Erasmus MC
Universitair Medisch Centrum Rotterdam

Ron van Schaik

Associate Professor Pharmacogenetics

Eur Clin Chem / Advisor EMA - PGWG

London, Oct 8-9, 2012

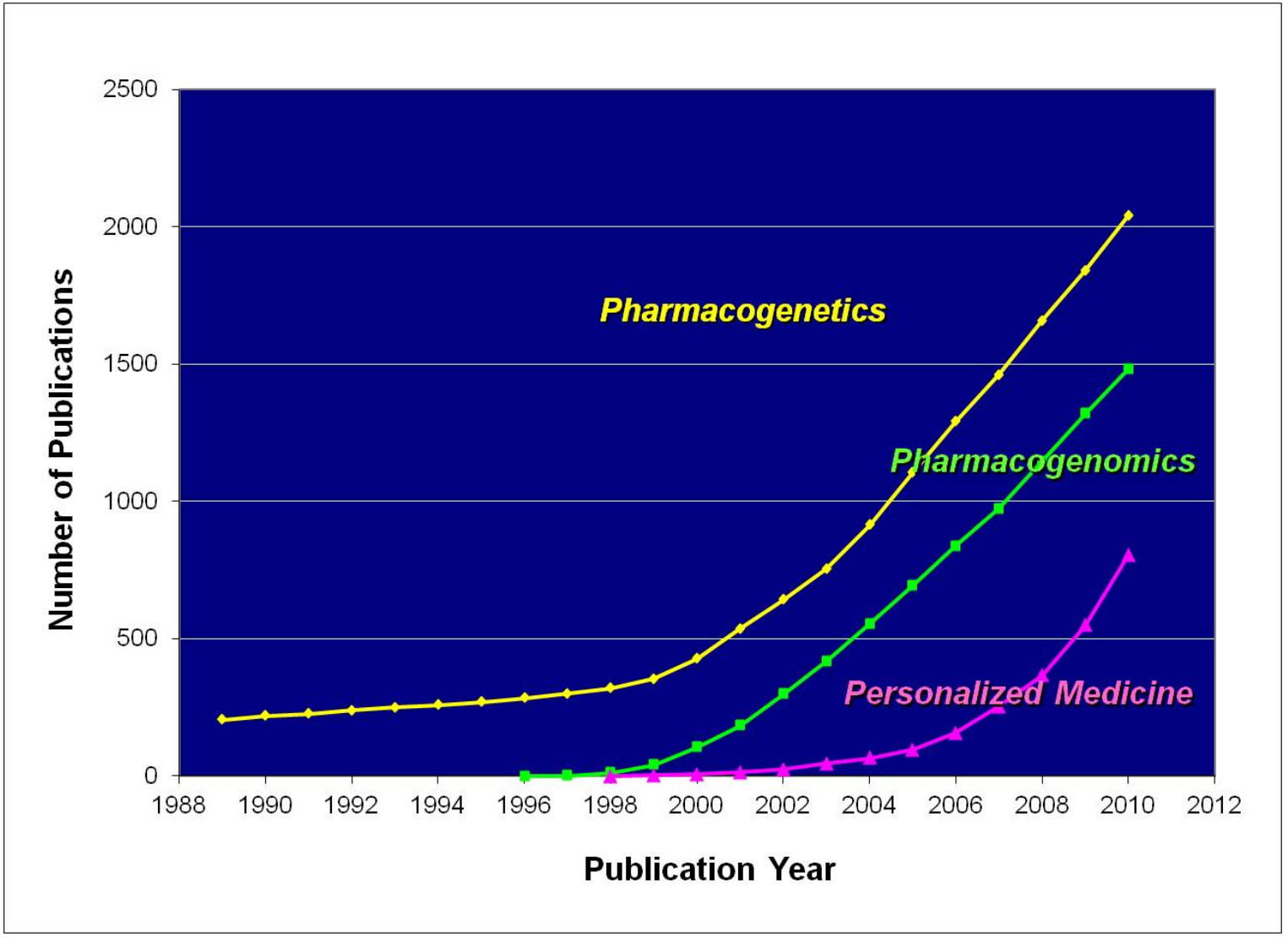
Pharmacogenetics

Clinical implementation: a 7 year experience

Pharmacogenetics Core Laboratory*
Dept. Clinical Chemistry
Erasmus MC Rotterdam

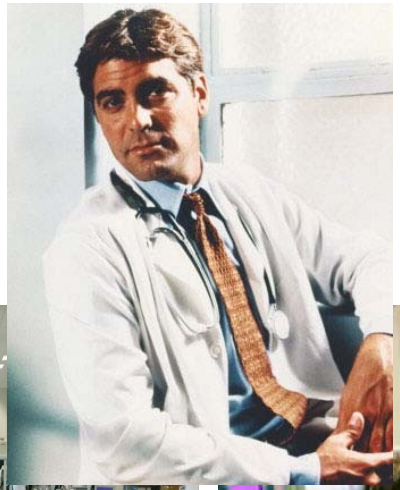
**IFCC Certified Reference Laboratory for Pharmacogenetics*

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH



(www.pganddrugresponse.blogspot.nl)

Bringing pharmacogenetics to the clinic



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"Here is my DNA sequence…"

(The New Yorker, 2000)

Hurdles to take…..

Proof of Principle
(Pharmacokinetics)

Proof of Efficacy
(Pharmacodynamics)

Availability of a test

Alternative treatment available?

Cost effectiveness

Uptake in Guidelines
(convincing clinicians)

Year
2012
2011
2010
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1980

TPMT gene and 6-MP or Azathioprine therapy

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UK questionare to 328 prescribers of AZA, 287 (70%) responded

otype

a

*1

*2

1

*3A

*3C

TPMT enzyme testing: 67% (91% prior to therapy)

dermatologists 94%
gastroenterologists 60%
rheumatologists 47%

DNA testing: 5%
(not routinely available(?)):

dermatologists 2%
gastroenterologists 2%
rheumatologists 1%

(Fargher EA et al 2007 J Clin Pharmacy and Ther 32: 187-195)

TPMT genotyping for 6-MP or AZA

Own experiences:

Succes
(although a bit late)

Dermatology:
always request for TPMT genotyping before start of AZA therapy
→ taken up in guidelines!

Gastro-Intestinal department:
request for TPMT genotyping before start of AZA therapy

Acute Lymphatic Leukemia:
Competition with national protocol for measuring enzyme activity

Turning point: awareness that genotype results were available in 3 days and cost only € 79.

www.erasmusmc.nl/farmacogenetica

Reason for genotyping request:

Drug
Dose
Conc.
Co-med.

- ☐ Screening prior to therapy
- ☐ High blood levels
- ☐ Low blood levels
- ☐ No effect
- ☐ Side effects

CONSULT

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email: r.vanschaik@erasmusmc.nl
(+31) 10 7033119

Prof. van Gelder
(internist-klinisch farmacoloog)
email: t.vangelder@erasmusmc.nl
(+31) 10 7033202

☐ Cito Fax

Gene to be tested:

- ☐ CYP1A2
- ☐ CYP2B6
- ☐ CYP2C8
- ☐ CYP2C9
- ☐ CYP2C19
- ☐ CYP2D6 - (33 variants, AmpliChip)
- ☐ CYP2D6 - (14 variants, DNA chip)
- ☐ CYP2E1
- ☐ CYP3A4
- ☐ CYP3A5
- ☐ ABCB1 (MDR-1)
- ☐ DPYD
- ☐ HLA-B*1502
- ☐ HLA-A*3301
- ☐ HLA-B*5701
- ☐ IL-28B
- ☐ SLCO1B1
- ☐ TPMT
- ☐ UGT1A1
- ☐ Pseudocholinesterase (BChE) (4)

☐ Unknown to me: please advice.
☐ Other gene, being:

Consulted with:

Niet invullen! T.b.v. interne registratie AKC:

☐ PORD DNAnr
PIDNR
Monsternr

www.erasmusmc.nl/farmacogenetica

Reason for genotyping request:

Drug

Dose

Conc.

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- ☐ CYP2C19
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- ☐ SLCO1B1
- ☐ TPMT
- ☐ UGT1A1
- ☐ Pseudocholinesterase (BChE) (4)

☐ Unknown to me: please advice.

X Other gene, being: **CYP450**

Consulted with:

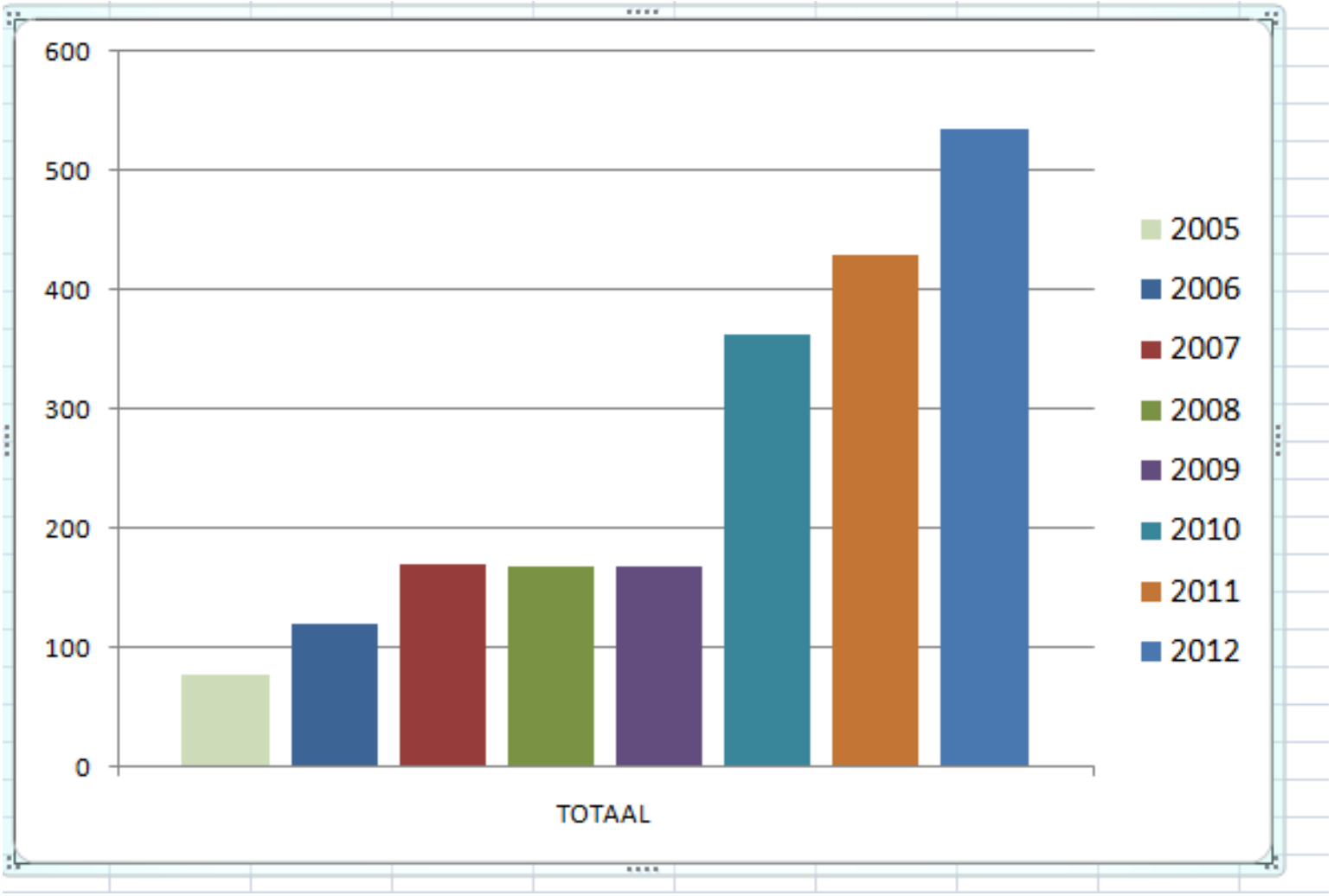
Niet invullen! T.b.v. interne registratie AKC:

☐ PORD DNAnr

PIDNR

Monsternr

PGx testing from 2005 → 2012



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CYP2D6
& antidepressants

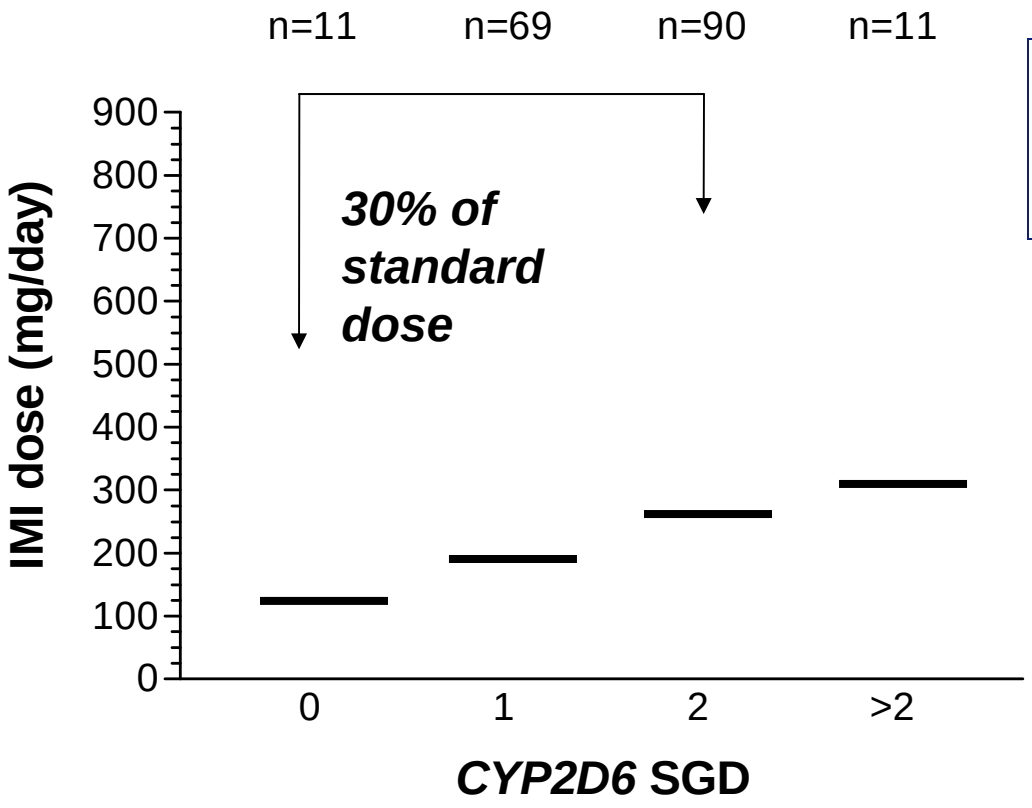
Psychiatry: Imipramine (antidepressive)

(Schenk et al 2008 Mol Psychiatry)

Psychiatry: Imipramine (antidepressive)

Imipramine —CYP2C19→ Desipramine —CYP2D6→ 2OH desipramine

Imipramine doses after reaching steady state



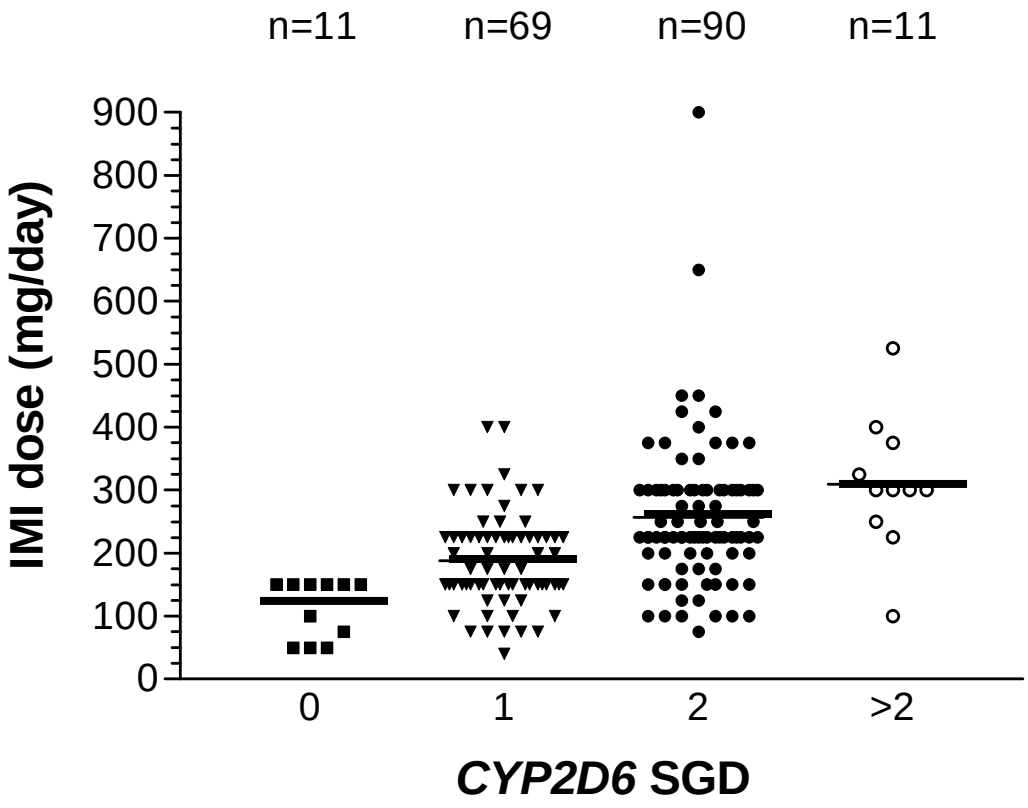
CYP2D6 genotyping:

*3, *4, *5, *6,

(Schenk et al 2008 Mol Psychiatry)

Imipramine (tricyclic antidepressant)

Imipramine doses after reaching steady state



(Schenk et al 2008 Mol Psychiatry)

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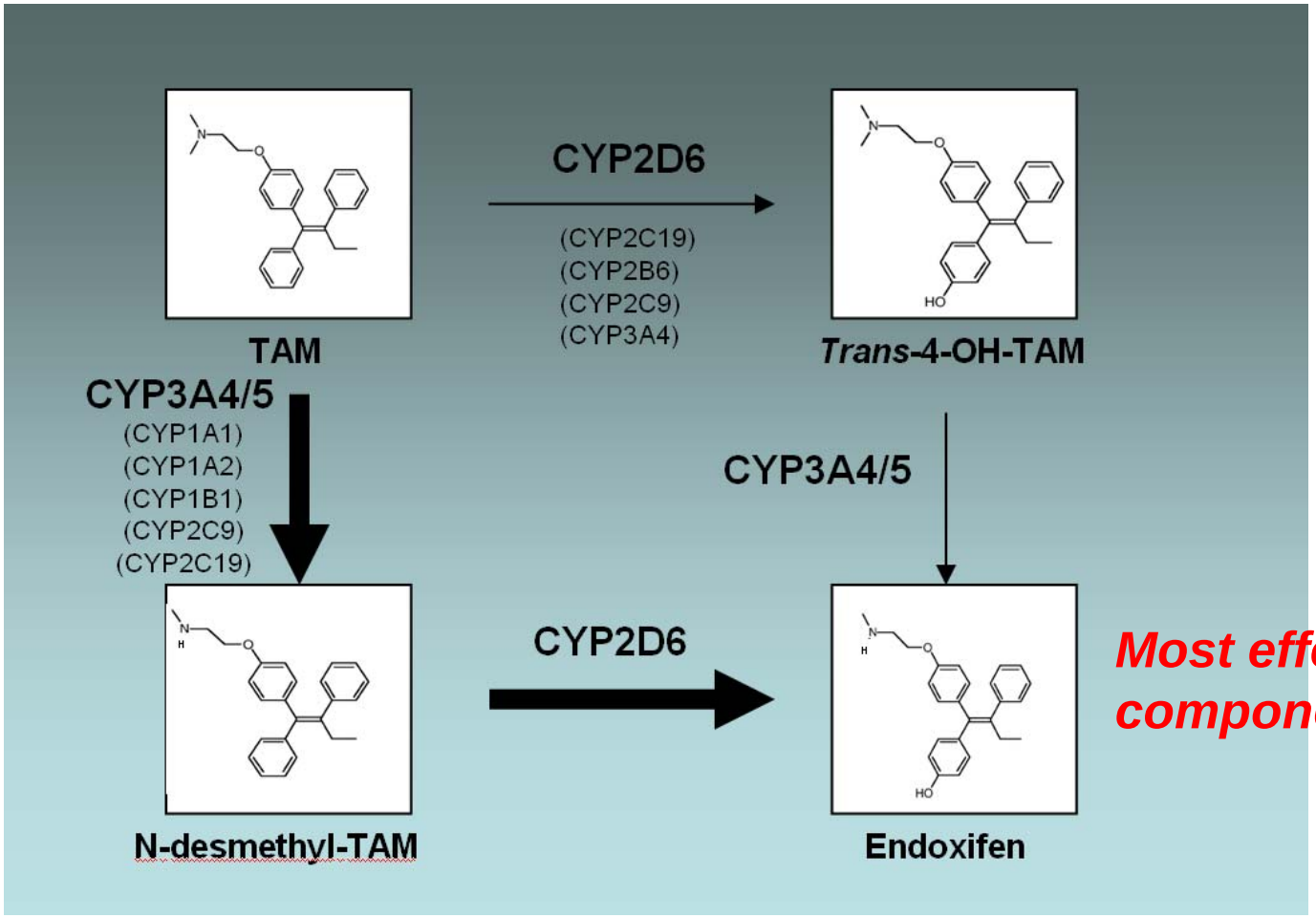
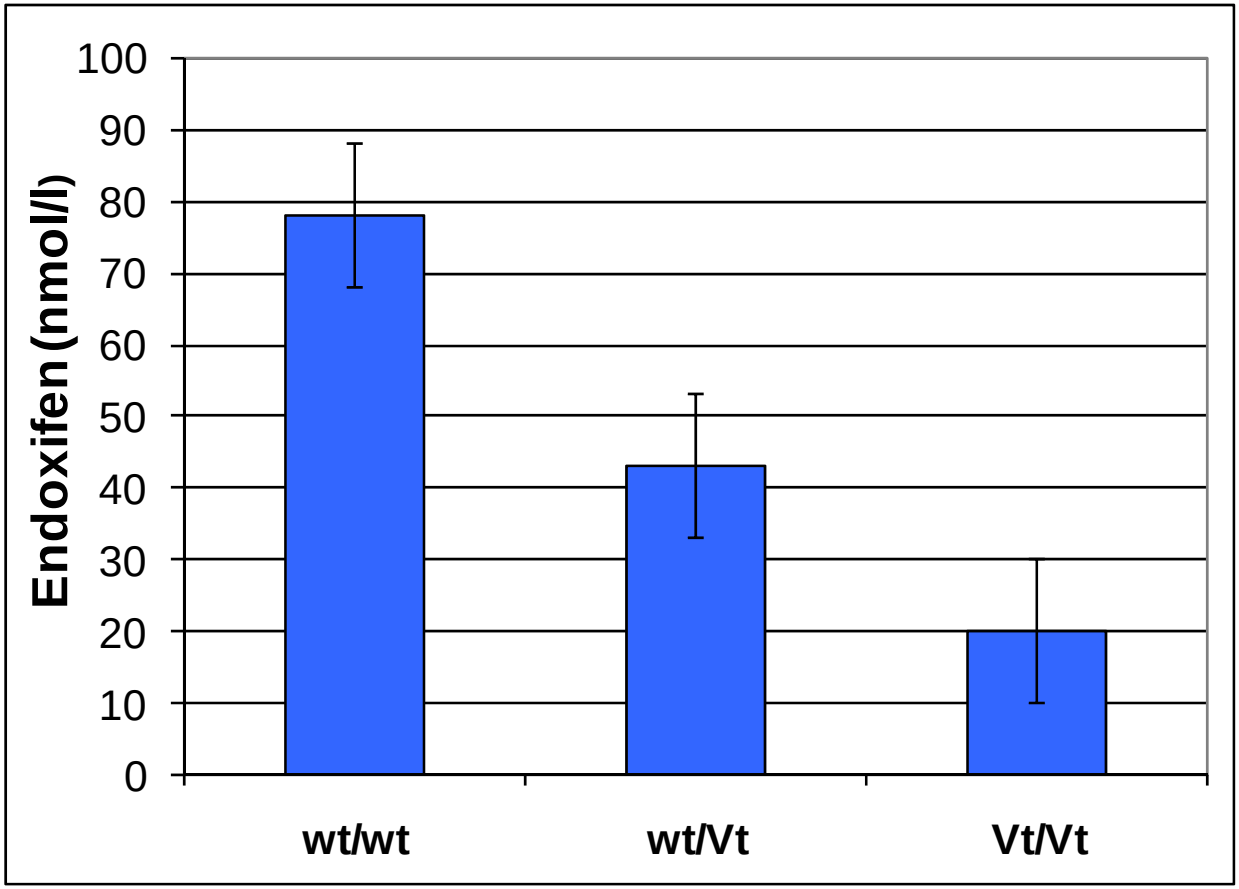
CYP2D6
& Tamoxifen

Tamoxifen metabolism & breast cancer



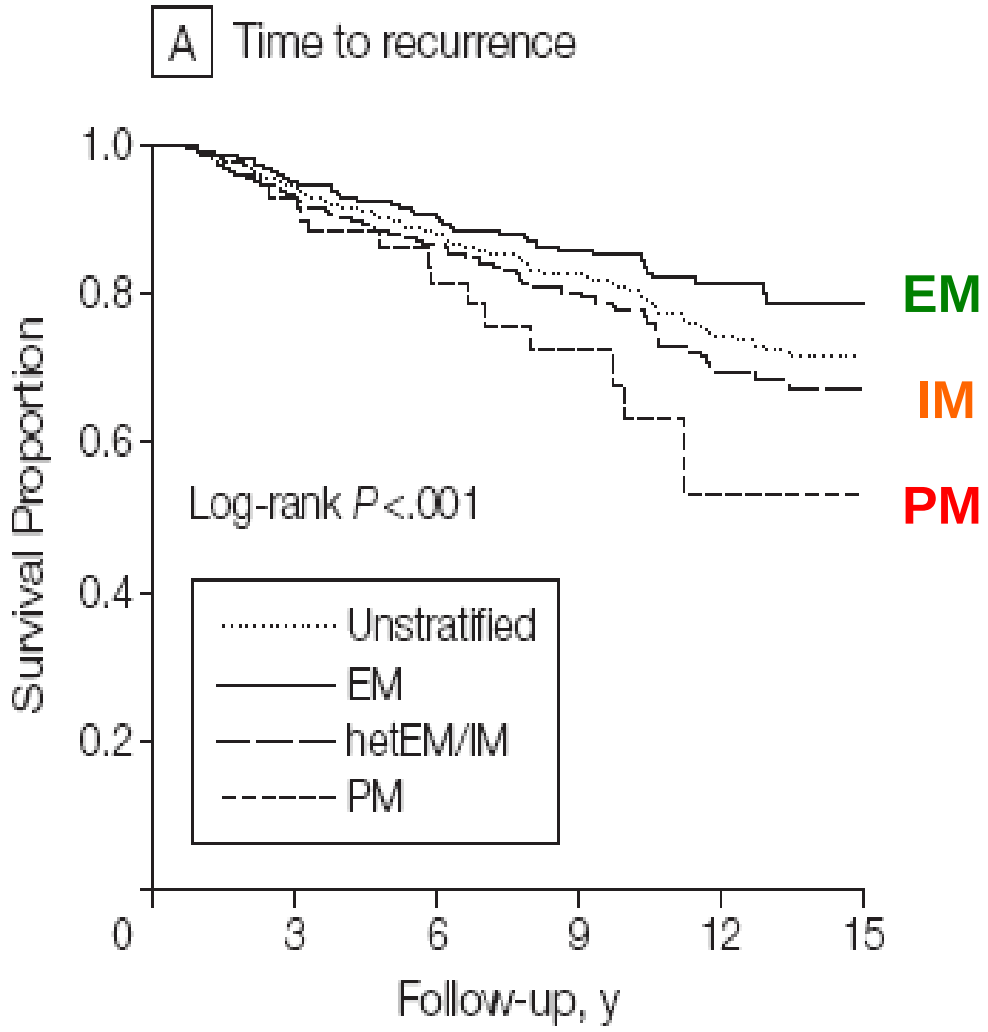
Most effective component

CYP2D6 genotype and endoxifen levels



(Vt=*4 = deficient; Based on Jin et al 2005)

CYP2D6 genotype and adjuvant TAM (n=1,325)



(Schroth et al 2009 JAMA (Oct 7))

Published Articles: contradictory results…..

Study	n	Genotyping	Endpoint	result
Kiyotani et al.Pharmacogen Genom 2010	167	*4, *5, *10, *21, *36, *41	RFS	+
Goetz et al. JCO 2005	190	*4	TTR, RFS	+
Schroth et al. JCO 2007	206	*4, *5, *10, *41	TTR, RFS	+
Lim et al. JCO 2007	21	*10	TTP	+
Ramon y Cajal et al. Breast Cancer Res Treat 2010	91	*4, *5, *41	DFS	+
Bijl et al. Breast Cancer Res Treat 2009	85	*4	OS	+
Schroth et al. JAMA 2009	**1325**	***3, *4, *5, *6, *10, *41**	**DFS**	+
Kiyotani et al. JCO 2010	282	*4, *5, *10, *10-*10, *14, *21, *36, *41	RFS	+
Lammers et al. Br J Cancer 2010	102	*3, *4, *5, *6, *10, *41	OS, TTP	+
Xu et al. Ann Oncol 2008	152	*10	DFS	+
Newman et al. Clin Cancer Res 2008	115	*3, *4, *5, *41	OS	+
Stingl et al. Curr Med Res Opin 2010	496	*4	TTP, PFS	-
Leyland-Jones et al. San Antonio 2010 (abstract)	**1243**	***4**	**DFS**	-
Rae et al. San Antonio 2010 (abstract)	**588**	***3, *4, *6, *10, *41**	**RR**	-
Okishiro et al. Cancer 2009	173	*3, *10	RFS	-
Toyama et al. JCO 2009	154	*10	OS	-
Dezentje et al. JCO 2010	747	?	DFS	-
Nowell et al. Breast Cancer Res Treat 2005	162	*3, *4, *6	PFS	-
Wegman et al. Breast Cancer Res 2005	76	*4	RR	invers
Wegman et al. Breast Cancer Res 2007	677	*4	DFS	invers

Slide courtesy of M Schwab

Current controversy…..

"Laboratory/Clin Pharmacology view"

1. CYP2D6 theoretically involved
2. Genotype proved to affect metabolism
3. Genotype proved to affect outcome

Ready for clinical implementation

"Oncology view"

1. Not all studes confirm the effect of CYP2D6 on outcome
2. There has been no randomized controlled trial available

Not ready for clinical implementation

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CYP2C19
& Clopidogrel

Clopidogrel: needs activation by CYP2C19 (3% PMs, 26% IMs)

N ENGL J MED 360;4 NEJM.ORG JANUARY 22, 2009

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Genetic Determinants of Response to Clopidogrel and Cardiovascular Events

Tabassome Simon, M.D., Ph.D., Céline Verstuyft, Pharm.D., Ph.D., Murielle Mary-Krause, Ph.D., Lina Quteineh, M.D., Elodie Drouet, M.Sc., Nicolas Méneveau, M.D., P. Gabriel Steg, M.D., Ph.D., Jean Ferrières, M.D., Nicolas Danchin, M.D., Ph.D., and Laurent Becquemont, M.D., Ph.D., for the French Registry of Acute ST-Elevation and Non–ST-Elevation Myocardial Infarction (FAST-MI) Investigators

CONCLUSIONS

Among patients with an acute myocardial infarction who were receiving clopidogrel, those carrying *CYP2C19* loss-of-function alleles had a higher rate of subsequent cardiovascular events than those who were not. This effect was particularly marked among the patients undergoing percutaneous coronary intervention. (ClinicalTrials.gov number, NCT00673036.)

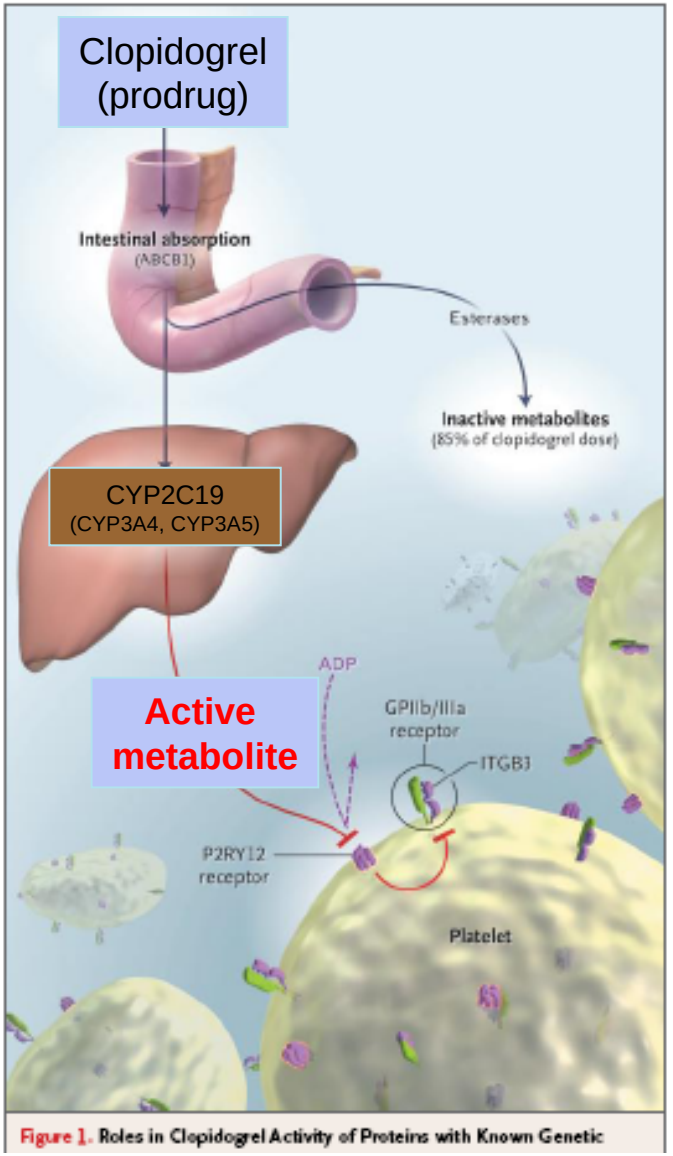


Figure 1. Roles in Clopidogrel Activity of Proteins with Known Genetic

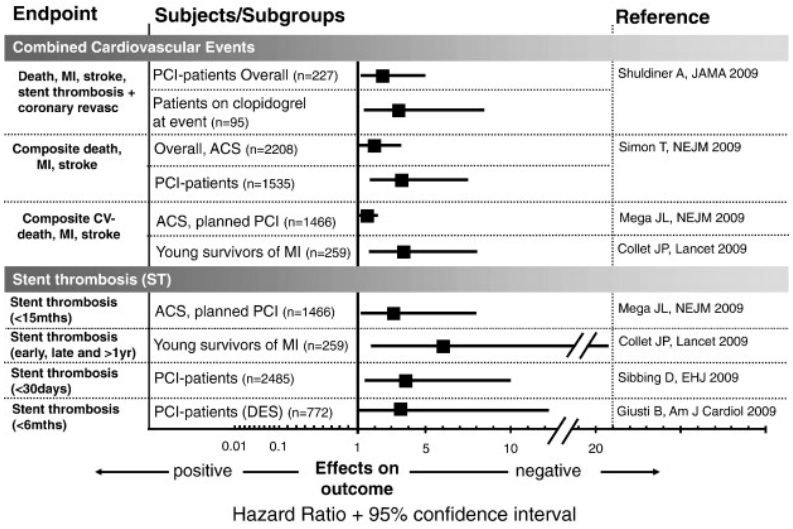
Clopidogrel: needs activation by CYP2C19 (3% PMs, 26% IMs)

Test for CYP2C19 variants:

Negative → clopidogrel
Positive → prasugrel

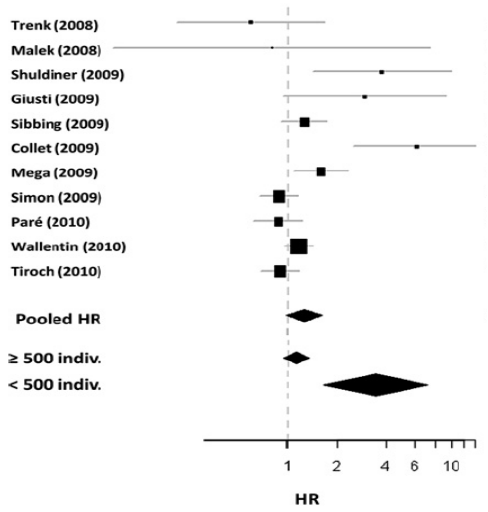
Meta-analysis
Geisler et al 2011 Pharmacol & Ther:

CY2C19*2 carriers are at risk



Meta-analysis
Zabalza et al 2012 BMJ:

Large studies fail to confirm risk



Antonius Hospital Nieuwegein

Erasmus MC

Hurdles to take…..

Proof of Principle
(Pharmacokinetics?)

Proof of Efficacy
(Do patients benefit?)

Availability of a test

Alternative treatment available?

Cost effectiveness

Uptake in Guidelines
(convincing clinicians)

Cost-effectiveness CYP2C9/VKORC1 testing

Ann Intern Med. 2009 Jan 20;150(2):73-83.

Cost-effectiveness of using pharmacogenetic information in warfarin dosing for patients with nonvalvular atrial fibrillation.

Eckman MH, Rosand J, Greenberg SM, Gage BF.

University of Cincinnati Medical Center, Cincinnati, OH 45267-0535, USA. mark.eckman@uc.edu

DESIGN: Markov state transition decision model.

DATA SOURCES: MEDLINE searches and bibliographies from relevant articles of literature published in English.

TARGET POPULATION: Outpatients or inpatients requiring initiation of warfarin therapy. The base case was a man age 69 years with newly diagnosed nonvalvular atrial fibrillation and no contraindications to warfarin therapy.

TIME HORIZON: Lifetime.

PERSPECTIVE: Societal.

INTERVENTION: Genotype-guided dosing consisting of genotyping for CYP2C9*2, CYP2C9*3, and/or VKORC1 versus standard warfarin induction.

OUTCOME MEASURES: Effectiveness was measured in quality-adjusted life dollars.

RESULTS: In the base case, genotype-guided dosing resulted in better outcomes, but at a relatively high cost. Overall, the marginal cost-effectiveness of testing exceeded $170 000 per QALY. On the basis of current data and cost of testing (about $400), there is only a 10% chance that genotype-guided dosing is likely to be cost-effective (that is, <$50 000 per QALY). Sensitivity analyses revealed that for genetic testing to cost less than $50 000 per QALY, it would ha at high risk for hemorrhage or meet the following optimistic criteria: prevent greater than 32% of maj available within 24 hours, and cost less than $200.

CONCLUSION: Warfarin-related genotyping is unlikely to be cost-effective for typical patients with nonvalvular atrial fibrillation, but may be cost-effective in patients a

PMID: 19153410 [PubMed - indexed for MEDLINE] Free Article

Cost of testing: $400 (!) (€300)

At Erasmus MC: €160

…cost effective if available within 24 hours and costs < $200 (€160)

…genotyping is unlikely to be cost effective

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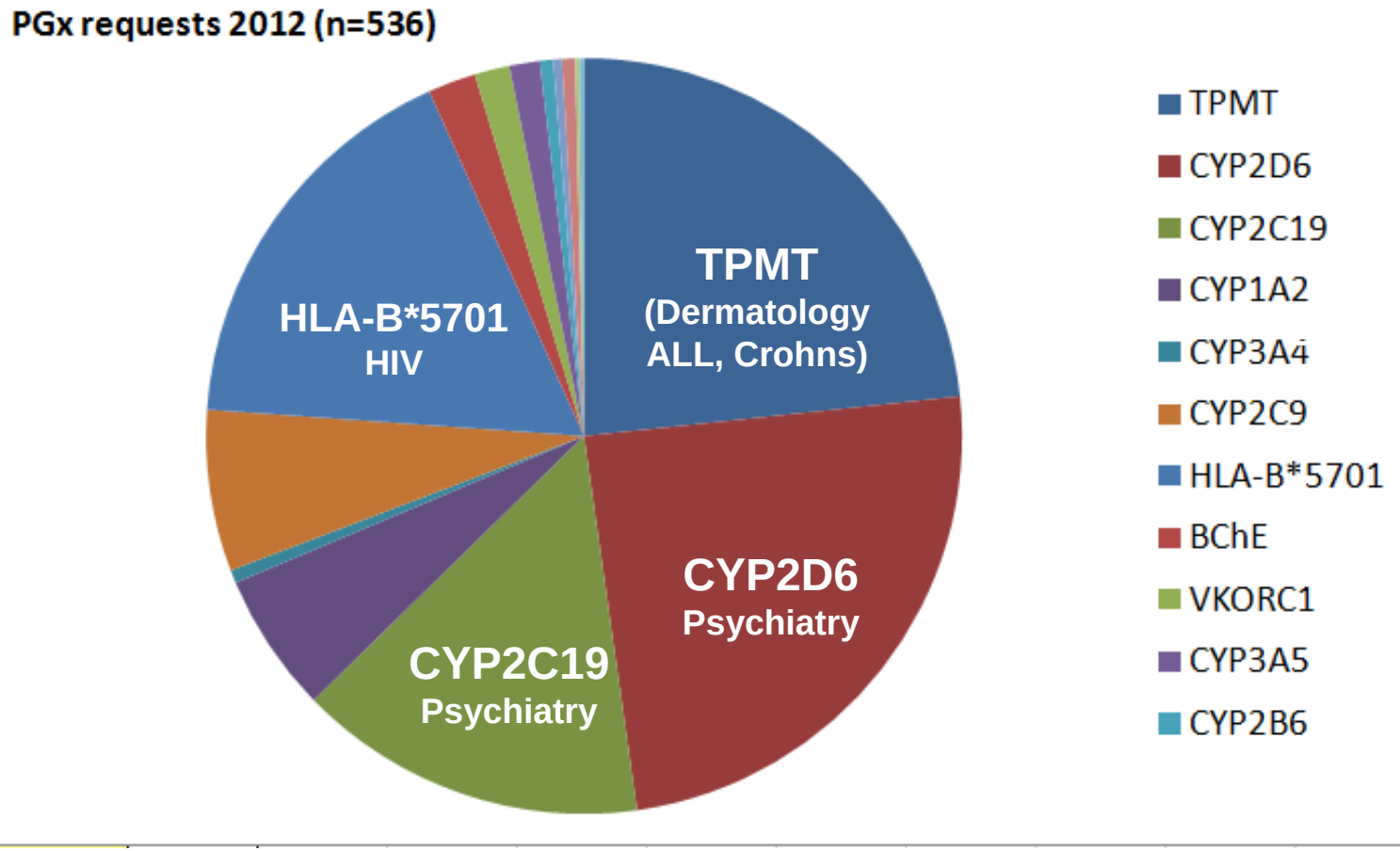
The laboratory

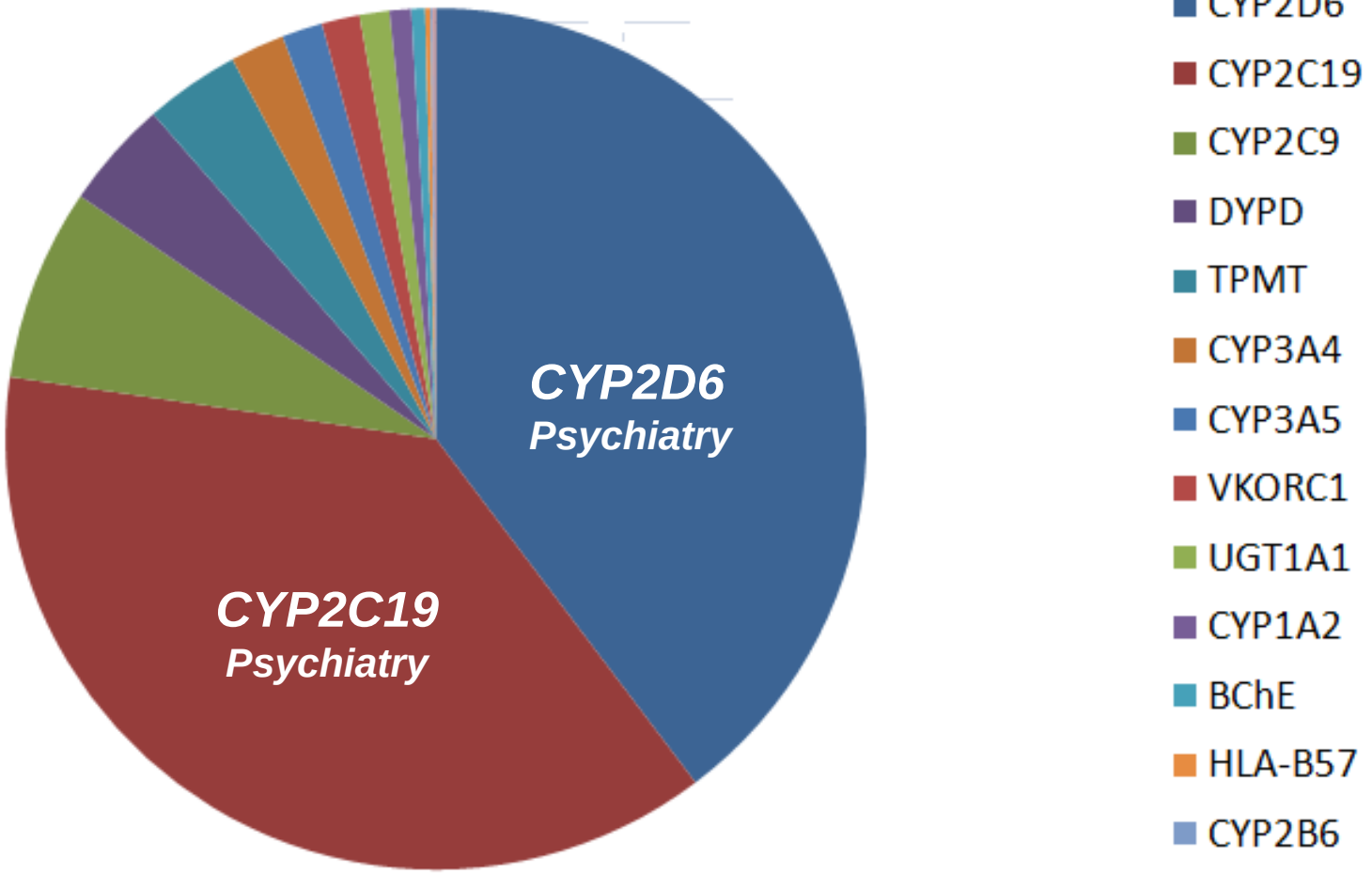
current diagnostics for patient care

PGx testing in 2012



Pharmacogenetic testing in The Netherlands 2011

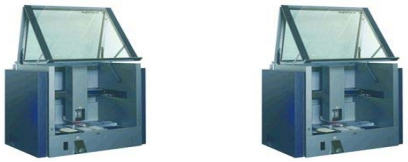
(6 labs: n=4,972)

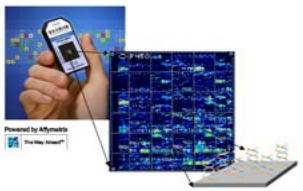


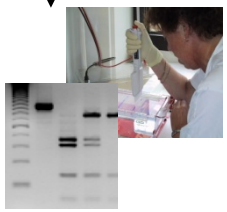
Procedures in PGX diagnostics

Reference
-80 C

DNA
isolation







SNP detection

Result

Report

Quality assurance:
Threeway split of sample

DNA isolation in duplicate

SNP analysis on two different platforms

Quality:
Participation in proficiency schemes

Speed:
Results in 4 dagen (24 hrs possible)

Support:
Specific dosing advice

Observations from 7 year two platform approach

- Rerun of 300 **TaqMan** samples (CYP3A5) by PCR-RFLP revealed 1% discrepancies. Sequencing proved the PCR-RFLP to be right.
- **Amplichip** missed twice a *6 in *1/*6 patients due to SNP under one of the primers. Detected because of discrepancy with TaqMan.
- 2 **PCR-RFLP** samples were called wrongly due to weak bands on gel: detected through comparing with TaqMan result.
- **Luminex** failed in 2/100 cases to give right genotype
- Discrepancy between direct **sequencing** and PCR-RFLP: sequencing was wrong (unequal allele amplification due to SNP close to seq-primer).
- Re-analysis of 6 reported **TMPT*3B** patients in 4 papers showed that none of these were actually TPMT*3B

Reporting

- Request: CYP2D6 genotyping
- Problem: side effects on 150 mg/day imipramine
- Material: EDTA blood
- Tested for: CYP2D6*2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *12, *14, *15, *16, *17, *19, *20, *25, *26, *29, *31, *35, *36, *40, *41 and geneduplication (AmpliChip)

- Result: CYP2D6*4/*4 (2 inactive alleles)
- Interpretation: Poor Metabolizer
- Advice: This genotype would fit with 30% of standard dose of imipramine

- Extra info on SNPs tested, duplicate analysis, techniques used, frequencies of predicted phenotype, limitations of the test
- Signed by Clinical Chemist and by Hospital Pharmacist

The future of pharmacogenetics….(?!?)

"Here is my sequence…"

(The New Yorker, 2000)

Royal Dutch Pharmacy (KNMP) initiative:

literature review by 15 experts

Rating evidence from literature and providing dose recommendations based on genotype

Chair:
Dr. Vera Deneer

www.kennisbank.knmp.nl

PharmGKB
Pharmacogenomics. Knowledge. Implementation.

www.pharmgkb.com

DRUG/SMALL MOLECULE:
imipramine

Dutch Pharmacogenetics Working Group Guideline - imipramine, CYP2D6

The Royal Dutch Pharmacists Association - Pharmacogenetics Working Group has evaluated therapeutic dose recommendations for imipramine based on CYP2D6 genotype (PMID:21412232). They recommend reducing the dose for poor and intermediate metabolizer patients, and selecting an alternative drug for ultra metabolizers.

Phenotype (Genotype)	Therapeutic Dose Recommendation	Level of Evidence	Clinical Relevance
PM (2 inactive alleles)	Reduce dose by 70% and monitor imipramine and desipramine plasma concentrations.	Published controlled studies of good quality* relating to phenotyped and/or genotyped patients or healthy volunteers, and having relevant pharmacokinetic or clinical endpoints.	Clinical effect (S): long-standing discomfort (48-168 hr) without permanent injury e.g. failure of therapy with tricyclic antidepressants, atypical antipsychotic drugs; extrapyramidal side effects; parkinsonism; ADE resulting from increased bioavailability of tricyclic antidepressants, metoprolol, propafenone (central effects e.g. dizziness); INR 4.5-6.0; neutropenia 1.0-1.5x10^9/l; leucopenia 2.0-3.0x10^9/l; thrombocytopenia 50-75x10^9/l
IM (2 decreased activity alleles, or 1	Reduce dose by 30% and monitor imipramine and	Published controlled studies of good quality* relating to	Minor clinical effect (S): QTc prolongation (<450 ms men, <470 ms women); INR increase < 4.5. Kinetic

Conclusions: hurdles in clinical practice

- Unawareness
- Competition with common practice
- Test accessibility
- Costs
- Turn-around-time
- Translation genotype to phenotype
- Difference in screening prior to therapy or diagnostic testing
- Convincing clinicians → uptake in guidelines (catch 22?)

Conclusions: hurdles in clinical practice (accompanying diagnostics)

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- Competition with common practice
- Test accessibility
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