Clinical implementation of pharmacogenomic markers to increase treatment safety in oncology

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U-PGx | Ubiquitous Pharmacogenomics

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Adverse Drug Reactions in pharmacological treatment

- Over 2 millions ADRs yearly in US, 100,000 resulting in death (*Inst Med, Nat Acad Press, 2000*)

- They are estimated to cost £1 billion in UK (Pirmohamed, Br Med J, 2004), and $4 billion annually in the US (*Lazarou J et al, JAMA, 1998)*

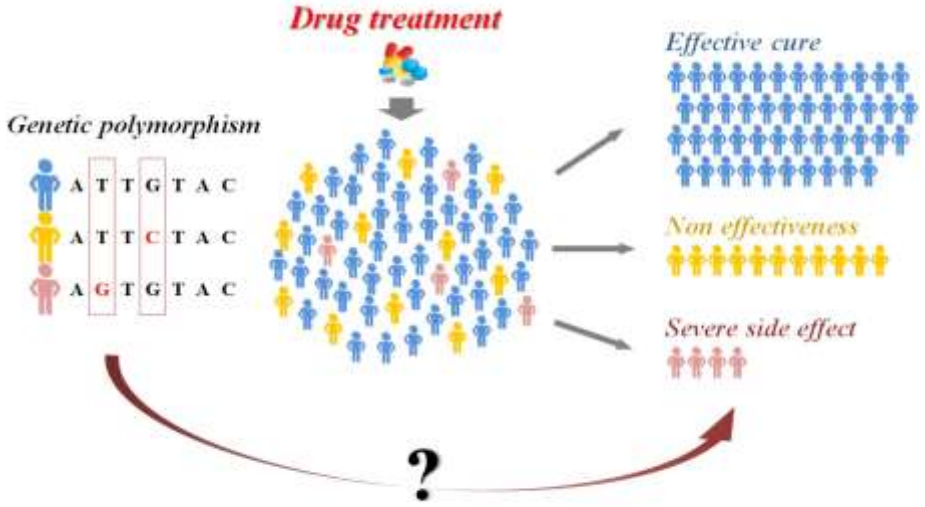
- A revision of 4,158 patients treated for mCRC in the US in 2014 pointed out that about 90% developed at least one ADR after the first cycle (66%>1 AE category) with a significant economic burden mainly related to severe hematological AE related to chemotherapy (*Latremouille et al, J Med Economics, 2016*)

"If it were not for the great variability among individuals, medicine might as well be a science and not an art"

Sir William Osler, 1892

Belfast, Northern Ireland, 24-26 October 2018
47th ESCP Symposium on Clinical Pharmacy

PGx approach to increase drug safety



Retrieved from http://xyc.ideaco.org/wp-content/uploads/2014/07/pharmacogenomics1.jpg

https://www.pharmgkb.org/
https://cpicpgx.org/guidelines/

Dosing Guidelines

CPIC® Clinical Pharmacogenetics Implementation Consortium

DPWG: Dutch Pharmacogenetics Working Group

FDA U.S. FOOD & DRUG ADMINISTRATION

Canadian Pharmacogenomics Network for Drug Safety

Gene-drug interactions currently included in the PGx guidelines for oncology

- *TPMT*/ 6-mercaptopurine
- *UGT1A1*28*/ irinotecan
- *DPYD*/ fluoropyrimidines
- *CYP2D6*/ tamoxifen

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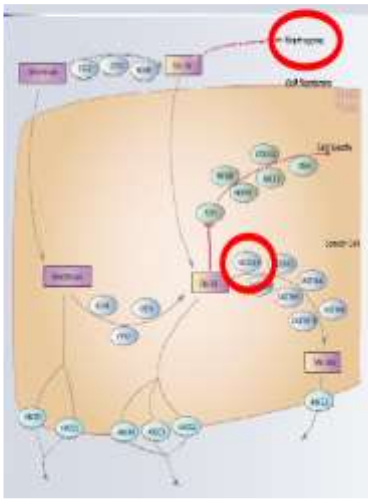
GENE-DRUG INTERACTION 1

UGT1A1-irinotecan

UGT1A1-Irinotecan

- Irinotecan is approved for the first line treatment of metastatic colorectal cancer and other solid tumors
- Exposure to the active irinotecan metabolite SN-38 is the major cause of adverse events
- Severe neutropenia and delayed diarrhea are the dose-limiting toxicities, with the sporadic occurrence of severe and occasionally life-threatening complications possibly causing the failure of the treatment
- UGT1A1 has a major role in SN-38 detoxification



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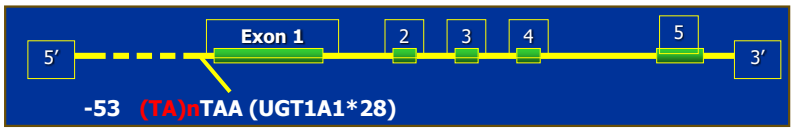
UGT1A1-Irinotecan



- *UGT1A1* encoding gene is polymorphic
- *UGT1A1*28* polymorphism is common in Caucasian population (10% is homozygous)
- *UGT1A1*28* polymorphism is related to lower UGT1A1 enzyme expression, therefore SN-38 glucuronidation could be less efficient

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Our **hypothesis external validation** in collaboration with Université Laval-Quebec

UNIVERSITÉ LAVAL

OUR RESULTS ON HAPLOTYPE II (as reported by us, all "defective" UGT1A alleles; Cecchin 2009) PREDICTIVE VALUE ON NEUTROPENIA (UNPUBLISHED DATA) WERE REPLICATED IN AN INDEPENDENT COHORT OF 167 CANADIAN mCRC PATIENTS TREATED WITH FOLFIRI-BASED REGIMENS.

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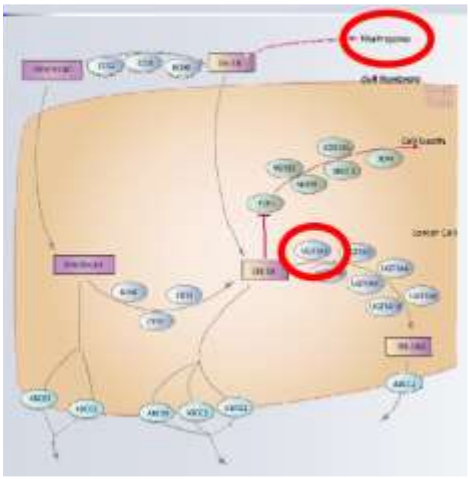
Lévesque E, Bélanger AS, Harvey M, Couture F, Jonker D,Innocenti F, Cecchin E, Toffoli G, and Guillemette C J Pharmacol and Exp Ther, 2013

Phase 1b studies based on the patient genotype for re-definition of MTD: the rational



•An impaired SN38 detoxification by an UGT1A1*28 polymorphic form increases the risk of acute severe toxicity

•We have demonstrated that *1/*1 or *1/*28 patients have lower toxicity (Toffoli et al, JCO, 2006)

•Registrative phase 1 studies for FOLFIRI regimen did not take patients genotype into account

•A re-definiton of proper irinotecan dose for *1/*1 or *1/*28 patients by genotype is requested

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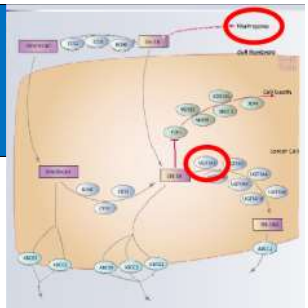
5x1000 AIRC – RICERCA DIACHEMO

Phase 1b studies based on the patient genotype for re-definition of MTD: the study design



Eligible mCRC patients

↓

Genotyping for UGT1A1 *28

↓

Stratification into two groups

GROUP 1:
UGT1A1 *1/*1
WILD TYPE PTS
IRI dose escalation

GROUP 2:
UGT1A1 *1/*28
HETEROZYGOUS PTS
IRI dose escalation

Patients with *28/*28 genotype excluded.
Clinical and pharmacokinetic monitoring

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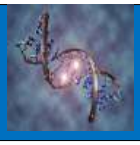
5x1000 AIRC – RICERCA DIACHEMO

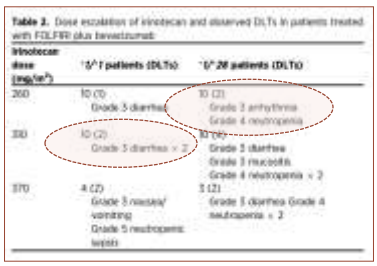
Phase 1b studies based on the patient genotype for re-definition of MTD: the results



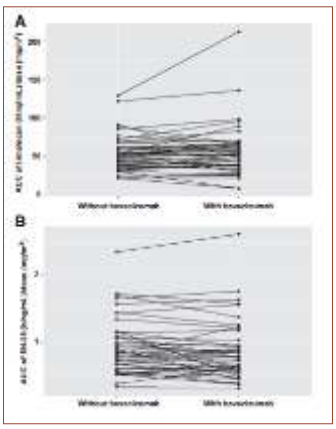
Therapy	*1/*1 genotype, Dose (mg/m²)	1/*28 genotype, Dose (mg/m²)	
FOLFIRI, standard dose	180	180	*Ducreux et al, J Clin Oncol, 1999*
FOLFIRI	370	310	*Toffoli et al, J Clin Oncol , 2010*
FOLFIRI plus BEVACIZUMAB	310	260	*Toffoli et al, Clin Cancer Res, 2016*
FOLFIRI plus CETUXIMAB	ongoing	ongoing	*ongoing*

The stratification of patients in FOLFIRI or FOLFIRI plus bevacizumab regimens according to *UGT1A1*28* genotype led to a higher MTD both in *UGT1A1*1/*28* and *UGT1A1*1/*1* patients.

Phase 1b studies based on the patient genotype for re-definition of MTD: the results



MTD is 260mg/m2 for *1/*28 and 310 for *1/*1 patients, lower than that of FOLFIRI alone (Toffoli et al JCO, 2010)



The interaction with bevacizumab is unlikely to be related to a PK interaction

Clinical Pharmacology & Therapeutics

ASCPT

ARTICLES

Cost Evaluation of Irinotecan-Related Toxicities Associated With the *UGT1A1*28* Patient Genotype

R Roncato[1], E Cecchin[1], M Montico[1], E De Mattia[1], L Gradini[2], A Buonadonna[2], V Soffiati[2], F Innocenti[3] and G Toffoli[1]

Spreading of *UGT1A1*28* pre-emptive genotyping to increase irinotecan safety is still limited. The definition of the cost consequences of patients genotype is one of the pending issues. A survey of the toxicity associated costs in 243 FOLFIRI treated mCRC

Table 3 Association between *UGT1A1*28* polymorphism and the costs for toxicity

UGT1A1 genotype	Number of patients	Mean predicted cost per patient[a] (95% CI) (Euro)		Regression coefficient	95% CI	P-value	Regression coefficient	95% CI	P-value
*1/*1	109	812	(653–970)	Ref[b]					
*1/*28	112	1,119	(885–1,353)	0.32	0.04–0.60	0.024	Ref[b]		
*28/*28	22	4,886	(2,611–7,160)	1.79	1.31–2.28	<0.001	1.47	0.99–1.95	<0.001

CI, Confidence Interval; Ref, Reference Category.
[a]By generalized linear model, adjusted by age, sex, adjuvant chemotherapy, and total number of chemotherapy cycles. [b]Reference category for regression coefficients calculation.

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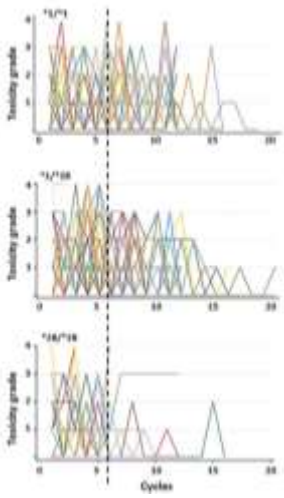
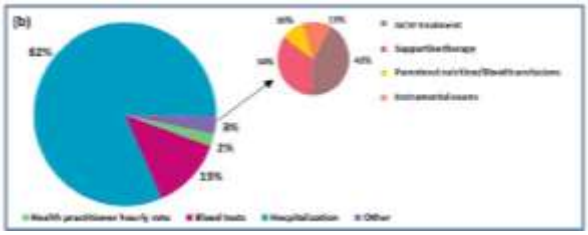
*Severe toxicity related to hospitalization costs (grade 4) are significantly more prevalent in patients that are carriers of *28 allele.*





Most of the costs are related to hospitalization (grade 4 toxicity). The risk of grade 4 toxicity is *28 allele dependent

Table 4 Occurrence of any kind of grade 4 toxicities based on *UGT1A1*28* polymorph

UGT1A1 genotype	Number of patients	Toxicity before the sixth cycle		OR (95% CI)	P-value
		G0-G3	G4 (%)		
*1/*1	109	108	1 (0.9)	Ref	
*1/*28	112	103	9 (8.0)	9.4 (1.2–75.6)	0.019
*28/*28	22	20	2 (9.1)	10.8 (0.9–124.8)	0.073
*1/*28/ and *28/*28	134	123	11 (8.2)	9.7 (1.3–76.1)	0.014

G, Grade; CI, Confidence Interval; Ref, Reference Category.

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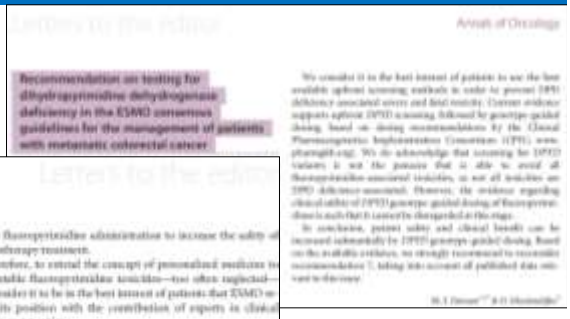
RONCATO R ET AL, CLIN PHARMACOL THER , 2017

GENE-DRUG INTERACTION 2

DPYD-fluoropyrimidines

Implementation of pre-emptive genotyping of the HOST for increasing treatment safety

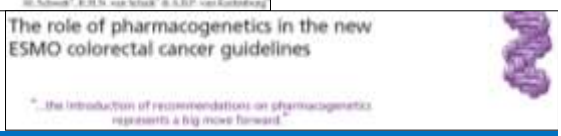
The old-new *DPYD* translational story


Annals of Oncology

Recommendation on testing for dihydropyrimidine dehydrogenase deficiency in the ESMO consensus guidelines for the management of patients with metastatic colorectal cancer


Annals of Oncology

Prevention of fluoropyrimidine toxicity: do we still have to try our patient's luck?


The role of pharmacogenetics in the new ESMO colorectal cancer guidelines

"...the introduction of recommendations on pharmacogenetics represents a big move forward."

Belfast, Northern Ireland, 24-26 October 2018
47th ESCP Symposium on Clinical Pharmacy

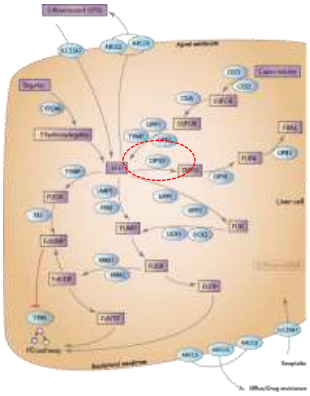
DPYD-Fluoropyrimidines

5-FU for 40 Yrs

- 5-FU bolus
- 5-FU infusion
  - 24 hrs
  - 48 hrs
  - 46 hrs
  - 120 hrs
  - ∞ hrs
- LV + 5-FU
- 5-FU + LV
- 5-FU + Lev
- 5-FU + everything
- .....

•FL are the mainstay of many chemotherapeutic schemes in different combination for different pathologies and settings

•10 to 26% of patients experiencing acute severe or life-threatening toxicity even in monotherapy regimens



DPYD IS A KEY ENZYME REPRESENTING A BOTTLENECK IN FL CELL DETOXIFICATION

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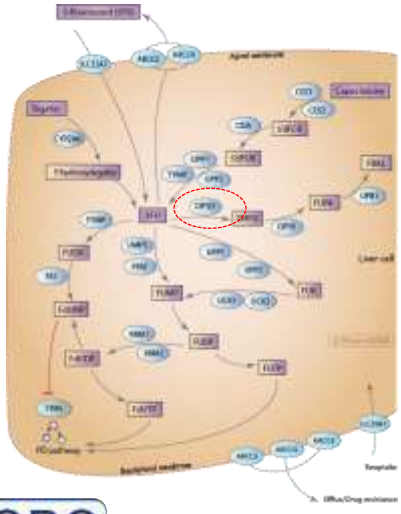
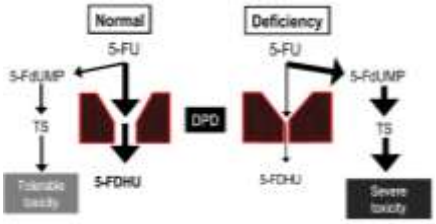
FL cellular metabolism





DPYD IS A KEY ENZYME REPRESENTING A BOTTLENECK IN FL CELL DETOXIFICATION

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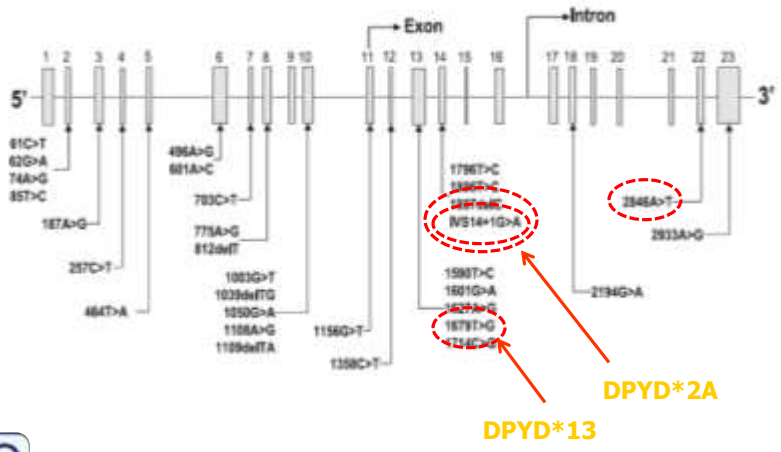
DPYD pharmacogenetics



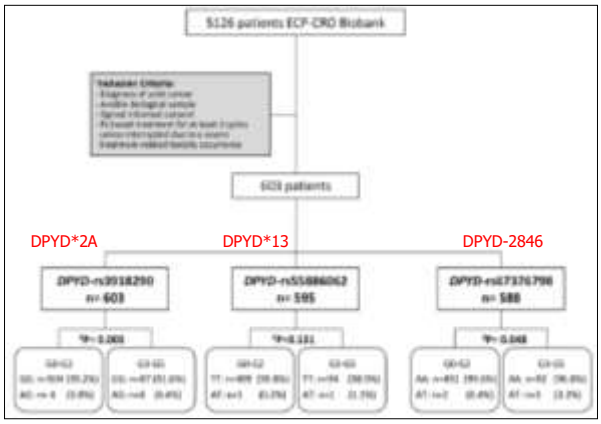

IJC International Journal of Cancer

IJC

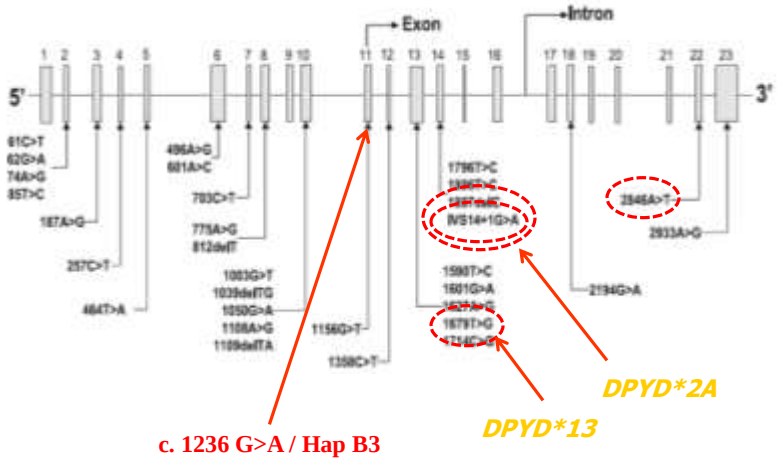
Clinical validity of a *DPYD*-based pharmacogenetic test to predict severe toxicity to fluoropyrimidines

- **603** solid cancer patients treated with FL-based regimen
- **Clinical End-Point**: Severe (≥G3) or lethal toxicity related to FL administration

- Frontline genotyping could have allowed the identification of 10 patients at risk for severe toxicity and 1 toxic death (**11.6% of severe toxic events**)
- The patient with toxic death was compound heterozygous for *DPYD*2A*, and *DPYD*13* and was treated in an adjuvant regimen for gastric cancer



DPYD pharmacogenetics



DPWG pharmacogenomics recommendations

Perspective

Pharmacogenomics

Translating *DPYD* genotype into DPD phenotype: using the *DPYD* gene activity score

Table 1. Values for activity assigned to alleles of *DPYD*.

Activity value	Alleles	Ref.
0	*DPYD*2A* (rs3918290)	[4,8,10,11,39,27,29–46]
	*DPYD*13* (rs55886062)	[4,19,30,32,33,46,49,50]
0.5	c.2846A>T (rs67376798)	[4,13,24,33,38,41,42,44,47]
	c.1236G>A/ HapB3 (rs56038477)	[34,15,42,44,46,47,49,50,56]
1	*DPYD*1* (wild-type)	

These values for both alleles of a patient are summed, leading to an individual gene activity score.

Table 2. Initial dose recommendation for *DPYD* gene activity score.

Gene activity score	% of standard dose
0	Alternative drug
0.5	25
1	50
1.5	75
2	100

Henricks et al, Pharmacogenomics, 2015

DPYD Gene Activity Score and Clinically Relevant Toxicity in 763 colorectal cancer patients

- **763** CRC patients treated with FL-based regimen
- **Study End-Point**: Severe (≥G3) non-hematological or (≥G4) hemathological toxicity related to FL administration

THE **CLINICALLY RELEVANT TOXICITY**: drives treatment decision and affects quality of life in cancer patients- this is what oncologists are more interested in

	N	%	**OR	95 % CI	OR	95 % CI
			Acute toxicity (<3cycles)		Overall toxicity	
4-SNP panel carriers	45/ 763	5.9	**2.69**	**1.33 – 5.41**	**2.67**	**1.42 – 5.04**
GAS						
2.0	718	94.1	1[a]		1[a]	
1.5	36	4.7	1.80	0.78 – 4.15	**2.08**	**1.02 – 4.27**
1.0	9	1.2	**10.12**	**2.55 – 40.2**	**7.09**	**1.69 – 29.65**
χ^2 for trend			***P* = 0.0007**		***P* = 0.0009**	

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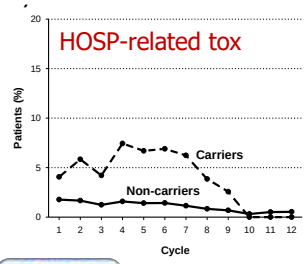
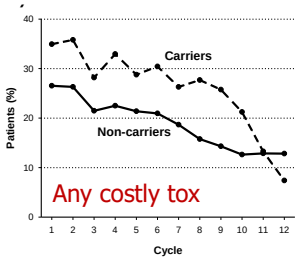
Dalle Fratte C et al; Paper IN PRESS, Journal of Molecular and Clinical Medicine

The genotype for DPYD risk variants in colorectal cancer patients and the related toxicity management costs in clinical practice Analysis on 550 patients from everyday clinical practice

4-SNP Panel				
Status	**n**	**[a]Mean (Euros)**	**95% CI**	
Non-carriers	513	817	779-854	
Carriers	37	2,972	2,456-3,505	p<0.0001
GENE ACTIVITY SCORE				
2	513	825	785-864	-
1.5	28	2,188	1,683-2,693	
1	9	5,414	2,268-8,561	-
0	-	-	-	p<0.0001





Most of the costs are related to hospitalization (grade 4 toxicity). The risk of grade 4 toxicity is *DPYD* allele dependent

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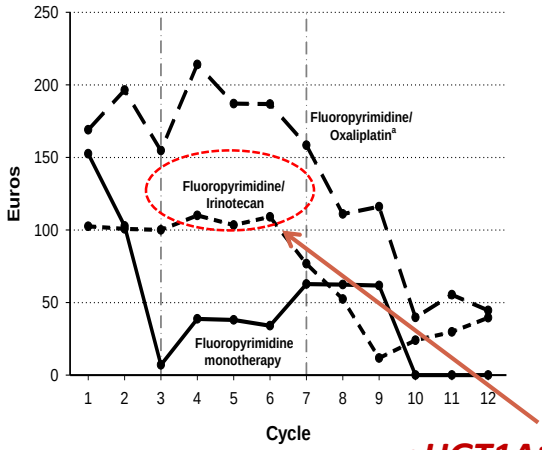
G Toffoli, F Innocenti, J Polesel , E De Mattia, M Guardascione, L Foltran, A Bignucolo, M Berretta, A De Paoli, R Roncato , and E Cecchin; Clin Pharmacol Ther, October 2018, e-pub

Colorectal cancer patient genotype for DPYD risk variants and related toxicity management: A cost-analysis from clinical practice data

Average cost for patients at each cycle

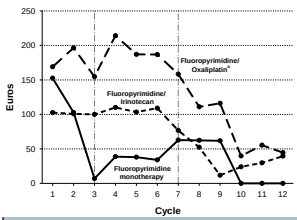


Combination treatment increases overall toxicity management cost. Integration of drug-specific pharmacogenetic markers of toxicity can improve identification of very costly patients

- ***UGT1A1*28*/irinotecan**

TOFFOLI, ET AL –CLIN PHARMACOL THER- OCT 2018- E-PUB

Colorectal cancer patient genotype for DPYD risk variants and related toxicity management: A cost-analysis from clinical practice data on 550 patients

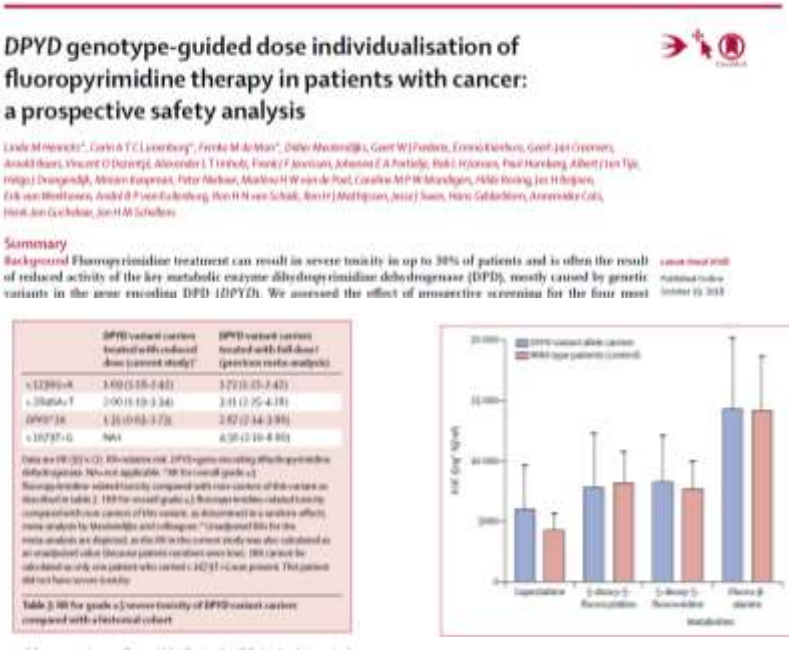


Integration of DPYD and UGT1A1*28 information can improve identification of very costly patients (in a subgroup of 265 patients treated with FL+IRI)

DPYD* variants or *UGT1A1*28/*28			Costs (Euro)		
	n	**(%)**	**Mean**	**95% CI**	**Anova**
Noncarriers	227	(85.7)	571	493-609	
Carriers	38	(14.3)	3,546	1,766-3,765	*P*<0.0001

Incremental cost of 2,975€ vs 2,155€ (without UGT)

TOFFOLI, ET AL –CLIN PHARMACOL THER- OCT 2018- E-PUB



DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis

Summary

1,103 patients were prospectively treated with FL containing regimens according to DPWG guidelines. The approach was demonstrated to be feasible and to increase treatment safety (as CRT occurrence)

- *UGT1A1*28* and *DPYD*2A, *13, DPYD-HAPB3* and *c.2846A>T* still not at the bedside.. further proofs of clinical validity and utility requested?

- Pharmacogenomics Clinical Implementation- Probably the best way to go

Prospective application of pharmacogenetic markers of CT-related toxicity at CRO

Up to date 562 cancer patients eligible for a therapy with FL and/or IRI were referred to the Pgx service prior to treatment

Based on the genotype data for DPYD and/or UGT1A1, a starting dose adjustment was suggested for 65 patients

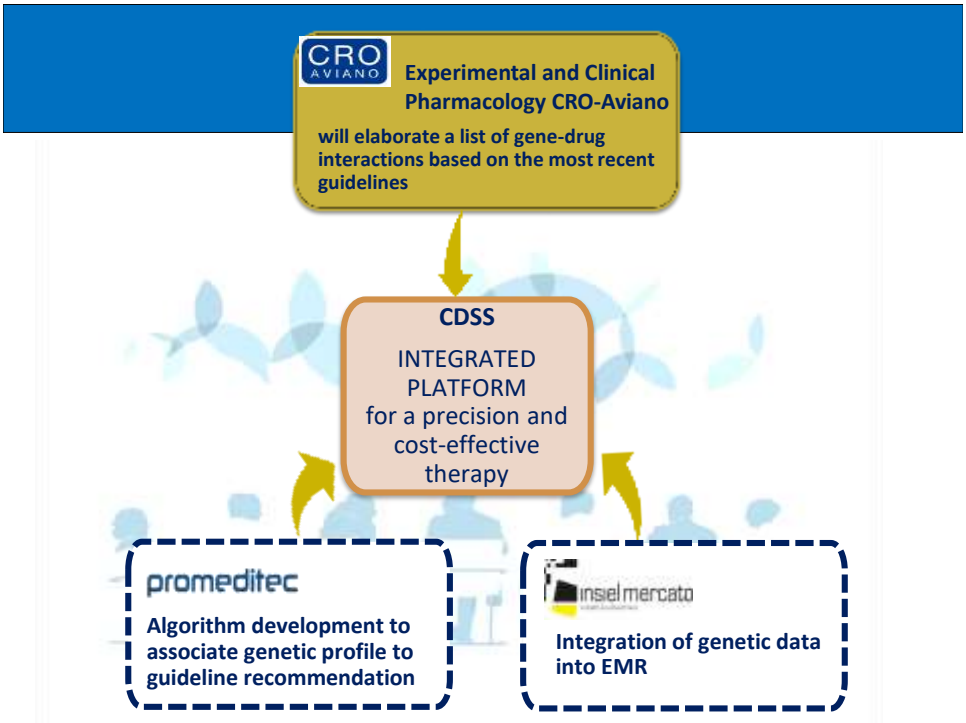
A PGx electronic report is embedded in the patient clinical record

Data collected could be used to design cost-effectiveness and HTA studies in order to clarify the real clinical utility of the test.

Pharmacogenetic electronic report

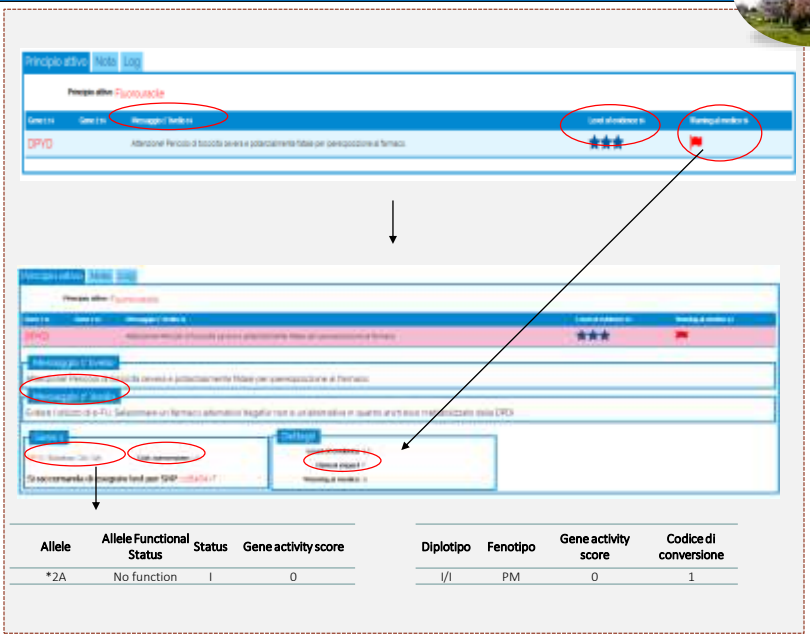
FARMAPRICE

DEVELOPMENT OF A CLINICAL DECISION SUPPORT SYSTEM FOR A PRECISION AND COST-EFFECTIVE THERAPY



FARMAPRICE- How does it work??

FARMAPRICE- How does it work??



Allele	Allele Functional Status	Status	Gene activity score
*2A	No function	I	0

Diplotipo	Fenotipo	Gene activity score	Codice di conversione
I/I	PM	0	1

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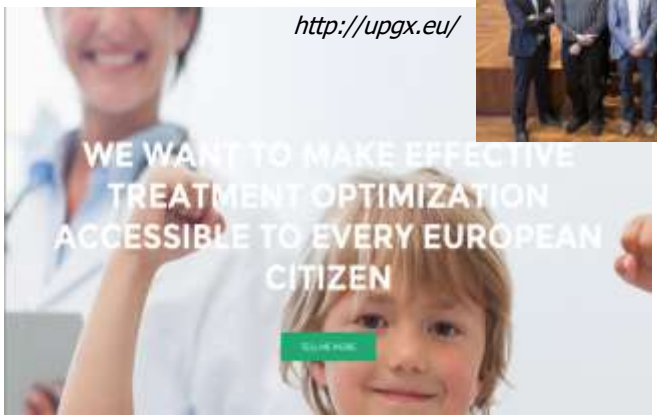
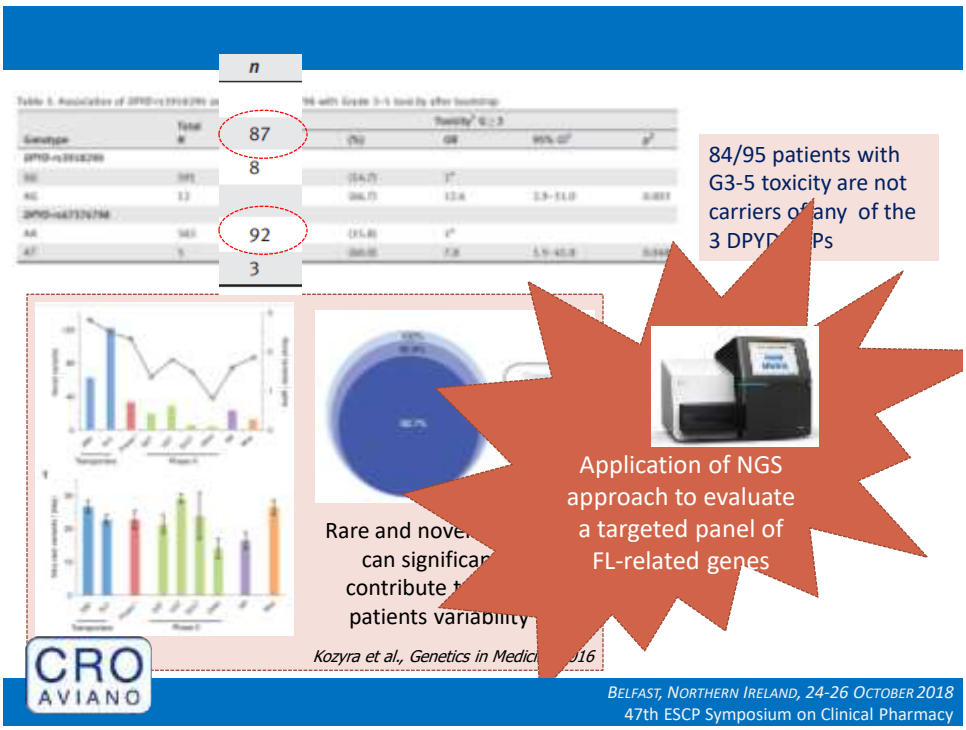
Coordinated by Leiden University-Prof HJ Guchelaar



C.H.van Der Wouden et al, Clin Pharmacol Ther, VOLUME 101 NUMBER 3 | MARCH 2017
E. Cecchin et al, Curr Pharm Biotech, 2017- VOLUME 18 ISSUE 3 / 204 - 209



Study Design

Patients treated with FL-based regimens included in our bio-bank

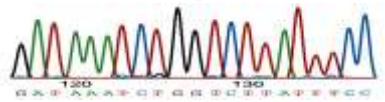
(n=1,122)

•Genotype data for *DPYD2A, *13, 2846, and HapB3**
•Toxicity data available

(n= 845)

INCLUSION CRITERIA
-Availability of biological material
-Clinical data
-Informed consent

INCLUSION CRITERIA
-Severe toxicity (≥G3)
-Absence of *DPYD* risk variants



130 patients selected for NGS analysis

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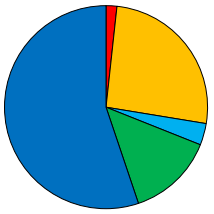
NGS panel design and analysis

Target Genes



- A list of **58 genes** was selected based on literature (PubMed, PharmGKB, Sphinx)
- The panel was designed to cover for each gene, the Coding sequence (**CDS**); the Untranslated regions (**5'UTR** and **3'UTR**); and the **Flanked splice junctions regions**
- A custom **enrichment** gene panel has been selected (SeqCap EZ, Roche)
- At present, the analysis was performed on **114/130** patients on **MiSeq** platform (**Illumina**)

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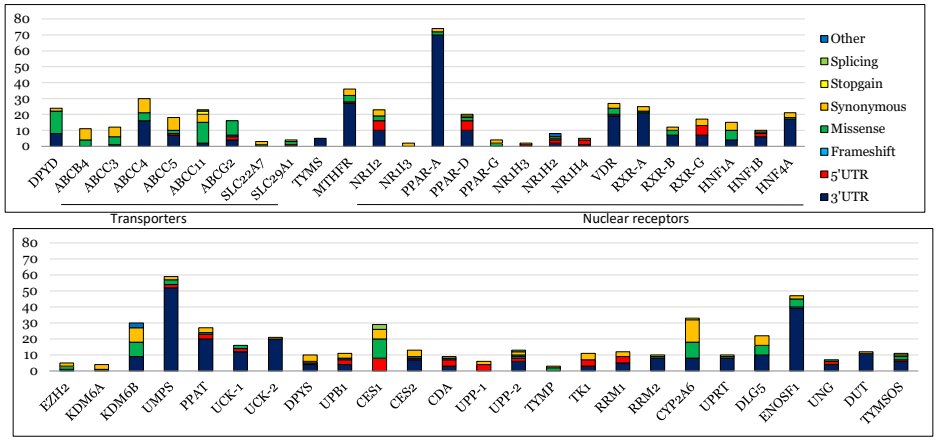
Preliminary Results I



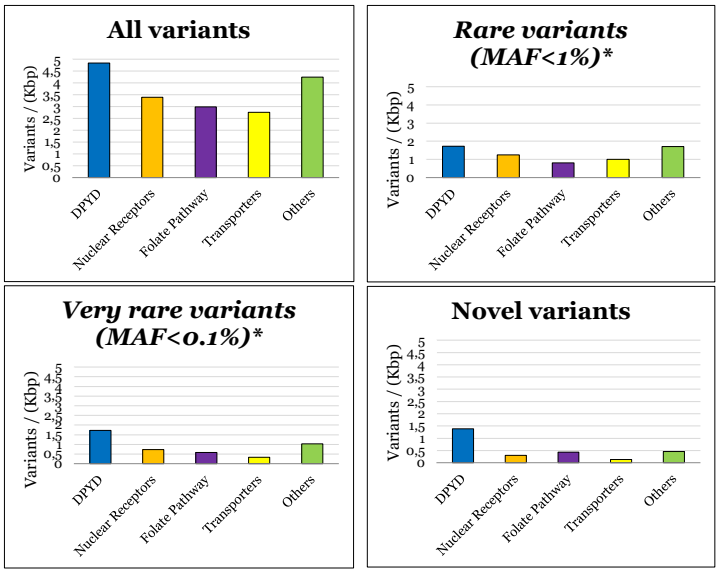
- High heterogenehity in distribution of numbers and class of variants
- *DPYD* was affected by an high number of exonic variants, the majority are missense

Preliminary Results II

The novel variants are 10.2% of all the detected variants

Number of variants normalized for the lenght of genes

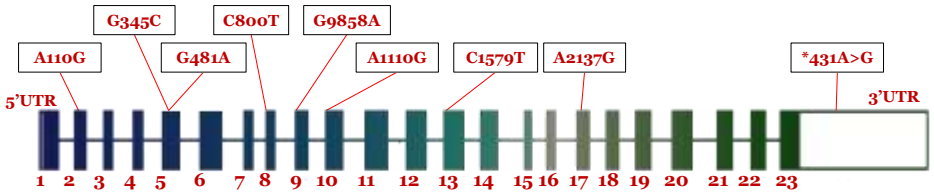
***DPYD* has an high variants rate per Kb**



** According to ExAC, 1000Genome and dbSNP.*

Preliminary Results II-DPYD novel and rare variants

Variant	Rs	Location	Functional Consequences	Freq ExAC_NFE	Observed Toxicity
c.G345C p.M115I	rs377169736	Exon5	Missense	0,0001	G4 Non Hematologic
c.G481A p.E161K	/	Exon5	Missense / Splice	/	G4 Hematologic
c.C800T p.T267I	/	Exon 8	Missense / Splice	/	G3 Non Hematologic
c.G958A p.G320R	/	Exon9	Missense / Splice	/	G4 Hematologic
c.A1110G p.I370M	/	Exon 10	Missense / Splice	/	G4 Non Hematologic
c.C1579T p.P527S	/	Exon13	Missense / Splice	/	G3 Non Hematologic
c.A2137G p.N713D	rs773407491	Exon 17	Missense	0.000008247	G4 Non Hematologic
c.*431A>G	/	UTR3	/	/	G4 Hematologic
c .-416A>G	/	Upstream	/	/	G4 Non Hematologic



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International Journal of *Molecular Sciences*

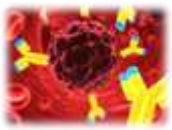
MDPI

Article

HLA-G 3′UTR Polymorphisms Predict Drug-Induced G3-4 Toxicity Related to Folinic Acid/5-Fluorouracil/ Oxaliplatin (FOLFOX4) Chemotherapy in Non-Metastatic Colorectal Cancer

Marica Garziera [1,*], Saverio Virdone [2], Elena De Mattia [1], Lucia Scarabel [1], Erika Cecchin [1], Jerry Polesel [2], Mario D'Andrea [3], Nicoletta Pella [4], Angela Buonadonna [5], Adolfo Favaretto [6] and Giuseppe Toffoli [1]

PGx studies



Immunosystem

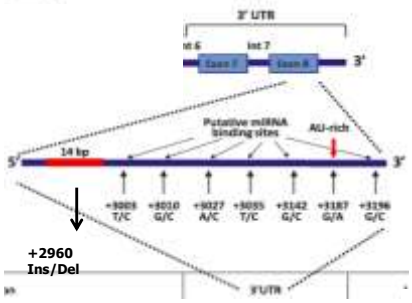
Exploratory research of new genetic markers of treatment sensitivity : the immunogenetic approach

CRO AVIANO

Int. J. Mol. Sci. 2017, 18, 1366; doi:10.3390/ijms18071366

Genetic polymorphism in HLA-G immunofactor

Human Leukocyte Antigen-G (HLA-G) **gene is involved in cancer immune tolerance, is over-expressed in CRC tumor tissue, and has a negative prognostic impact.**



Amodio *et al.*, *Tissue Antigens* 2014

144 patients with stages II-III CRC treated with adjuvant FOLFOX (5-FU/OXALI/LV) monitored for 8 SNPs in the HLA-G 3'UTR previously related with HLA-G plasma level

Int. J. Mol. Sci. 2017, 18, 1366; doi:10.3390/ijms18071366

Significant associations between HLA-G 3'UTR haplotypes and G3-4 toxicities

Toxicity Type	Haplotype	14-bp	+3003	+3010	+3027	+3035	+3142	+3187	+3196	n	Significant Genetic Models			Bootstrap
											Model	OR (95%-CI) *	p	p
Hematological	UTR-2 Het Hom	Ins	T	C	C	C	G	A	G	23 10	Rec	5.46 (1.14-33.52)	0.020	0.074
Hematological	UTR-4 Het Hom	Del	C	G	C	C	C	A	C	4 1	Dom	0.35 (0.12-0.98)	0.046	0.098
Neutropenia	UTR-2 Het Hom	Ins	T	C	C	C	G	A	G	22 10	Rec	3.92 (1.20-12.07)	0.017	0.059
Neutropenia	UTR-6 Het Hom	Del	T	G	C	C	C	A	C	6 8	Dom	4.77 (1.07-21.20)	0.040	0.058
Neurotoxicity	UTR-2 Het Hom	Ins	T	C	C	C	G	A	G	3 4	Rec	11.29 (1.84-69.40)	0.009	0.079

Immunogenetic germline variants can interact with the effect of chemotherapy and identify profiles of pateints at high risk of severe

Garziera et al., Int. J. Mol. Sci. 2017, 18, 1366

Conclusion

- Current scientific evidence supports the use of some germline genetic markers as *UGT1A1*28, DPYD*2A, DPYD*13, DPYD-HapB3, and c.2846A>T* to increase treatment safety in cancer and to save economical resources
- Clinical implementation of these genetic markers is still lagging behind and the development of new IT tools as well as the development of large scale randomized clinical trials could support this process
- Further advancement in the field of genome technology will allow new more comprehensive sequencing approaches to consider also the contribute of rare genetic variants in the definiton of the patients ADME phenotype
- New exploratory markers of chemotherapy related toxicity could be highlighted by a pathway based approach exploring the field of the immunosystem related genetics

Acknowledgements

U-PGx | Ubiquitous Pharmacogenomics

Experimental and Clinical Pharmacology CRO AVIANO

Director Giuseppe Toffoli

Pgx group: Francesco Angelini, Alessia Bignucolo, Francesco Comello, Lisa Dal Cin, Chiara Dalle Fratte, Elena De Mattia, Tania Diraimo, Eva Dreussi, Fabrizio Ecca, Marica Garziera, Michela Guardascione , Silvia Mezzalira, Loredana Romanato, Rossana Roncato, Franca Sartor

Quebec University- Prof Chantal Guillemette

University of Chapel Hill- Prof Federico Innocenti

U-Pgx European Consortium- Prof HJ Guchelaar

van der Wouden CH, Cambon-Thomsen A, Cecchin E, Cheung K, Dávila-Fajardo CL, Deneer VH, Dolžan V, Ingelman-Sundberg M, Jönsson S, Karlsson MO, Kriek M, Mitropoulou C, Patrinos GP, Pirmohamed M, Samwald M, Schaeffeler E, Schwab M, Steinberger D, Stingl J, Sunder-Plassmann G, Toffoli G, Turner RM, van Rhenen MH, Swen JJ

Univ of Padova- Istituto Oncologico Veneto
Univ di Trieste- Burlo Garofolo- Prof G De Corti, Prof G Stocco
Univ of Florence- Prof E Mini, Dr S Nobili
Hospital San Filippo Neri –Roma- Dr. M D'Andrea
Hospital Ca Foncello- Treviso- Dr. A Favaretto