutropenia after a 300- to 350-mg/m2 infusion of irinotecan.

IRINOTECAN

❖ By excluding patients with a *UGT1A1*28* homozygous genotype from receiving this standard dose of irinotecan, the incidence of grade 4 neutropenia would have fallen from 10.1% (6 of 59 patients with 6/6, 6/7, or 7/7 genotype) to 5.7% (3 of 53 patients with 6/6 or 6/7 genotype).

❖ This incidence could drop to 0% with exclusion of all patients with a *UGT1A1*28* allele (ie, 6/7 and 7/7 patients), but this would also mean that more than 50% of all eligible patients would be denied irinotecan therapy at this dose schedule.

❖ Increase the priority for commercial development of this assay, providing the same wide access that is now available for the use of *TPMT* testing to avoid severe mercaptopurine and azathioprine toxicity.

❖ More trials to determine predictive toxicity values of genotype according to drug dose, administration scheme or drug combinations with irinitecan

The Invader® UGT1A1 Molecular Assay (FDA Approved in 2005) for In Vitro Diagnostic Use

(Third Wave Technologies)

- First IVD pharmacogenetic test using the patented Invader® Chemistry.
- Determines UGT1A1*28 genotype as recommended in the recent label update for the chemotherapeutic irinotecan, which is approved as first-line therapy for metastatic colorectal cancer.
- Identifies patients at risk for severe toxicity when treated with Irinotecan therapy.
- Studies ongoing to determine clinical utility for UGT1A1*28 genotyping for new and existing drugs metabolized by UDP glucuronosyltransferase.

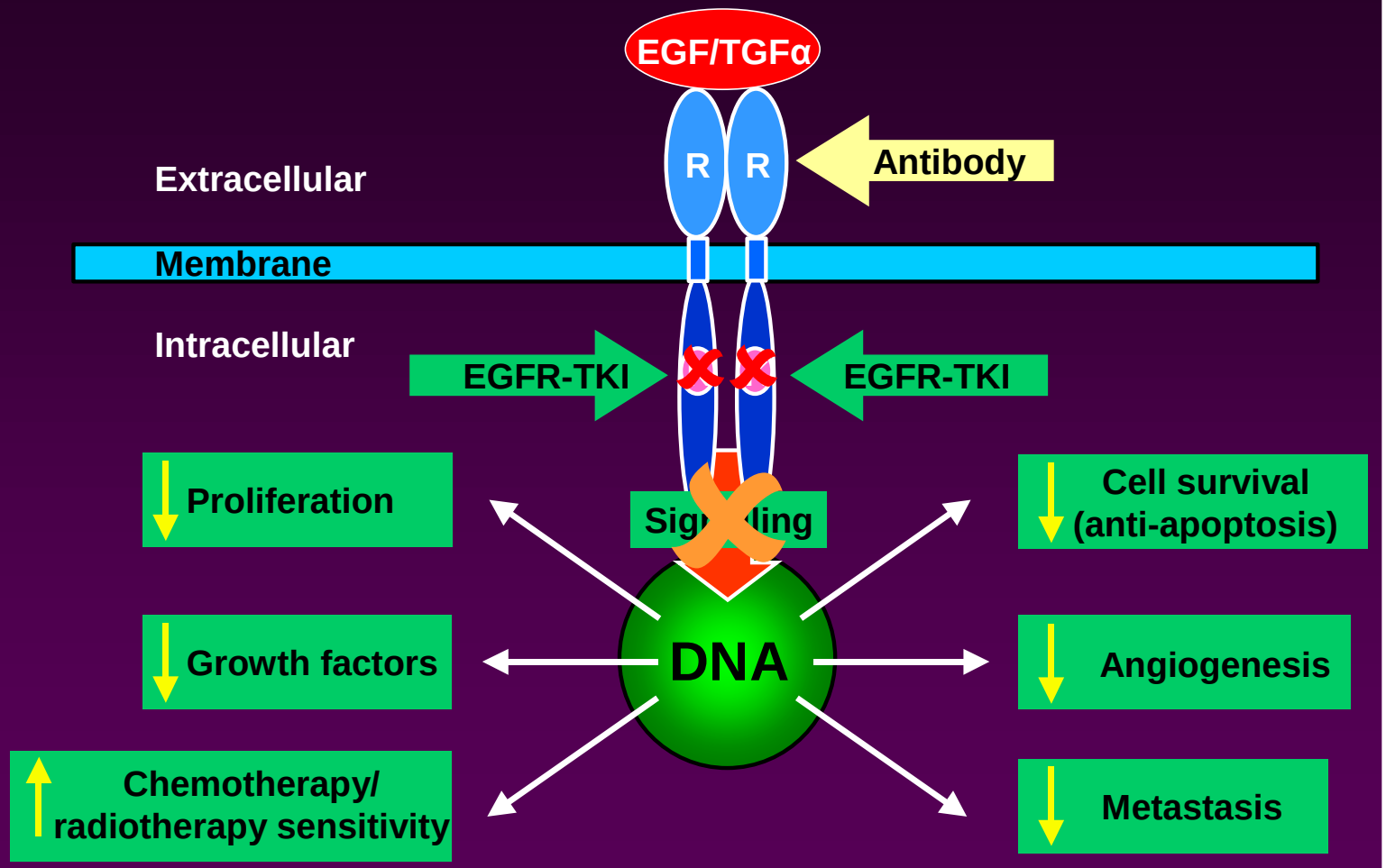
Patient Group	Assay Result	Prevalence	Risk of Neutropenia
All Patients	No Test	- - -	10 in 100
Wildtype *1/*1 Genotype	6/6	50%	0 in 100
Heterozygous-deficient *1/*28 Genotype	6/7	40%	12 in 100
Heterozygous-deficient *28/*28 Genotype	7/7	10%	50 in 100

RECREO!!!!!!

Novel biological approaches

- **Inhibitors of the EGFR family**
  - **small molecule TKIs of EGFR, eg gefitinib, erlotinib**
  - **monoclonal antibodies to EGFR, eg cetuximab**
  - **monoclonal antibodies to HER2, eg trastuzumab**
- **Farnesyl transferase inhibitors**
- **Inducers of apoptosis, eg cyclooxygenase-2 (COX-2) inhibitors, inhibitors of protein kinase C, gene therapy, bcl-2 antisense oligonucleotide**

Mode of action of EGFR inhibitors



Clinical development of anti-EGFR agents in NSCLC

- **Gefitinib**
  - **Phase II studies of once-daily, oral gefitinib in NSCLC (Kris et al 2002; Fukuoka et al 2003)**
    - **antitumour activity, symptom relief, favourable safety profile**
  - **Phase III first-line combination studies in stage III/IV NSCLC (Giaccone et al 2002; Johnson et al 2002)**
    - **no added benefit over combination chemotherapy alone**
- **Erlotinib**
  - **Phase II study in EGFR-positive, previously treated stage IIIB/IV NSCLC (Perez-Soler et al 2001)**
    - **antitumour activity, favourable safety profile**
  - **Phase III first-line combination and third-line monotherapy studies ongoing in NSCLC**
- **Cetuximab**
  - **Phase I study of cetuximab alone and in combination with cisplatin in patients with EGFR-positive advanced tumours**
  - **Phase II cetuximab combination studies ongoing in EGFR-positive NSCLC**

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Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib

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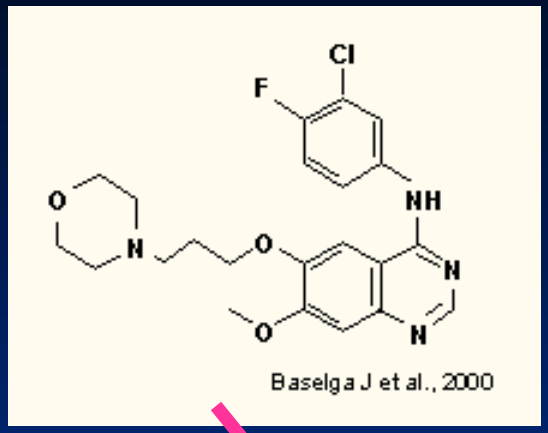
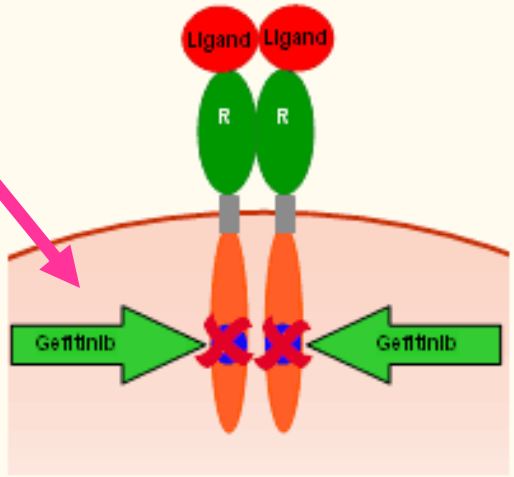
Gefitinib: Laboratorio Astra- Zeneca (Iressa)



Baselga J et al., 2000



Gefitinib (ZD1839)

- Chinazolinderivat
- Oral verfügbar
- Potenter und selektiver Inhibitor der EGFR-Tyrosin- kinase
  - EGFR IC_{50} = 0.023-0.079 µM
  - erbB2 IC_{50} = 1.2-3.7 µM
- Inhibiert liganden-induziertes Zellwachstum
  - IC_{50} = 0.080 µM

Background

❖ Most patients with non–small-cell lung cancer have no response to the tyrosine kinase inhibitor gefitinib, which targets the epidermal growth factor receptor (EGFR). However, about 10 percent of patients have a rapid and often dramatic clinical response. The molecular mechanisms underlying sensitivity to gefitinib are unknown.

Methods

We searched for mutations in the *EGFR* gene in primary tumors from patients with non– small-cell lung cancer who had a response to gefitinib, those who did not have a response, and those who had not been exposed to gefitinib.

The functional consequences of identified mutations were evaluated after the mutant proteins were expressed in cultured cells.

Results

❖Somatic mutations were identified in the tyrosine kinase domain of the *EGFR* gene in eight of nine patients with gefitinib-responsive lung cancer, as compared with none of the seven patients with no response ($P<0.001$).

❖Mutations were either small, in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the tyrosine kinase domain. Similar mutations were detected in tumors from 2 of 25 patients with primary non–small-cell lung cancer who had not been exposed to gefitinib (8 percent).

❖All mutations were heterozygous, and identical mutations were observed in multiple patients, suggesting an additive specific gain of function. In vitro, EGFR mutants demonstrated enhanced tyrosine kinase activity in response to epidermal growth factor and increased sensitivity to inhibition by gefitinib.

Table 1. Characteristics of Nine Patients with Non–Small-Cell Lung Cancer and a Response to Gefitinib.

Patient No.	Sex	Age at Beginning of Gefitinib Therapy	Pathological Type*	No. of Prior Regimens	Smoking-Status†	Duration of Therapy	Overall Survival‡	*EGFR* Mutation§	Response¶
		yr				*mo*			
1	F	70	BAC	3	Never	15.6	18.8	Yes	Major; improved lung lesions
2	M	66	BAC	0	Never	>14.0	>14.0	Yes	Major; improved bilateral lung lesions
3	M	64	Adeno	2	Never	9.6	12.9	Yes	Partial; improved lung lesions and soft-tissue mass
4	F	81	Adeno	1	Former	>13.3	>21.4	Yes	Minor; improved pleural disease
5	F	45	Adeno	2	Never	>14.7	>14.7	Yes	Partial; improved liver lesions
6	M	32	BAC	3	Never	>7.8	>7.8	Yes	Major; improved lung lesions
7	F	62	Adeno	1	Former	>4.3	>4.3	Yes	Partial; improved liver and lung lesions
8	F	58	Adeno	1	Former	11.7	17.9	Yes	Partial; improved liver lesions
9	F	42	BAC	2	Never	>33.5	>33.5	No	Partial; improved lung nodules

* Adenocarcinoma (Adeno) with any element of bronchoalveolar carcinoma (BAC) is listed as BAC.
† Smoking status was defined as former if the patient had not smoked any cigarettes within 12 months before entry and never if the patient had smoked less than 100 cigarettes in his or her lifetime.
‡ Overall survival was measured from the beginning of gefitinib treatment to death.
§ *EGFR* denotes the epidermal growth factor receptor gene.
¶ A partial response was evaluated with the use of response evaluation criteria in solid tumors; major and minor responses were evaluated by two physicians in patients in whom the response could not be measured with the use of these criteria.

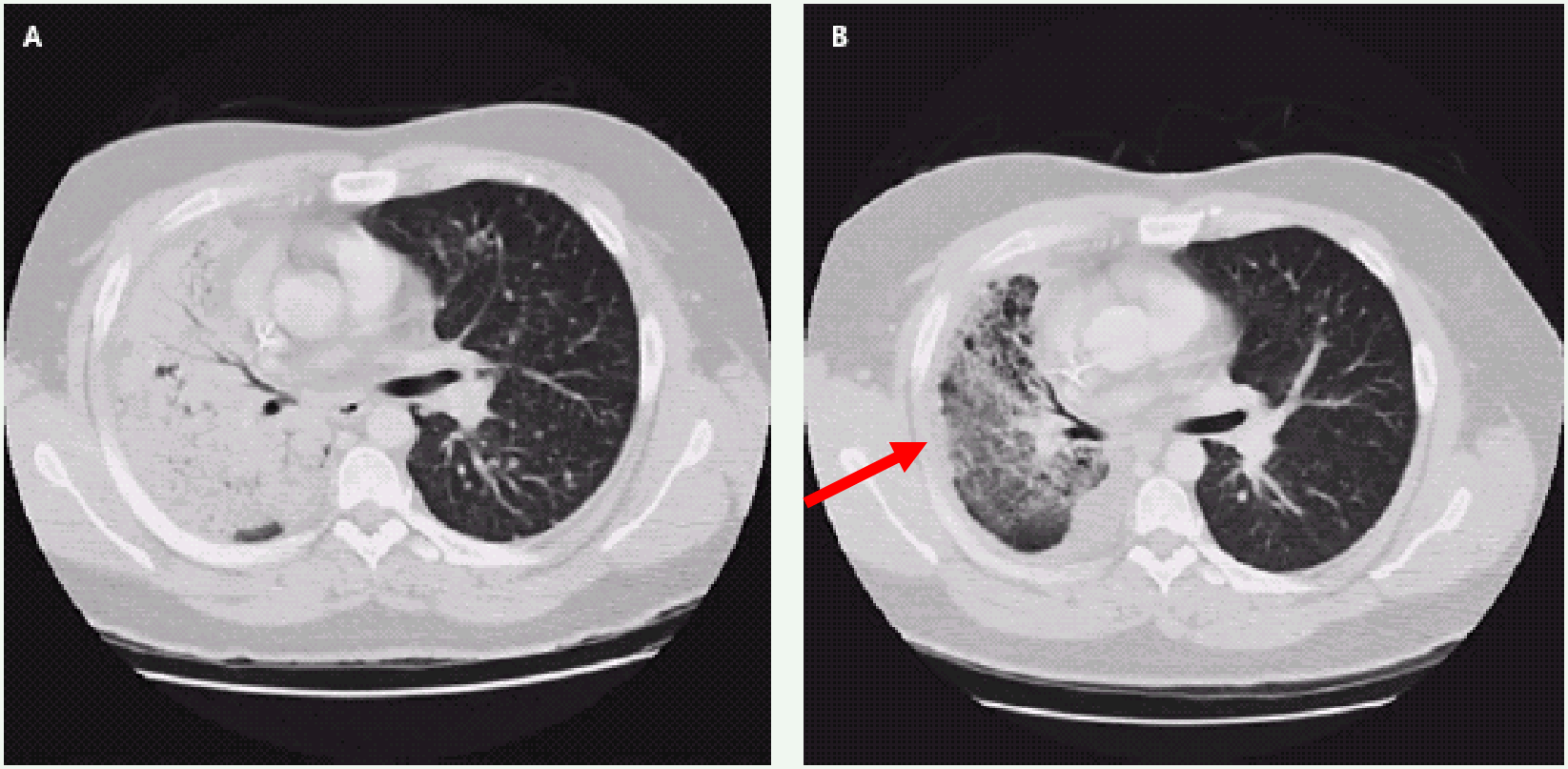


Figure 1. Example of the Response to Gefitinib in a Patient with Refractory Non–Small-Cell Lung Cancer.

A computed tomographic scan of the chest in Patient 6 shows a large mass in the right lung before treatment with gefitinib was begun (Panel A) and marked improvement six weeks after gefitinib was initiated (Panel B).

Table 2. Somatic Mutations in the Tyrosine Kinase Domain of EGFR in Patients with Non–Small-Cell Lung Cancer.

Patient	Mutation	Effect of Mutation
Patients with a response to gefitinib		
Patient 1	Deletion of 15 nucleotides (2235–2249)	In-frame deletion (746–750)
Patient 2	Deletion of 12 nucleotides (2240–2251)	In-frame deletion (747–751) and insertion of a serine residue
Patient 3	Deletion of 18 nucleotides (2240–2257)	In-frame deletion (747–753) and insertion of a serine residue
Patient 4	Deletion of 18 nucleotides (2240–2257)	In-frame deletion (747–753) and insertion of a serine residue
Patient 5	Substitution of G for T at nucleotide 2573	Amino acid substitution (L858R)
Patient 6	Substitution of G for T at nucleotide 2573	Amino acid substitution (L858R)
Patient 7	Substitution of A for T at nucleotide 2582	Amino acid substitution (L861Q)
Patient 8	Substitution of T for G at nucleotide 2155	Amino acid substitution (G719C)
Patients with no exposure to gefitinib*		
Patient A	Deletion of 18 nucleotides (2240–2257)	In-frame deletion (747–753) and insertion of a serine residue
Patient B	Deletion of 15 nucleotides (2235–2249)	In-frame deletion (746–750)

* Among the 25 patients with no exposure to gefitinib (15 with bronchoalveolar cancer, 7 with adenocarcinoma, and 3 with large-cell carcinoma), 2 (Patients A and B) — both of whom had bronchoalveolar cancer — had *EGFR* mutations. No mutations were found in 14 lung-cancer cell lines representing diverse histologic types: non–small-cell lung cancer (6 specimens), small-cell lung cancer (6 specimens), bronchus carcinoid (1 specimen), and an unknown type (1 specimen). Polymorphic variants identified within *EGFR* included the following: the substitution of A for G at nucleotide 1562, the substitution of A for T at nucleotide 1887, and a germ-line variant of unknown functional significance, the substitution of A for G at nucleotide 2885, within the tyrosine kinase domain.

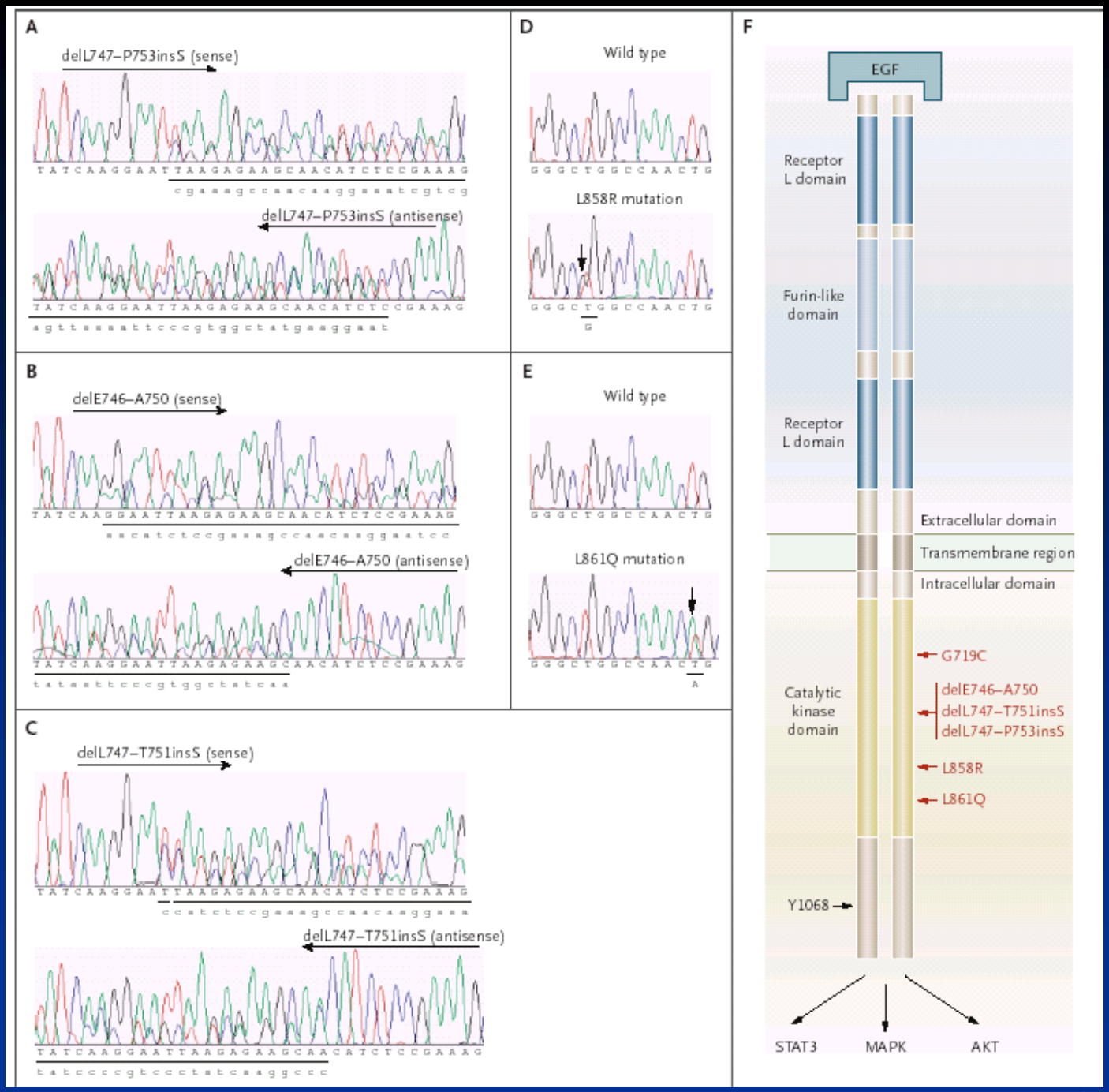
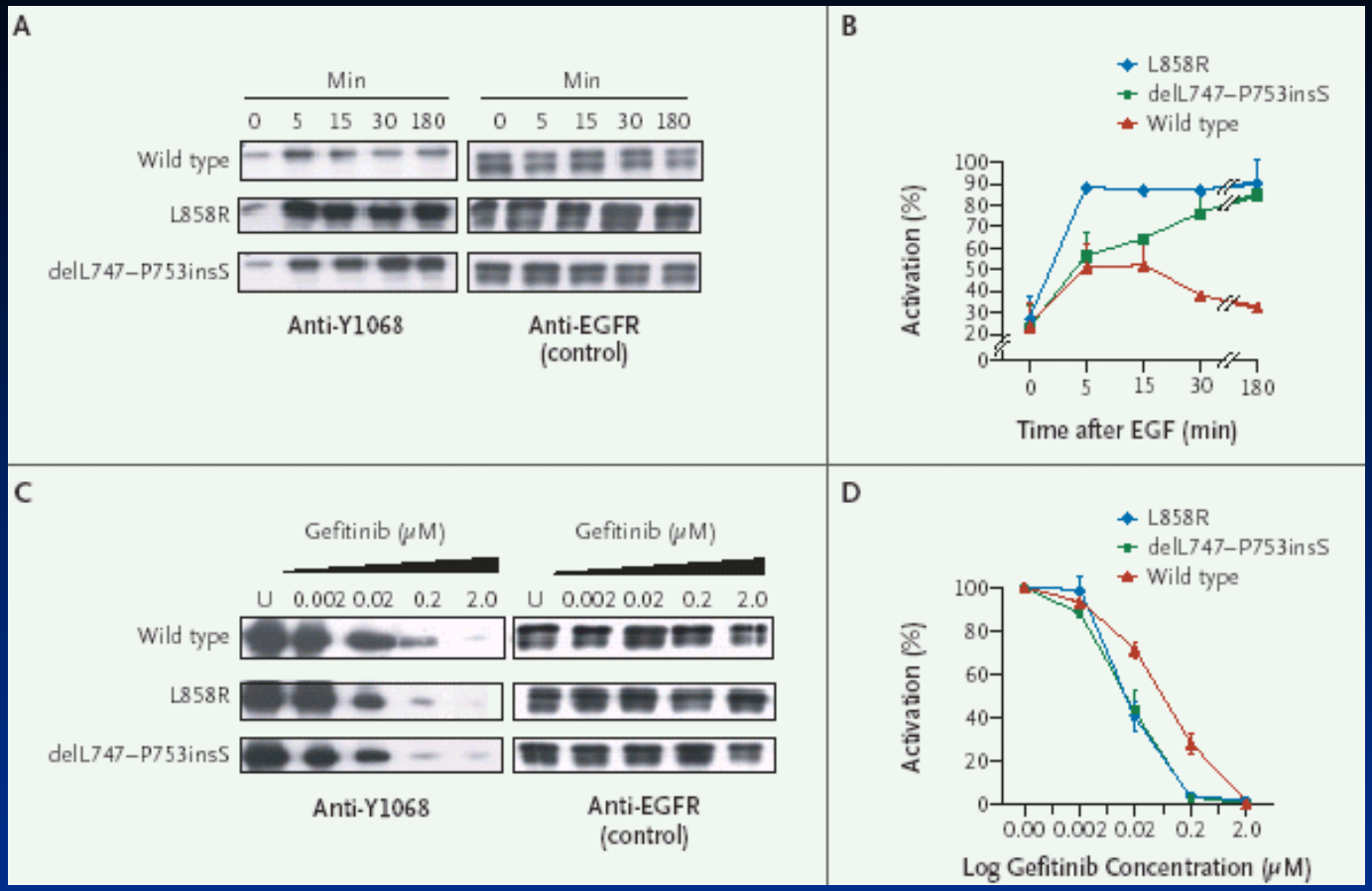

A
delL747–P753insS (sense)
delL747–P753insS (antisense)
B
delE746–A750 (sense)
delE746–A750 (antisense)
C
delL747–T751insS (sense)
delL747–T751insS (antisense)
D
Wild type
L858R mutation
E
Wild type
L861Q mutation
F
EGF
Receptor L domain
Furin-like domain
Receptor L domain
Extracellular domain
Transmembrane region
Intracellular domain
Catalytic kinase domain
G719C
delE746–A750
delL747–T751insS
delL747–P753insS
L858R
L861Q
Y1068
STAT3
MAPK
AKT

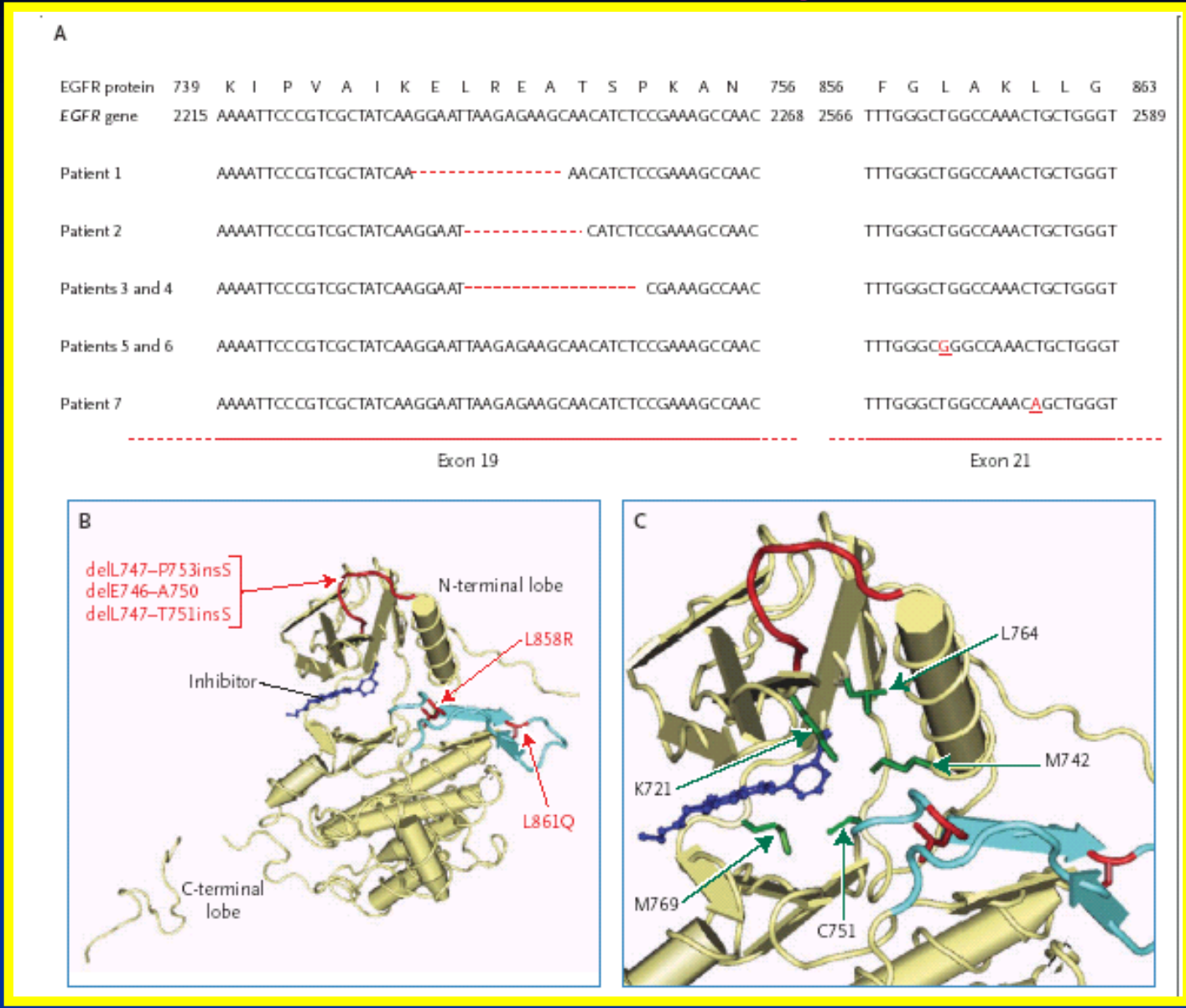
Figure 2. Mutations in the *EGFR* Gene in Gefitinib-Responsive Tumors.
Panels A, B, and C show the nucleotide sequence of the *EGFR* gene in tumor specimens with heterozygous in-frame deletions within the tyrosine kinase domain (double peaks). Tracings in both sense and antisense directions are shown to demonstrate the two breakpoints of the deletion; the wild-type nucleotide sequence is shown in capital letters, and the mutant sequence is in lowercase letters. The 5' breakpoint of the delL747–T751insS mutation is preceded by a T-to-C substitution that does not alter the encoded amino acid. Panels D and E show heterozygous missense mutations (arrows) resulting in amino acid substitutions within the tyrosine kinase domain. The double peaks represent two nucleotides at the site of heterozygous mutations. For comparison, the corresponding wild-type sequence is also shown. Panel F shows dimerized EGFR molecules bound by the EGF ligand. The extracellular domain (containing two receptor ligand [L] domains and a furin-like domain), the transmembrane region, and the cytoplasmic domain (containing the catalytic kinase domain) are highlighted. The position oftyrosine 1068 (Y1068), a site of autophosphorylation used as a marker of receptor activation, is indicated, along with downstream effectors activated by EGFR autophosphorylation — STAT3, MAP kinase (MAPK), and AKT. The locations of tumor-associated mutations, all within the tyrosine kinase domain, are shown in red.

Enhanced EGF–Dependent Activation of Mutant EGFR and Increased Sensitivity of Mutant EGFR to Gefitinib



A)Time course of ligand-induced activation of the delL747–P753insS and L858R EGFR mutants, after the addition of EGF to serum-starved cells. The autophosphorylation of EGFR is used as a marker of receptor activation, with the use of Western blotting to recognize the phosphorylated tyrosine 1068 (Y1068) of EGFR (left side), and compared with the total concentrations of EGFR expressed in Cos-7 cells as control (right side). Autophosphorylation of EGFR is measured at intervals after the addition of EGF (10 ng per milliliter). **B) EGF-induced phosphorylation of wild-type and mutant EGFR**.The intensity of EGFR phosphorylation has been adjusted for the total protein expression mean (±SD) % activation of the receptor. **C) Dose-dependent inhibition of the activation of EGFR by gefitinib**. Autophosphorylation of EGFR tyrosine 1068 is demonstrated by Western blot analysis of Cos-7 cells expressing wild-type or mutant receptors and stimulated with 100 ng of EGF per milliliter for 30 minutes. Cells were untreated (U) or pretreated for three hours with increasing concentrations of gefitinib (left side). Total amounts of EGFR expressed are shown on the right side (control). D)**Mean (±SD) inhibition of EGFR by gefitinib.** Concentrations of phosphorylated EGFR were adjusted for total protein expression.

Clustering of Mutations in the *EGFR* Gene at Critical Sites within the ATP-Binding Pocket



Conclusions

❖A subgroup of patients with non–small-cell lung cancer have specific mutations in the *EGFR* gene, which correlate with clinical responsiveness to the tyrosine kinase inhibitor gefitinib

❖Responders were mainly:
Female, non-smokers, asiatic, adenocarcinoma, carriers of EGFR mutations

❖These mutations lead to increased growth factor signaling and confer susceptibility to the inhibitor.

❖ Screening for such mutations in lung cancers may identify patients who will have a response to gefitinib.

❖ The authors postulate that the mutations result in repositioning of these critical residues, stabilizing their interaction with both ATP and its competitive inhibitor gefitinib.

❖ Such a mechanism would explain both the increased receptor activation after ligand binding and the enhanced inhibition induced by gefitinib. (Also design of more potent inhibitors targeting the mutant receptors)

❖These data suggest that EGFR tyrosine kinase mutations can be used to identify the subgroup of patients with non–small-cell lung cancer in whom this growth factor receptor may be essential to tumor growth, whereas the overexpression of EGFR in the absence of mutations may reflect the less critical role played by this factor in the majority of cases.

Science. 2004 304(5676):1497-500.

EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy

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Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways.

❖ The authors reported that EGFR mutants selectively activate Akt and signal transduction and activator of transcription (STAT) signaling pathways, which promote cell survival, but have no effect on extracellular signal-regulated kinase signaling, which induces proliferation.

❖NSCLC cells expressing mutant EGFRs underwent extensive apoptosis after small interfering RNA-mediated knockdown of the mutant EGFR or treatment with pharmacological inhibitors of Akt and STAT signaling and were relatively resistant to apoptosis induced by conventional chemotherapeutic drugs.

❖Thus, mutant EGFRs selectively transduce survival signals on which NSCLCs become dependent; inhibition of those signals by gefitinib may contribute to the drug's efficacy.

Sordella et al Science. 2004 Aug 20;305(5687):1163-7

Mecanismos de resistencia a Gefitinib en NSCLC

1- Mutaciones en el target (resistencia primaria y secundaria)
2- Adquisición de otra vìa de proliferación downstream del EGFR o redundante (mutación de KRAS, resistencia primaria)
3- Amplificación de alelo EGFR normal
4- Alteración en el tráfico del receptor
5- Amplificación de MET (resistencia secundaria)- produce la activación de PI3K dependiente de ERBB3

Se están desarrollando EGFR-TKIs de segunda generación:
- Se unen irreversiblementee a EGFR
-Inhiben varios miembros de la familia EGFR o múltiples vías de transducción de señales.

KRAS and kinase inhibitors in CCR

•Cetuximab and panitumumab are anticancer monoclonal antibodies that target the EGFR)and are used to treat metastatic colorectal cancer as monotherapy or in combination regimens.

•KRAS is a signaling molecule downstream from growth factor receptors. Data from clinical trials suggest that patients who have KRAS mutations detected in codon 12 or 13 do not benefit from therapy with EGFR-inhibitors.

•The availability of KRAS mutation testing in accredited laboratories, combined with results of relevant trials, have prompted ASCO to issue a Provisional Clinical Opinion recommending that all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations.

•ASCO recommends that patients in whom KRAS mutation in codon 12 or 13 is detected should not receive anti-EGFR antibody therapy as part of their treatment, a recommendation subsequently accepted by regulatory authorities in the United States and Europe.

Tumor profiling for breast cancer

Assays of genetic expression in tumor tissue have been proposed as prognostic indicators in the treatment of breast cancer.

These gene expression profiles include:

- MammaPrint® 70-gene assay (Molecular Profiling Institute (MPI) Inc., Phoenix, AZ and Agendia BV, Amsterdam, The Netherlands)
- Oncotype DX™ 21-gene assay (Genomic Health, Redwood City, CA)
- Rotterdam Signature 76-gene panel (Veridex LLC, a Johnson & Johnson Co, Warren, NJ)

MammaPrint®, a Breast CA Molecular Prognostic Test

Agendia (Amsterdam, the Netherlands),

-FDA approved in 2007.

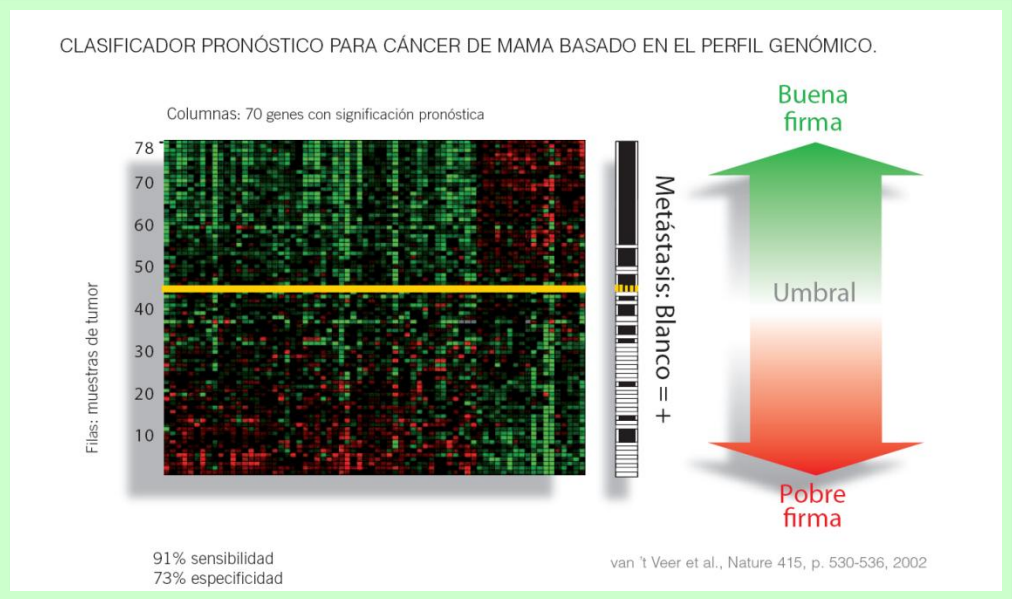
- "Test that determines the likelihood of breast cancer returning within five to 10 years after a woman's initial cancer."(the first cleared product that profiles genetic activity).

- MammaPrint® is a DNA micro array-based in vitro diagnostic laboratory service that measures **the activity of 70 genes**, providing information about the likelihood of tumour recurrence. The test measures the level of expression of each of these genes in a **sample of a woman's surgically-removed breast cancer tumour** and then uses a specific formula or algorithm to produce a score that determines whether the patient is deemed low risk or high risk for spread of the cancer to another site.

-The result may help a doctor in planning appropriate follow-up for a patient when used with other clinical information and laboratory tests.

MammaPrint® Specifications After Agendia receives the fresh tumor sample

- Isolation of RNA from **frozen tumor tissue**
- DNAse treatment of isolated RNA
- Transcription into cDNA and then into cRNA
- Fluorescent-labeling of tumor and reference cRNA
- cRNA purification; hybridization of the cRNAs of tumor and reference sample to the MammaPrint®
- microarrayScanning the MammaPrint® microarray and data acquisition
- Calculation and determination of the risk of recurrence in breast cancer patients



Mammaprint

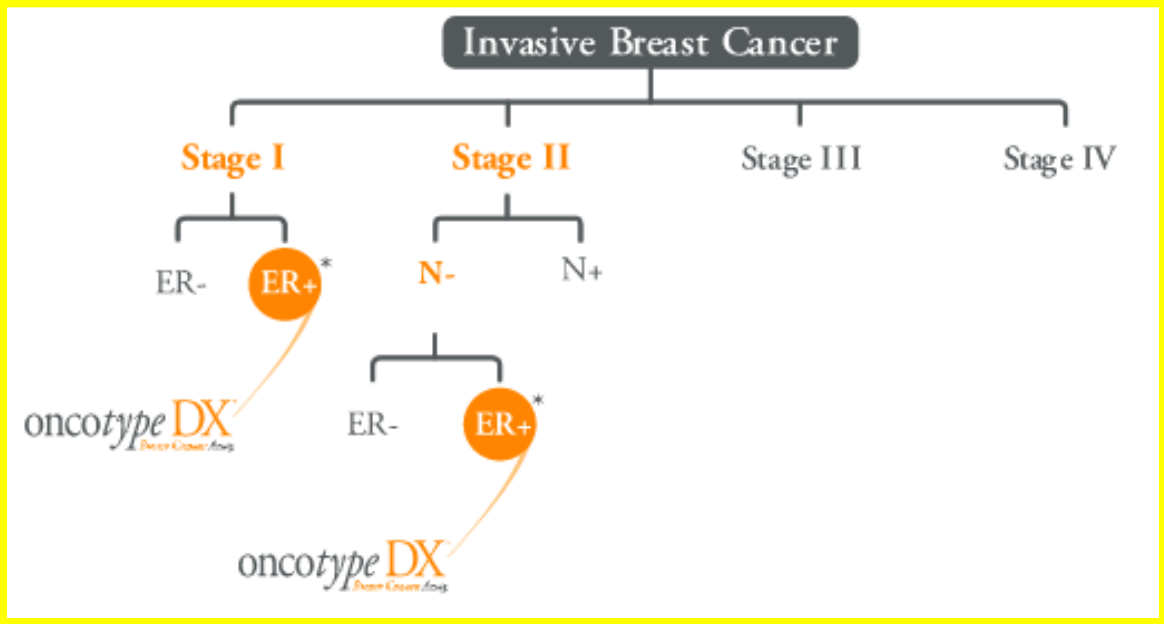
- Pacientes con cá. mama en el mismo estadío pueden presentar marcadas diferencias en la respuesta al tratamiento y en la evolución posterior. Los más importantes predictores de recaída a distancia, status ganglionar axilar y grado histológico, fallan en la óptima clasificación de los tumores de mama en relación a su comportamiento clínico.

-Este perfil de expresión genética ha sido validado en un grupo de al menos 700 pacientes y ha demostrado mejorar los parámetros clínicos usados actualmente para pronosticar la evolución de la enfermedad.

La tecnología MammaPrint® proporciona los recursos para seleccionar las pacientes que pueden beneficiarse con el tratamiento adyuvante y las que evitarán el tratamiento innecesario.

Oncotype DX (Genomic Health)

Onco*type* DX™ is a diagnostic assay that quantifies the likelihood of breast cancer recurrence in women with newly diagnosed, early stage breast cancer. In addition to predicting distant disease recurrence, Onco*type* DX also assesses the benefit from chemotherapy.[1] The assay — performed using **formalin-fixed, paraffin-embedded tumor tissue** — analyzes the expression of **a panel of 21 genes** and the results are provided as a Recurrence Score™ (0-100). The gene panel was selected and the Recurrence Score calculation was derived through extensive laboratory testing and multiple independent clinical development studies.



Assumes patient will be treated with tamoxifen. [1] The data on chemotherapy benefit is derived from the NSABP Study B-20 which compared hormonal therapy alone versus CMF based chemotherapy and hormonal therapy.

Panel de 21 genes usados por ONCO*TYPE* DX

Gen	Grupo
Ki67	Proliferaciòn
STK15	Proliferación
Survivin	Proliferaciòn
Cyclin B1	Proliferación
MYBL2	Proliferación
GRB7	HER2
HER2	HER2
ER	Estrógeno
PGR	Estrógeno
BCL2	Estrógeno
SCUBE2	Estrógeno
MMP11	Invasión
CTSL2	Invasión
GSTM1	Independiente
CD68	Independiente
BAG1	Independiente

normalizaciòn

ACTB	**referencia**
GAPDH	**referencia**
RPLPO	**referencia**
GUS	**referencia**
TFRC	**referencia**

- Extracción de RNA de taco de parafina
- PCR-real time (triplicado)
- Normalización con referencia
- cálculo de score de recurrencia (RS)

Oncotype Dx

1- Onco*type* DX is clinically validated to assess the likelihood of distant recurrence in women with newly diagnosed, stage I or II, node-negative, estrogen receptor-positive breast cancer who will be treated with tamoxifen.

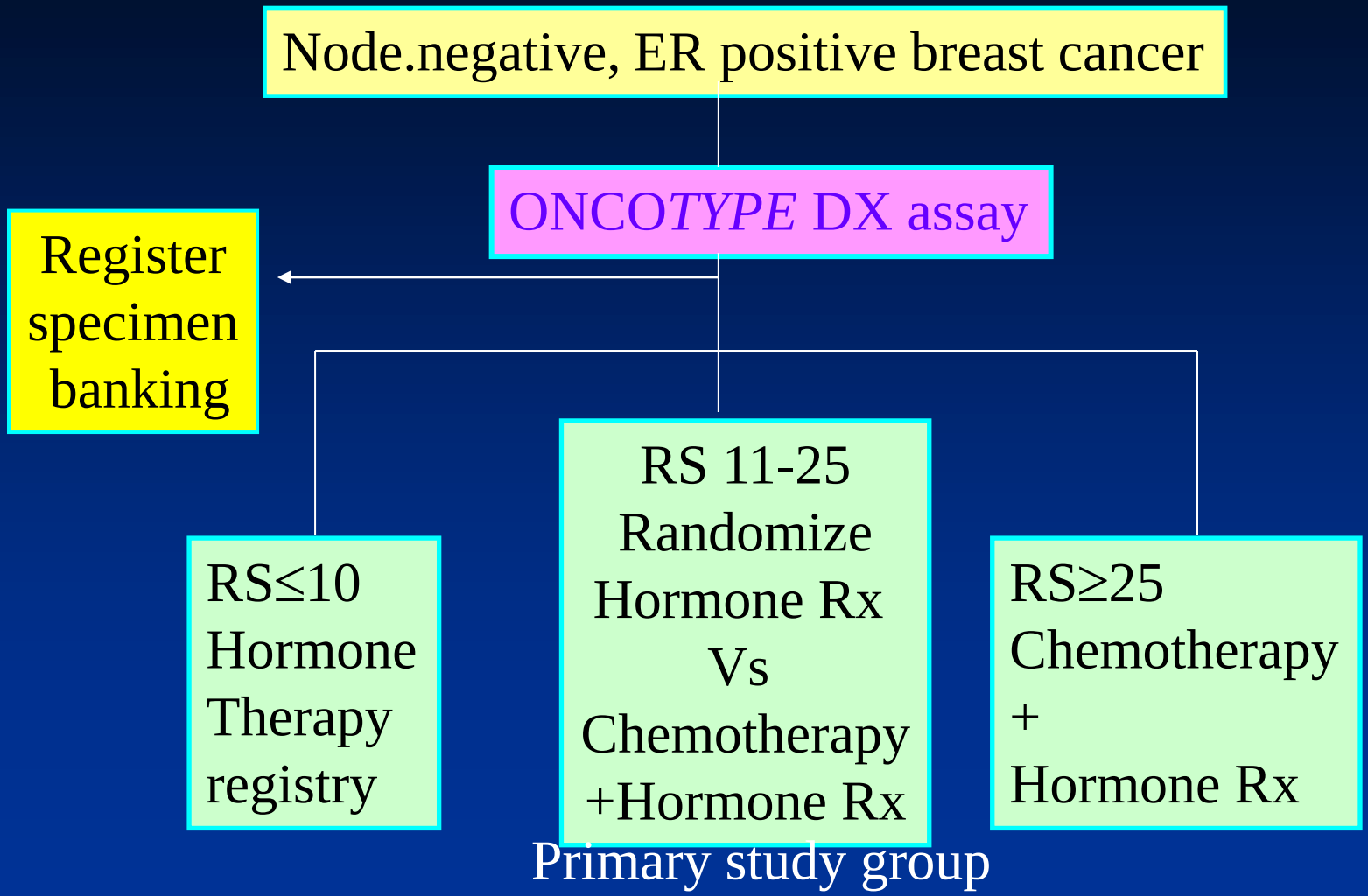
2- The ability of the Onco*type* DX assay to predict the treatment benefit in breast cancer was demonstrated in a study of 651 eligible patients from the tamoxifen alone arm (n = 227) and tamoxifen plus chemotherapy treatment arm (CMF/MF) (n = 424) of NSABP Study B-20. Results showed that the Recurrence Score™ (RS) is a significant predictor of chemotherapy benefit (p-value for interaction = 0.038) and that not all patients benefit equally from chemotherapy:

- Patients with tumors that had low Recurrence Scores (RS < 18) derived minimal, or no benefit from chemotherapy

- Patients with tumors that had high Recurrence Scores (RS ≥ 31) had a large absolute benefit from chemotherapy

Phase III clinical trial TAILORx

(sponsored by the National Cancer Institute and administered by the Eastern Cooperative Oncology Group)



TAILORx is one of the largest clinical trials that will be conducted in women with early-stage breast cancer and will include over 1,000 sites and is expected to accrue over 10,000 patients

Rotterdam Signature 76-Gene Panel

-The Rotterdam signature 76-gene panel was developed to assist physicians

to predict the likelihood that a patient with early-stage breast cancer will develop a metastasis.

- This is the first proposed assay that represents a prognostic molecular marker that could be used with all lymph node negative (LNN) breast cancer patients, regardless of age, tumor size and grade, or ER status.

- Analysis of the 76-gene signature classifies patients as having a gene expression signature associated with either a low or high risk of developing metastatic disease.

- The test is not yet commercially available.

Predicting drug sensitivity and resistance: Profiling ABC transporter genes in cancer cells

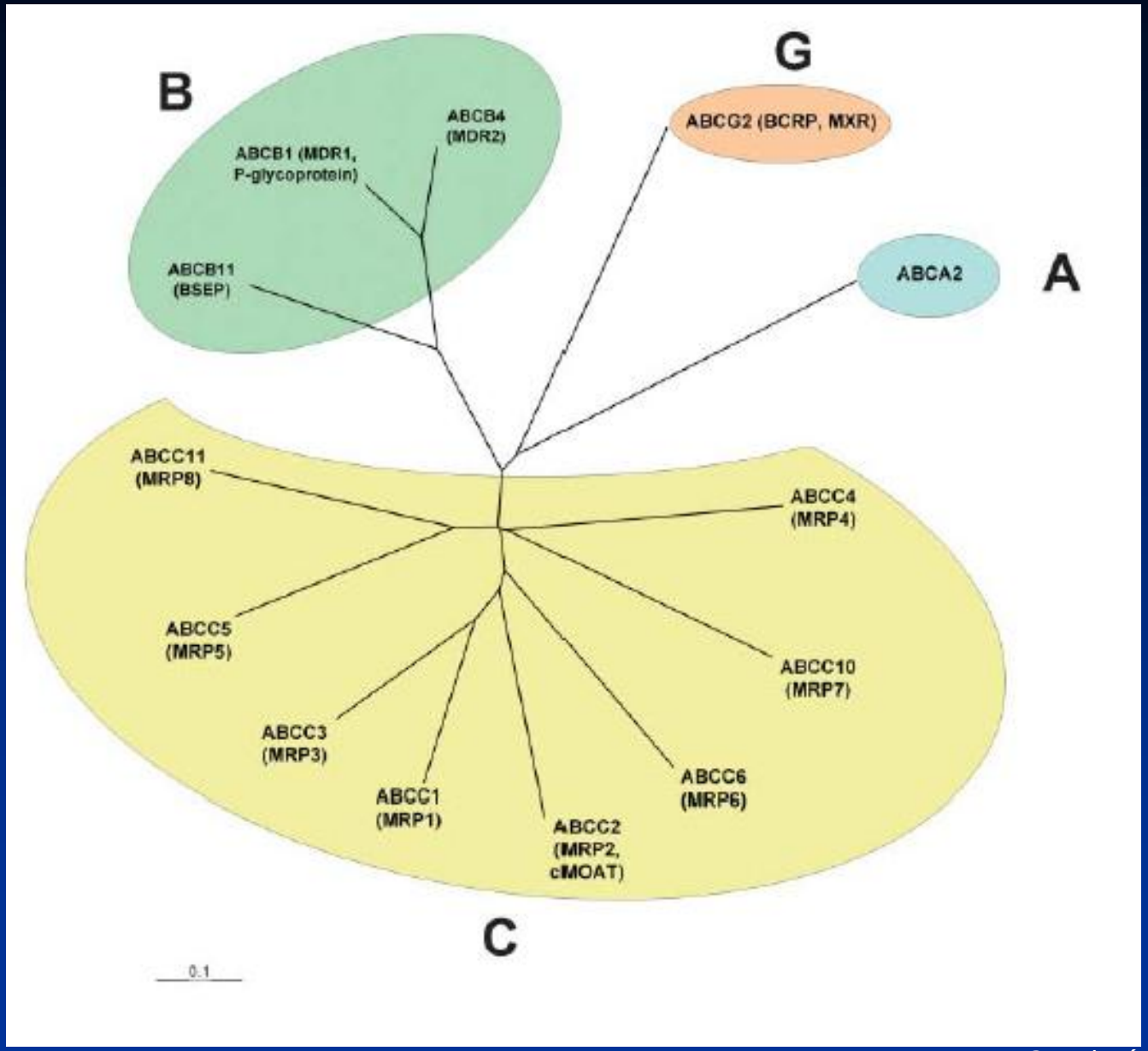
Transporter proteins

- Cancer cells utilize transporters, notably members of the ATP binding cassette (ABC) superfamily, to enhance their survival and chemoresistance.

- Comprised of seven families designated A through G, certain ABC transporters couple the hydrolysis of ATP to move drugs and xenobiotics unidirectionally out of cells, thereby effecting drug resistance.(49 members)

- Solute carrier (SLC). ·60 transporters in 46 families (SLC1 to SLC46)

ABC transporters currently known or suspected to cause resistance to cancer chemotherapeutic drugs



Representative antineoplastic drugs known or suspected to be subject to attenuation by these transporters

ABCA2, estramustine;
ABCB1(P-glycoprotein or MDR1), anthracyclines, etoposide,imatinib, taxanes, vinca alkaloids
ABCB4 (MDR2),paclitaxel, vinblastine
ABCB11 (BSEP), paclitaxel
ABCC1 (MRP1), anthracyclines, etoposide, methotrexate,
but not taxanes (a point of distinction from ABCB1drug resistance spectrum);
ABCC2 (MRP2, cMOAT), cisplatin,doxorubicin, etoposide, methotrexate, mitoxantrone,vinca alkaloids,
ABCC3 (MRP3), cisplatin,doxorubicin, etoposide, methotrexate, vinca alkaloids
ABCC4 (MRP4), methotrexate, thiopurines;
ABCC5 (MRP5), 6-mercaptopurine, 6-thioguanine;
ABCC6 (MRP6), anthracyclines, etoposide, teniposide;
ABCC10 (MRP7), docetaxel, paclitaxel, vinca alkaloids;
ABCC11 (MRP8), purine and pyrimidine nucleotide analogs, NSC671136
ABCG2 (BCRP, MXR), mitoxantrone,methotrexate, topotecan, irinotecan SN-38, imatinib, flavopiridol,anthracyclines (if mutation present at codon 482).

Los polimorfismos genéticos en los transportadores pueden afectar:

- la absorción oral de las drogas
- la eliminación en el hígado y el riñón
- el influjo/eflujo de la Droga en la célula target
  (relevante para la terapia antitumoral).

-Los transportadores más estudiados en los últimos años son:

ABCB1 (Glicoproteína- P)
ABCG2 (BCRP, breast cancer resistance protein)

ABCB1

-Codificada por gen MDR-1
-Expresada en hígado, riñón, intestino delgado eflujo/eliminación de varios sustratos endógenos y exógenos
-Expresado en Placenta (barrera materno-fetal)
-Expresado vasculatura del SNC (barrera hematoencefálica)
-Células Tumorales (mecanismo de resistencia múltiple a drogas)

Agentes quimioterápicos sustratos de ABCB1:

-Actinomicina D
-Daunorubicina, Docetaxel, Doxorubicina
-Etopósido,Paclitaxel
-Vinblastina y Vincristina

Table 11 Ethnic frequency (%) of allelic variants in the *ABCB1* gene

Allelic variant	Caucasians	African-Americans	Asians	Africans	Middle Easterns
C1236T			62		
G2677T	42–48		37		
G2677A	2		19		
C3435T	33–65	14–16	37–47	17–27	45

A pesar de la buena correlación de los SNPs con la expresión y funcionalidad del transportador ABCB1 los resultados son contradic-torios respecto de la farmacocinética de las drogas antineoplásicas salvo para algunos casos de absorción oral

ABCG2

- Inicialmente descubierto en líneas celulares de cáncer de mama resistentes a antraciclinas
- Expresado en placenta, SNC, hígado, glándula adrenal, próstata, testículo y útero.
- Expresión en tejido mamario (normal o tumor) no tiene significación Clínica.
-Distintas drogas quimioterápicas son sustratos del ABCG2:
  - mitoxantrona
  - metotrexate
  - flavopiridol
  - topotecan
  - irinotecan (y SN-38)
  - imatinib
  - gefitinib

Table 12 Ethnic frequencies (%) of allelic variants in *ABCG2* gene

Allelic variant	Caucasians	African-Americans	Asians	Hispanics	Africans	Middle Easterns
V12M	2	4	20–45	40		5
Q141K	11–14	2.3–5.0	15–35	10	1.0	13
I206L	0	0	0	10		0
N590Y	1					

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