RÉSEAU NATIONAL DE PHARMACOGÉNÉTIQUE

Pharmacogenetics-based personalized therapy: *levels of evidence and recommendations from the* ***French national network of pharmacogenetics*** *(RNPGx)*

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Equipe 14 « Dose individualization of anticancer drugs », CRCT INSERM U1037

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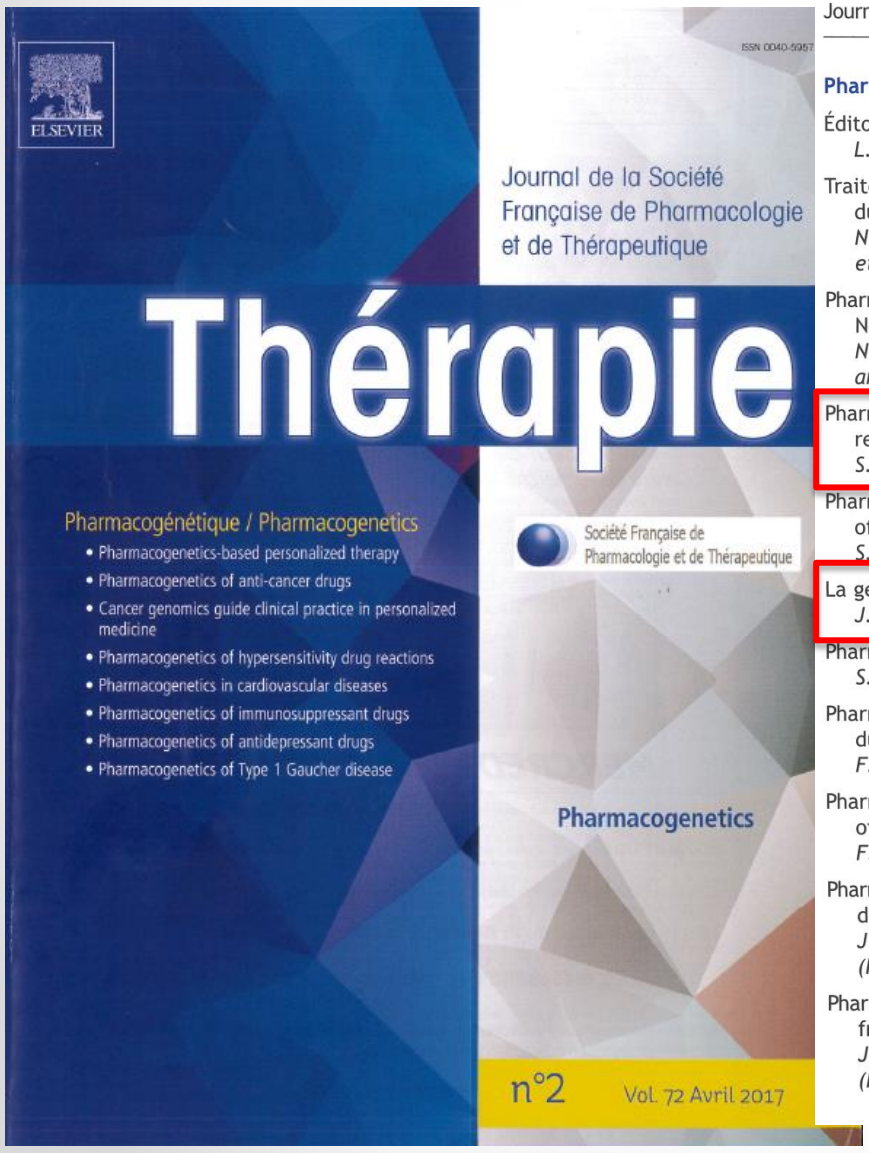
Conflicts of Interest and Source of Funding: none

Thematic issue about PGx



SOMMAIRE

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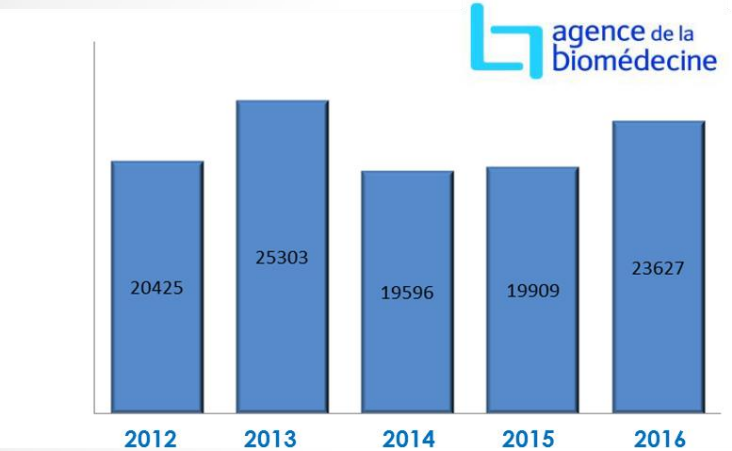
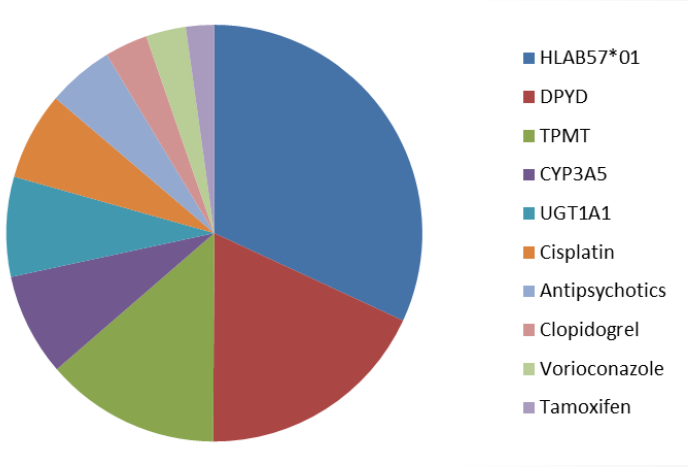
Pharmacogénétique / Pharmacogenetics

PGx activity in France

- 50 laboratories offer PGx testing: pharmacogenetic testing is available for a variety of drug-gene pairs.
- 23627patients have been explored in 2016 (germline PGx)





- Select the most appropriate drug option or to adjust drug dose
- Tests are not always mentioned in drug information labels and the information provided is generally insufficient to know exactly how they can be useful.

Background

- Examples of information available in selected French drug information labels (accessed in 2016)

Capecitabine	Contraindication: ‘‘In patients exhibiting **a known complete deficiency of dihydropyrimidine dehydrogenase (DPD) activity**’’
Irinotecan	Pharmacodynamics: Data from a meta-analysis indicate that **persons who are homozygous for the *UGT1A1*28* allele (Gilbert’s syndrome) have a higher risk of hematological toxicity** (grades 3 and 4) after irinotecan administration at moderate to high doses (150 mg/m2). (…) **Patients known to be homozygous for *UGT1A1*28* should be given the normally indicated irinotecan dose**. However, these patients should be carefully monitored to detect potential hematological toxicity. **The exact reduction for the initial dose in this population of patients has not been established (…) Data are currently insufficient to conclude concerning the clinical usefulness of the UGT1A1 genotype’’**

Methodology

- **Elaboration of a classification for PGx testing, which integrates :**
  - The functional impact of genetic variations
  - The nature of the phenotype concerned
  - The clinical evidences available
  - The existence of non-genetic options for treatment personalization

Results

- Elements that can be used to assess the level of evidence relative to the functionality of a variant in PGx

Level of evidence	Description of elements concerning the variant
Demonstrated (avéré) effect on function	Direct functional impact on the expression or activity of a "pharmacogene" product demonstrated *in vitro*, with *ex vivo* data in humans corroborating this functional impact Indirect functional impact on the expression or activity of a "pharmacogene" product (existence of a linkage disequilibrium (LD) within a haplotype containing the deleterious genetic variation), with *ex vivo* data in humans corroborating this functional impact
Probable effect	Direct (demonstrated *in vitro*) or indirect (by LD) functional impact on the expression or activity of a "pharmacogene" product, without *ex vivo* data in humans, or a functional impact that has not been the object of an in vitro demonstration
Potential (à confirmer) effect	*In silico* predicted functional impact (change in protein sequence, localization in a functional domain, modeling)

Results

- **Levels of recommendations for PGx testing: proposed classification**

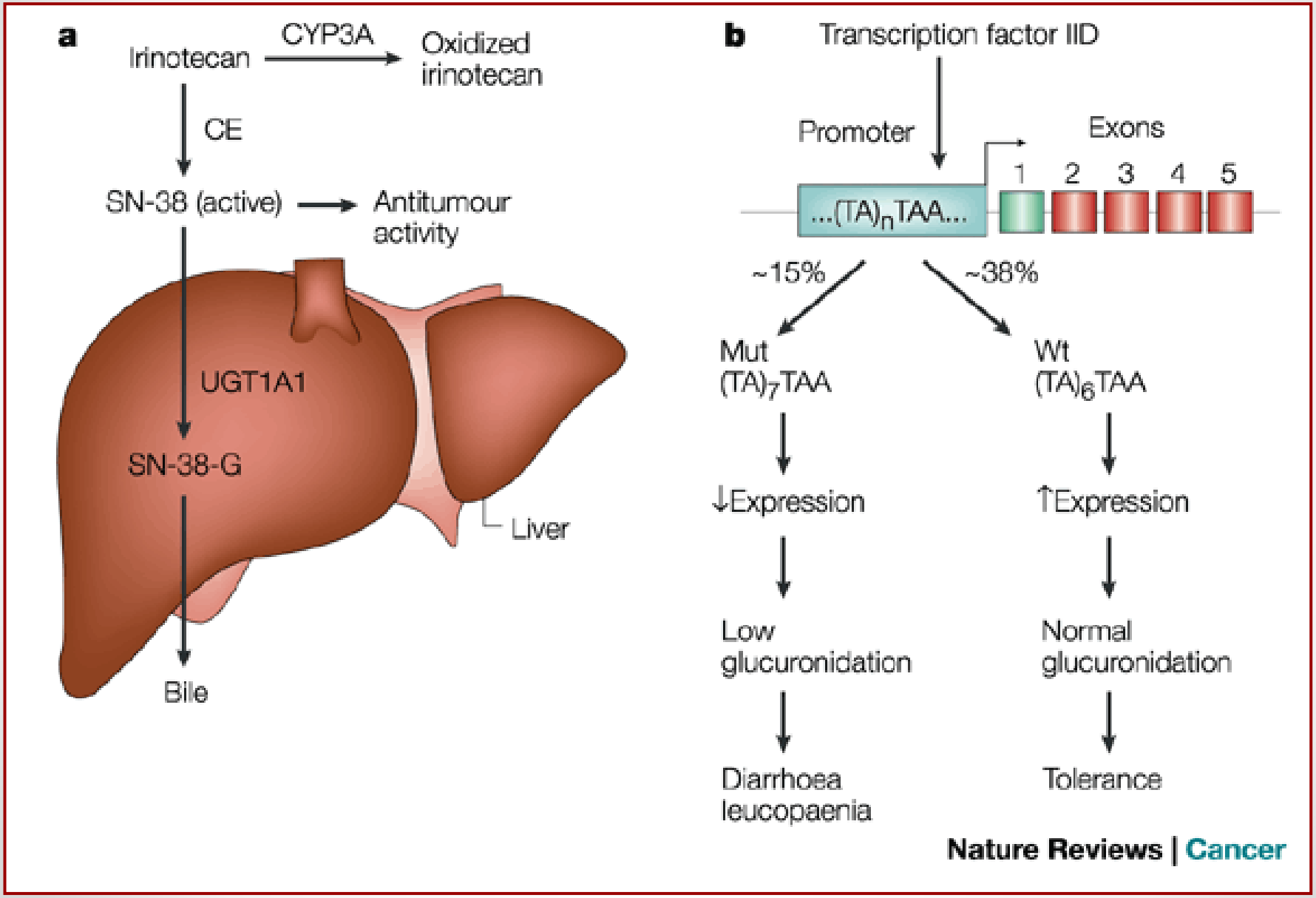
Essential / indispensable	**Demonstrated or probable functionality** Demonstrated impact on a **clinical phenotype of major importance** [response (efficacy, resistance)/toxicity] for therapeutic management; **difficult or impossible to predict with a non-genetic approach;** having led to expert agreement in favor of systematic testing
Advisable / conseillé	**Demonstrated functionality** Demonstrated impact on **clinical phenotype of major importance**, but **predictable by a non-genetic approach** (phenotyping), having led to **expert agreement** in favor of testing as a complement to phenotyping or when phenotyping is not possible as a first-intention approach Or **Demonstrated functionality** Demonstrated impact on an **intermediary non-clinical phenotype** but that is important to predict drug exposure (e.g. pharmacokinetics) for therapeutic management, having led to **expert agreement** in favor of testing.
Possibly useful / Eventuellement utile	**Demonstrated or probable functionality** Probable impact that remains to be demonstrated on a **clinical phenotype** or on an **intermediary** (non-clinical) **phenotype** having led to **expert consensus** in favor of testing, **case-by-case**, depending on the clinical context (unusual response to a drug, specific disease)

Based on CPIC Guidelines and PharmGKB

Application in the case of selected drugs in **ONCOLOGY**

Gene (allele)	Drug	Context	RNPGx LOR
*UGT1A1 (*28)*	Irinotecan	Before treatment initiation with a standard dose (180 – 230 mg/m^2)	**Advisable**
		Before treatment initiation with an intensified dose (> 240 mg/m^2)	**Essential**
*DPYD (*13, *2A, c.2846A>T)*	Fluoropyrimidines	Before treatment initiation, especially in patients at risk of toxicity (bolus or high-doses, toxicity in a family member), or in an adjuvant situation	**Advisable / Essential** *depending on phenotyping availability*
*TPMT (*2, *3A, *3B, *3C)*	6-mercaptopurine (azathioprine)	Before treatment initiation	**Essential**
CYP2D6 (multiple alleles)	Tamoxifen	Case-by-case	**Possibly useful**
SLCO1B1 (rs4149081,rs11045879) *MTHFR (c.677C>T, c.1298A>C)*	Methotrexate	Case-by-case	**Possibly useful**

UGT1A1 and irinotecan



Relling & Dervieux *Nature Reviews Cancer* **1**, 99-108 (November 2001)

RNGPx recommendations

Collective work with Groupe de Pharmacologie Clinique Oncologique (GPCO-Unicancer)

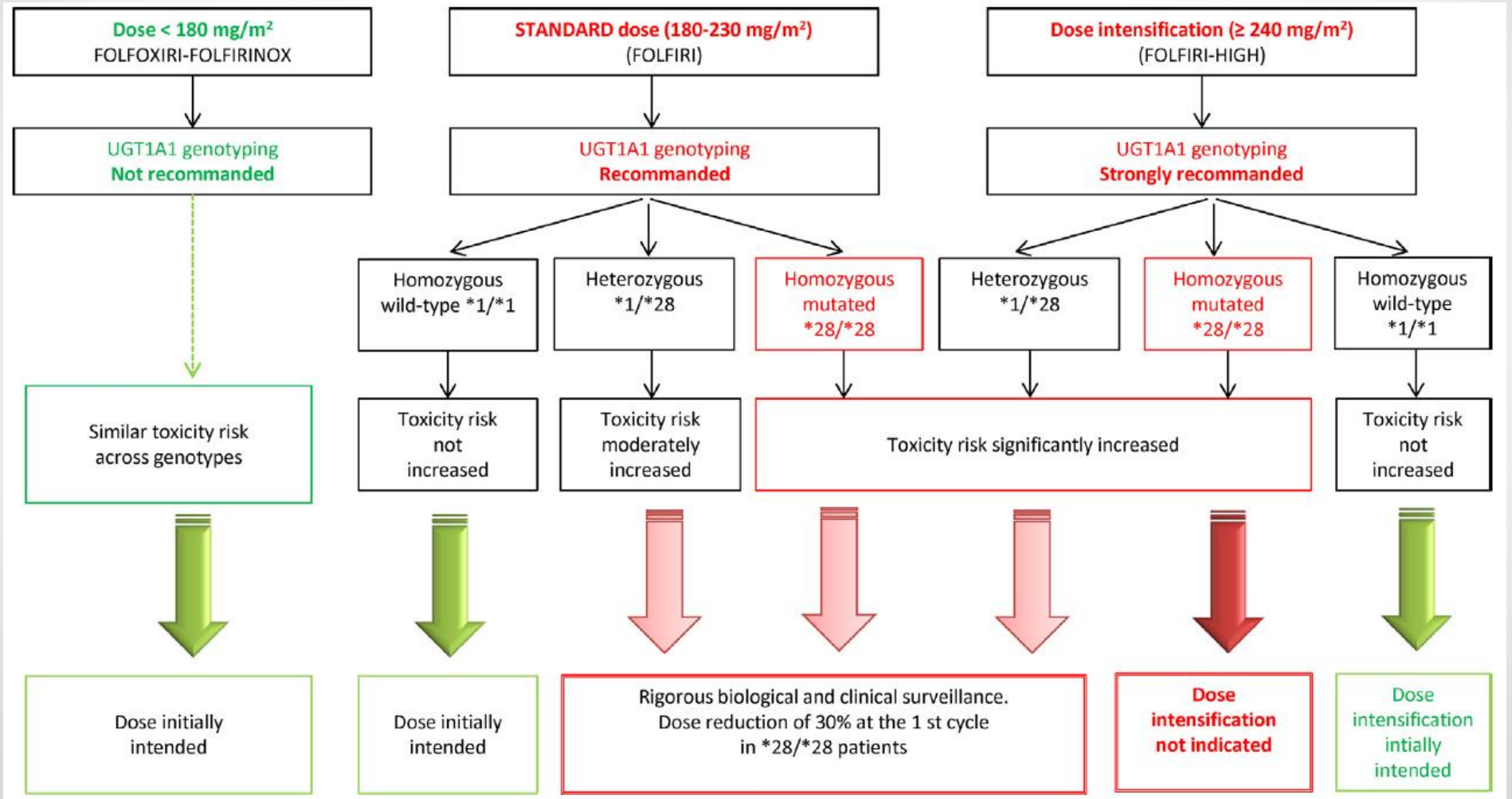


Figure 1. Decision tree for irinotecan prescription, reproduced from Boyer et al. [41]. With courtesy Fundamental and Clinical Pharmacology.

Etienne-Grimaldi et al. Fundamental Clin Pharm, 2015

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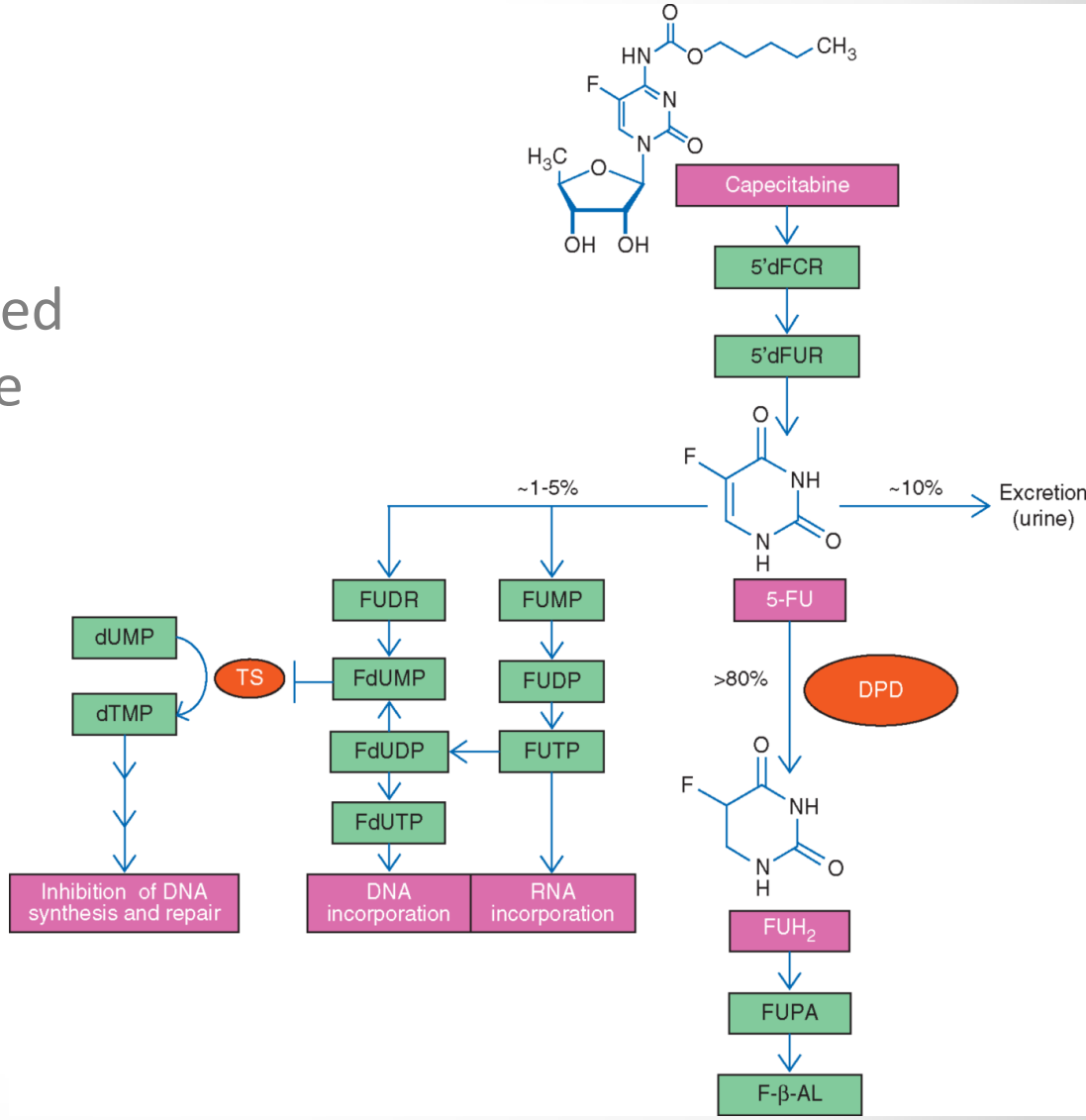
DPD (**D**ihydro**p**yrimidine **d**ehydrogenase)

- ✓ Fluoropyrimidines:
  - ✓ 5-fluorouracil (5-FU)
  - ✓ capecitabine
- 15% to 30% of patients treated with 5-FU will develop severe toxicity



From: DPYD genotype-guided dose individualization to improve patient safety of fluoropyrimidine therapy: call for a drug label update. Heinricks et al. Ann Oncol. Published online August 02, 2017.

DPD (**D**ihydro**p**yrimidine **d**ehydrogenase)

→ 3 (4?) main genetic variants

Table 1 Recommendations concerning genetic variants of *DPYD*.

Gene[a]	Name[a]	Polymorphism[a]	rs identifier	Allele symbol	Frequency of the minority allele in the Caucasian population (%)	Enzymatic activity	
						In vitro functionality	*In vivo* functionality
DPYD	Dihydropyrimidine dehydrogenase	c.1679T>G	rs55886062	*DPYD*13*	0.1	≈ ↓° 75%	Reduced
		c.1905+1G>A	rs3918290	*DPYD*2A*	0.5	No activity	No activity
		c.2846A>T	rs67376798		0.7%	≈ ↓° 40%	Reduced
		c.1236G>A	rs56038477	HapB3	2.4	NA	NA

NA: data not available.

[a] HNGC-approved nomenclature (see http://www.genenames.org).

DPD (**d**ihydro**p**yrimidine **d**ehydrogenase)

CPIC (Clinical Pharmacogenetics Implementation Consortium) guidelines - 2013

Table 2 CPIC dose recommendations for fluoropyrimidines as a function of DPD based on the presence of three variants (**2A*, **13* and c.2846A>T) [10].

DPD genotype/phenotype DPD	Example of diplotype	Dose recommendation
Homozygous for wild allele or high DPD activity level	**1/*1* (wild-type)	Standard dose
Heterozygous genotype or intermediary DPD activity level	**1/*2A* **1/*13* **1*/c.2846T	Start with 50% of standard dose then adapt dose to toxicity or pharmacokinetic assay (if available)
Homozygous genotype for mutated allele or complete DPD deficiency	**2A/*2A* **13/*13* c.2846T/T	Formal contraindication for 5-FU or capecitabine

CPIC: Clinical Pharmacogenetics Implementation Consortium; DPD: dihydropyrimidine dehydrogenase.

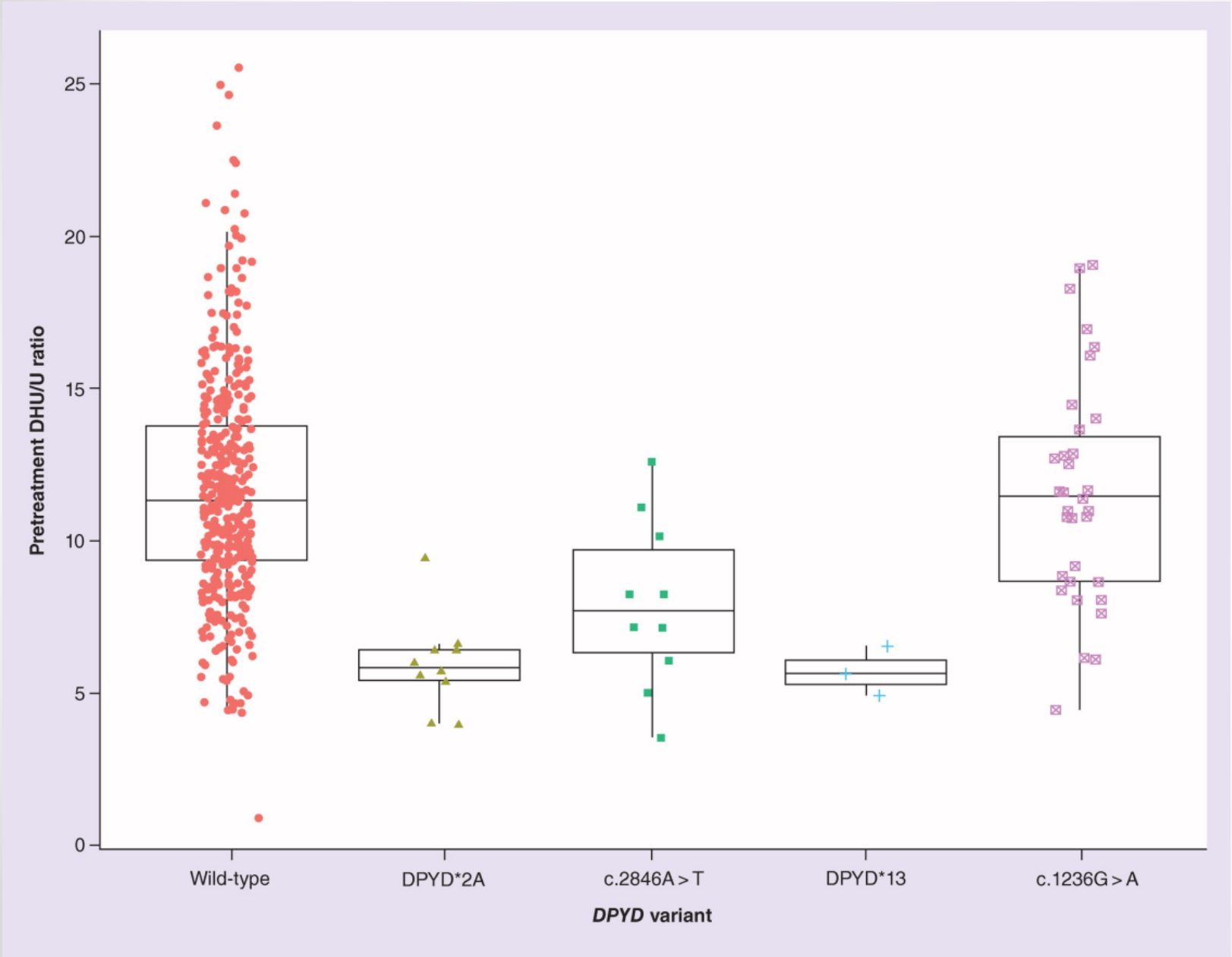
Henricks et al. Pharmacogenomics – 2015 – The Netherlands

Table 3 Recommended initial dose for fluoropyrimidines as a function of the DPD activity level according to Henricks [15] based on the presence of four variants (**2A*, **13*, c.2846A>T and HapB3).

Activity score of the DPD diplotype	Example of diplotype	% of standard dose
0	**2A/*2A*; **13/*13*	Alternative treatment
0.5	**2A/c.2846T*; **13/c.2846T*	25
1	**1/*2A*; **1/*13*; c.2846T/c.2846T	50
1.5	**1/c.2846T*, **1/HapB3*	75
2	**1/*1* (wild-type)	100

Each allele is associated with an activity score computed according to *in vitro* [26,27] and/or *in vivo* [28] functionality. *DPYD*2A*: 0; *DPYD*13*: 0; c.2846A>T: 0.5; HapB3: 0.5. DPD: dihydropyrimidine deshydrogenase.

UH2/U *vs.* genotype

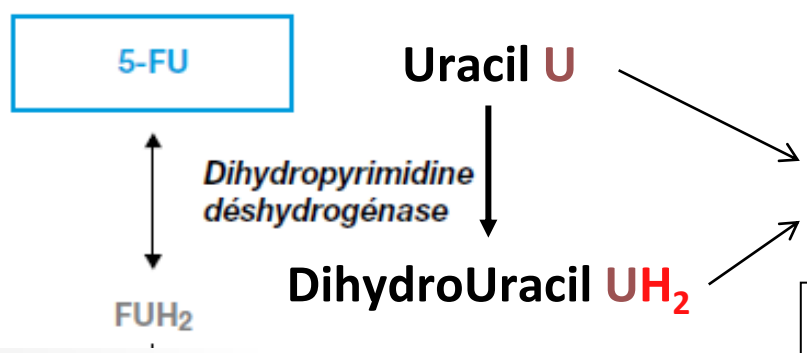


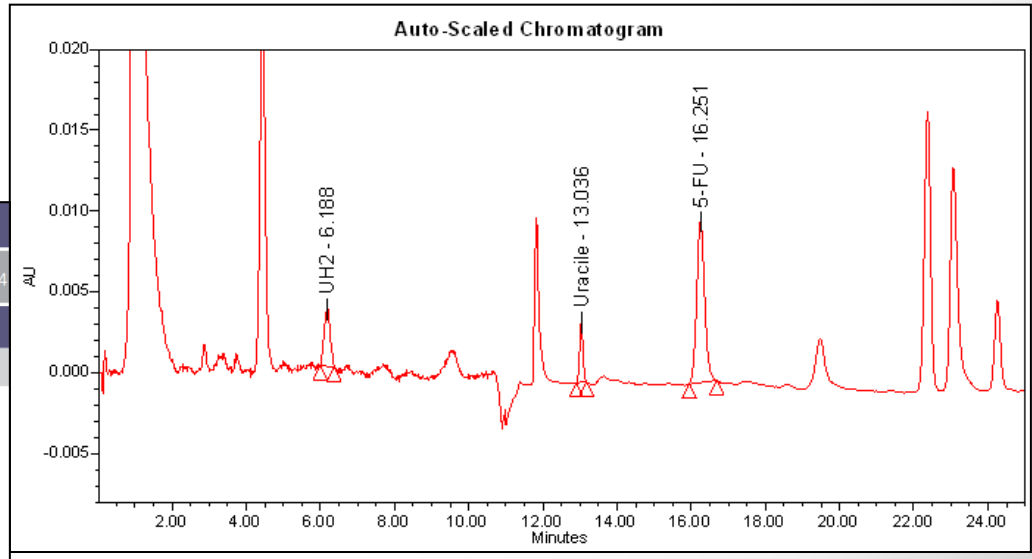
Henricks et al. Pharmacogenomics. 2015;16(11):1277-86.

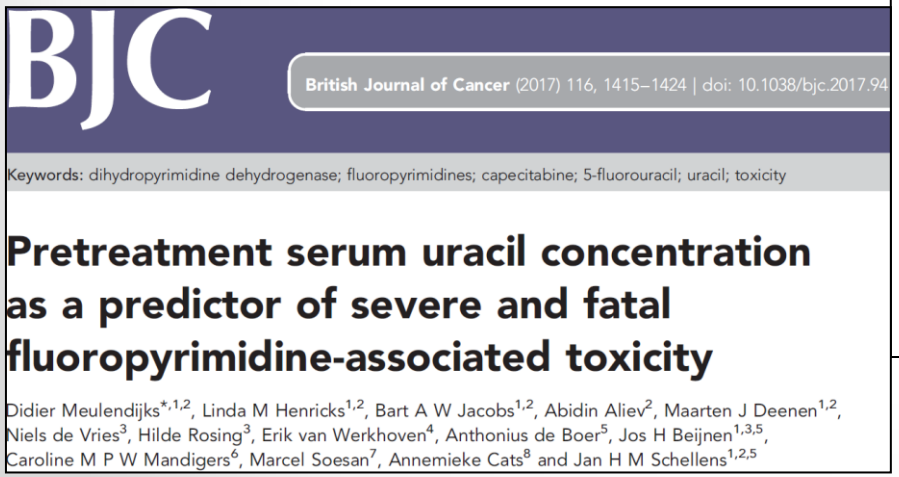
DPD (**d**ihydro**p**yrimidine **d**ehydrogenase)

- DPD Phenotyping test : measurement of enzyme activity

  → **UH_2/U ratio** by HPLC or UPLC/MS







BJC

British Journal of Cancer (2017) 116, 1415–1424 | doi: 10.1038/bjc.2017.94

Keywords: dihydropyrimidine dehydrogenase; fluoropyrimidines; capecitabine; 5-fluorouracil; uracil; toxicity

Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity

Didier Meulendijks[*,1,2], Linda M Henricks[1,2], Bart A W Jacobs[1,2], Abidin Aliev[2], Maarten J Deenen[1,2], Niels de Vries[3], Hilde Rosing[3], Erik van Werkhoven[4], Anthonius de Boer[5], Jos H Beijnen[1,3,5], Caroline M P W Mandigers[6], Marcel Soesan[7], Annemieke Cats[8] and Jan H M Schellens[1,2,5]

Genotyping of a Family With a Novel Deleterious *DPYD* Mutation Supports the Pretherapeutic Screening of DPD Deficiency With Dihydrouracil/Uracil Ratio

F Thomas[1,2], I Hennebelle[1,2], C Delmas[1,2], I Lochon[1,2], C Dhelens[3], C Garnier Tixidre[4], A Bonadona[5], N Penel[6], A Goncalves[7], JP Delord[2,8], C Toulas[9] and E Chatelut[1,2]

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 99 NUMBER 2 | FEBRUARY 2016

We report the **genetic and phenotypic analyses** of DPD in

- ✓ a patient who died after a first cycle of 5-FU (FOLFOX).
- ✓ family members (n=6 healthy volunteers)

⇨ to determine the **functional consequences** of the mutations and study the **genotype/phenotype correlation**.

Results of the patient

- **DPD phenotype:**

Individual	Dihydrouracil (ng/ml)	Uracil (ng/ml)	UH_2/U	Type of deficiency
patient	83.4	786.2	0.1	Complete

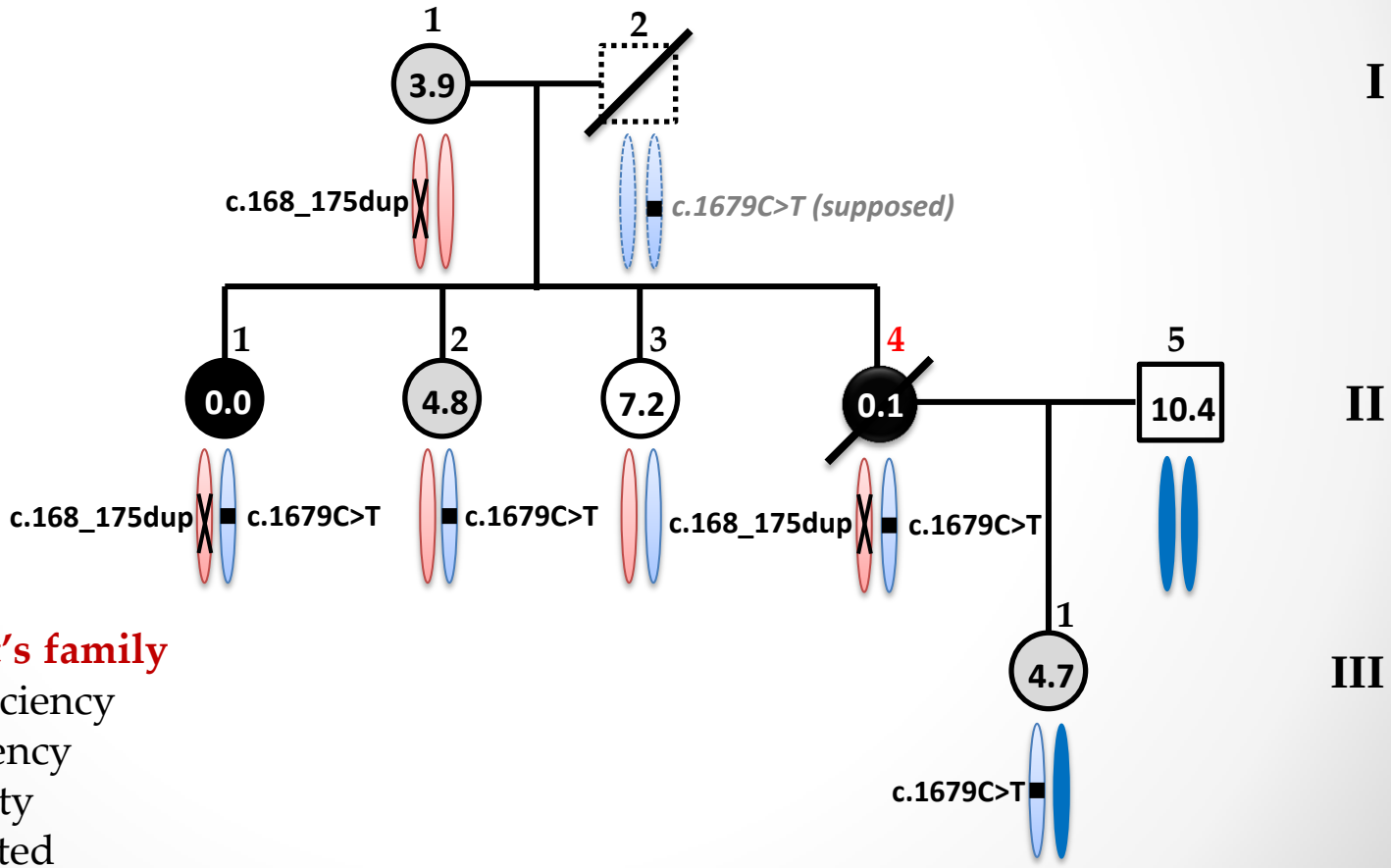
$UH_2/U > 6$: normal, $1<UH_2/U<6$: partial deficiency, $UH_2/U <1$: complete deficiency

- **First genetic screening** (3 *DPYD* variants: c.1905+1G>A, c.1679T>G, and c.2846A>T)
  - Heterozygous for c.1679T>G (exon 13)

- **Full *DPYD* sequencing**
  - heterozygous insertion of 8 nucleotides in exon 3
  - Never described
  - Truncated protein

Results of the family members

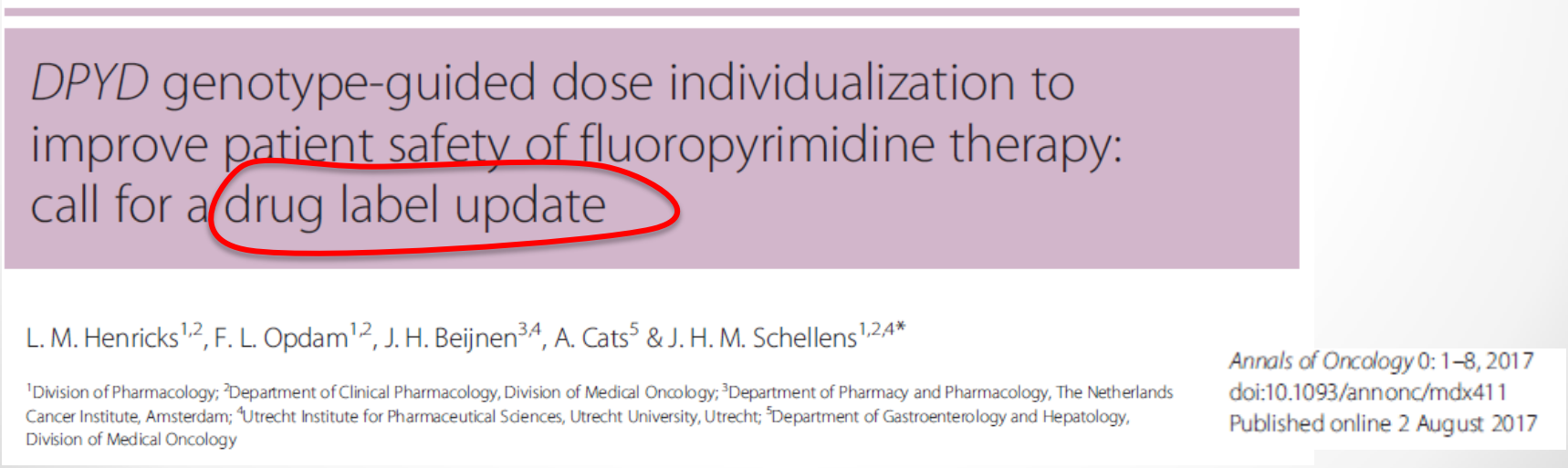
- Describe the pattern of inheritance and study the correlation phenotype/genotype:



Pedigree of the patient's family

● = complete DPD deficiency
○ = partial DPD deficiency
○ = normal DPD activity
▭ = individuals not tested

- Large amount of proof showing the implication of DPD deficiency and fluoropyrimidines toxicity
- FUSAFE (Dr Etienne-Grimaldi): meta-analyses to compare the performance of DPD genotyping *vs DPD phenotyping vs their combined approach, for predicting severe FU toxicity, and surveys addressed to French health professionals to evaluate needs and practices*
- RNPGx: essential (advisable if phenotyping test available)



DPYD genotype-guided dose individualization to improve patient safety of fluoropyrimidine therapy: call for a drug label update

L. M. Henricks[1,2], F. L. Opdam[1,2], J. H. Beijnen[3,4], A. Cats[5] & J. H. M. Schellens[1,2,4*]

[1]Division of Pharmacology; [2]Department of Clinical Pharmacology, Division of Medical Oncology; [3]Department of Pharmacy and Pharmacology, The Netherlands Cancer Institute, Amsterdam; [4]Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht; [5]Department of Gastroenterology and Hepatology, Division of Medical Oncology

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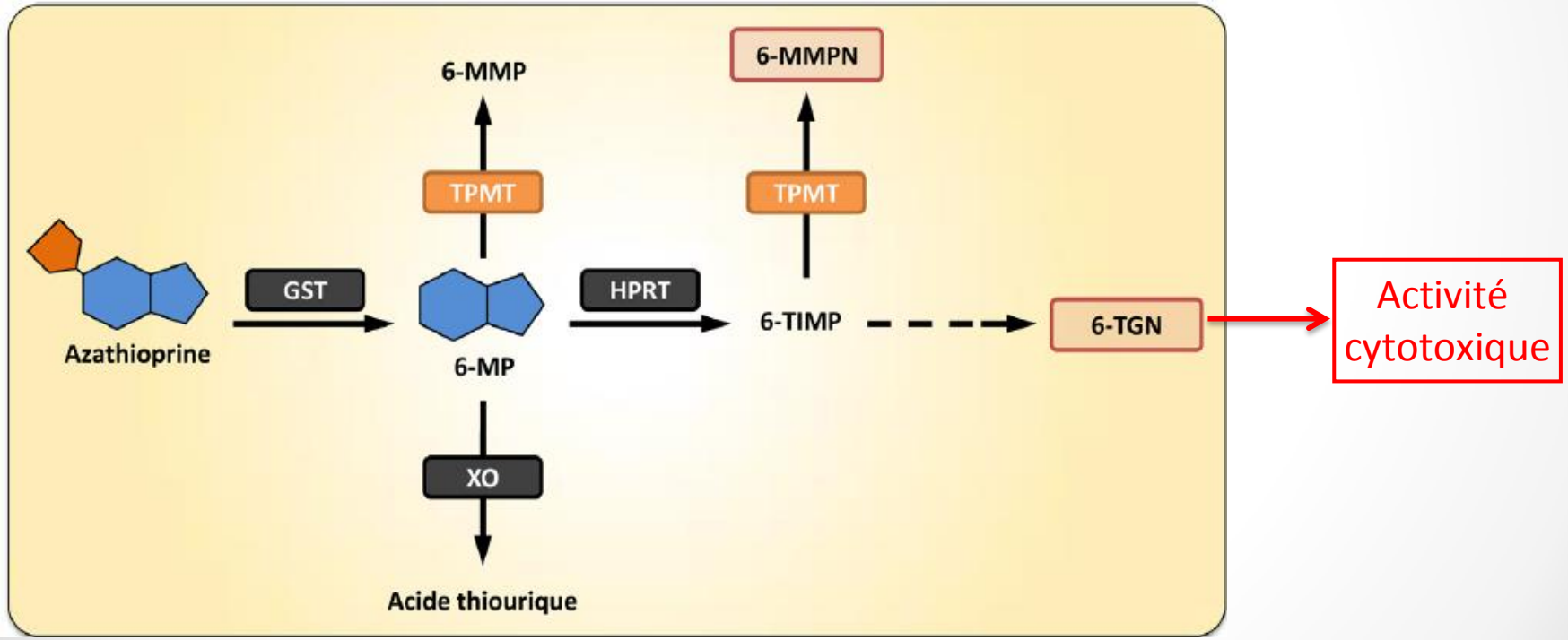
6-mercaptopurine et TPMT

- Chimiothérapie en hémato-oncologie



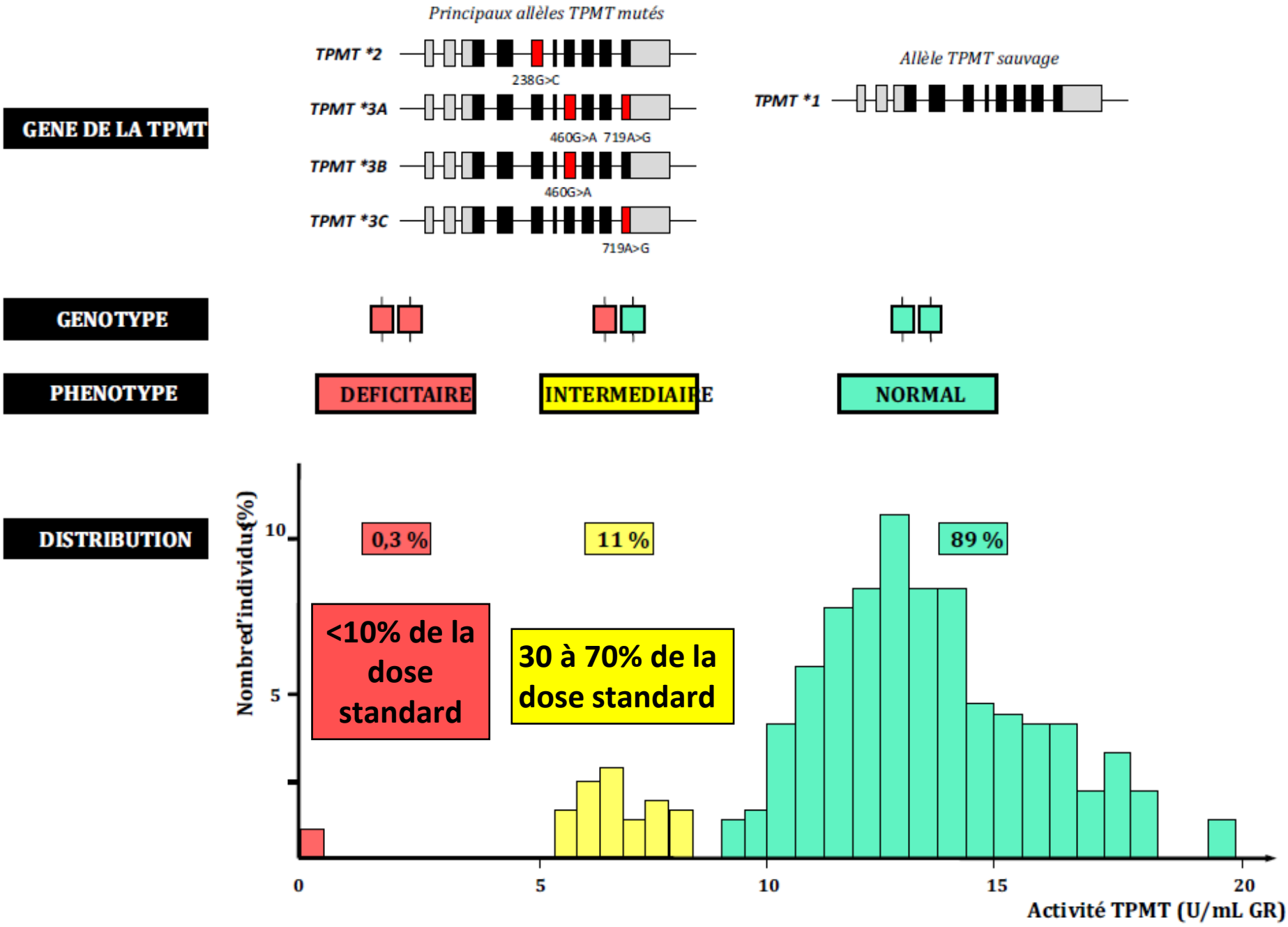


Figure 3. Polymorphismes génétiques de la TPMT et distribution d'activité dans la population générale. Reproduction avec l'aimable autorisation d'Edimark© La lettre du pharmacologue 2013;27(3):72. GR : globules rouges ; TPMT : thiopurine S-méthyltransférase.

Conclusion & Perspective

- The classification proposed will be applied to drug-gene pairs in other important therapeutic domains in order to provide recommendations.

- The objective of RNPGx is to reach consensual recommendations in order to rationalize and promote PGx testing.

Thank you for your attention