Challenges in the design and conduct of clinical pharmacogenomic studies

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NCI Cancer Pharmacogenomics Workshop

Bethesda, MD

July 21, 2009

Goals of clinical pharmacogenomic studies

- Evaluation of association of candidate polymorphism(s) with phenotype(s)
- Discovery of novel polymorphisms associated with phenotype(s)
- Replication of positive findings from another clinical study

Considerations in study design

- Phenotyping
  - Prospective vs. retrospective
- Treatment
  - Controlled vs. observational
- Sample size
  - Planned vs. convenience
- DNA source
  - Blood vs. saliva/buccal vs. tissue samples
- Gene selection
  - Candidates vs. pathway vs. genome-wide
- Variant selection
  - Functional vs. tag SNPs
- Control of type I error
  - Exploratory vs. corrected

Considerations in study design - phenotyping

- Continuous vs. discrete endpoints
  - ANC nadir vs. grade 4 neutropenia (Y/N)
- Incorporation of endophenotypes (e.g., plasma concentrations or pharmacodynamic biomarkers)
- Minimization of phenotyping error

Iyer, Pharmacogenomics J, 2002 (n = 20 pts at 300 mg/m^2)

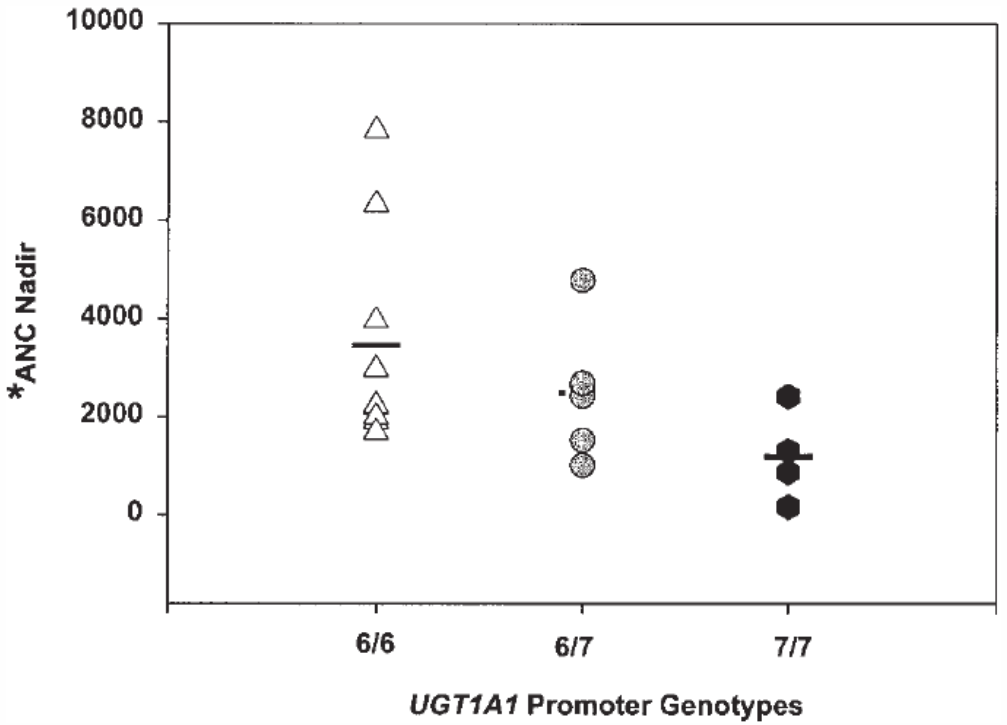
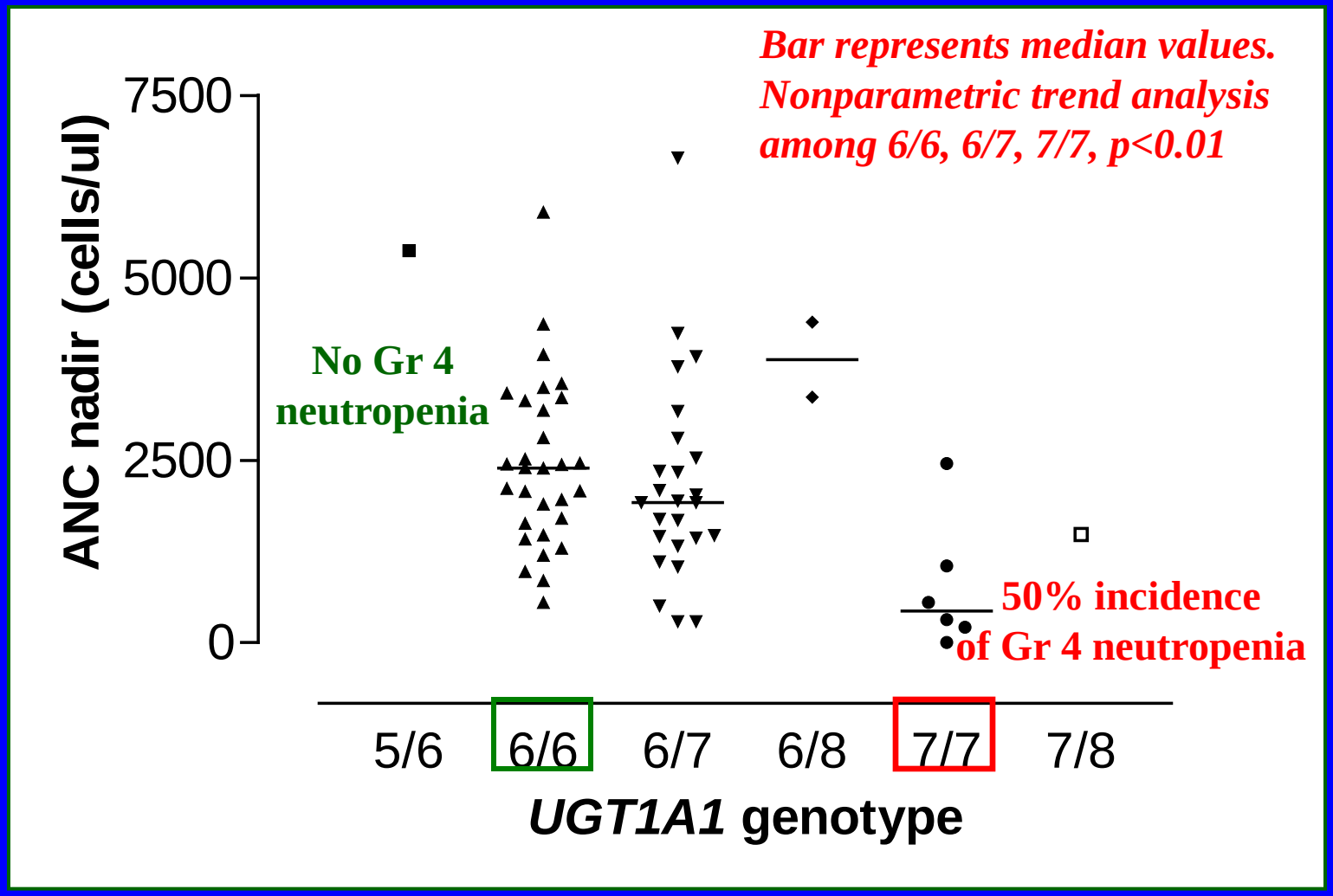


Figure 2 Correlation between absolute neutrophil count (ANC) and *UGT1A1* promoter genotypes in patients after irinotecan treatment. *Significant trend in ANC nadirs (6/6>6/6>7/7, $z = -2.05$, $P = 0.04$, non-parametric trend analysis).

Neutropenia (q3 wk schedule) is Correlated with *UGT1A1* Genotype (*28)



Innocenti F, et al. *J Clin Oncol.* 2004; 22(8):1382-8. Reprinted with permission.

Irinotecan pharmacokinetics and TA indel genotype

	Irinotecan AUC (μg*h/ml)	SN-38 AUC (ng*h/ml)	SN-38G AUC (μg*h/ml)	Glucuronidation ratio
6/6 (n=30)	24.4±7.8	336±168	2.0±1.4	6.5±4.0
6/7 (n=25)	26.1±10.8	458±380	1.9±1.7	5.6±4.8
7/7 (n=6)	25.4±6.7	542±195	1.8±1.3	3.6±2.8

Nonparametric trend analysis, p=0.03

Innocenti F, et al: *J Clin Oncol.* 2004;22(8):1382-8.

Repeated measures of intraocular pressure result in a higher heritability and greater power in genetic linkage studies

Francis Carbonaro MD MRCOphth.[1], Toby Andrew PhD[1], David A Mackey MD FRANZCO[2], Terri L Young[3], Tim D Spector MD FRCP [1], Christopher J Hammond MD MRCP FRCOphth.[1,4]

- **Using 4 measures versus 1 resulted in**
  - **Increased absolute heritability (10-14%)**
  - **Decreased required sample size (29-48%)**

Carbonaro F, et al: *Invest Ophthalmol Vis Sci.* 2009; 50(11):5115-9.

Power for Association With Error

Phenotype Edition

Specify the analysis you would like to perform — Help

- (•) Power calculations for a fixed sample size
- ( ) Sample size calculations for a fixed power

Specify how to generate the genotype frequencies — Help

- (•) Genetic model free method
- ( ) Genetic model based method

Specify Pr(observed control | true affected) = 1 - sensitivity — Help

[ ]

Specify Pr(observed case | true unaffected) = 1 - specificity — Help

[ ]

Specify the Significance Level of the test between 0 and 1 — Help

[ ]

Specify the disease prevalence between 0 and 1 — Help

[ ]

Reset | Next Page

Laboratory of Statistical Genetics
The Rockefeller University

http://linkage.rockefeller.edu/pawe/paweph.htm

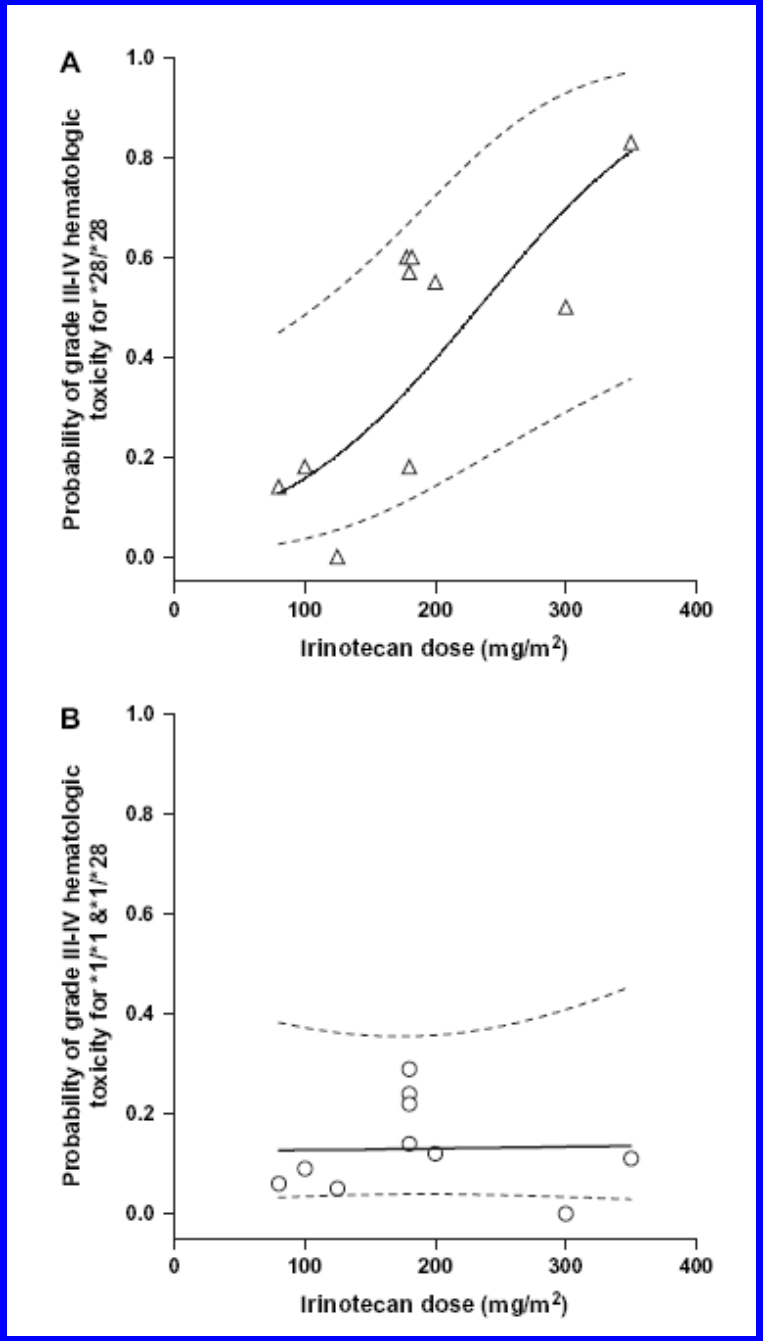
Considerations in study design - treatment

- Prospective studies minimize confounding
  - treatment is assigned in an unbiased manner
    - Not confounded by risk factors apparent to experienced clinicians
    - Dose is controlled
- Observational approaches are best utilized in the context of replication of positive findings from prospective studies

COMMENTARY

UGT1A1*28 Genotype and Irinotecan-Induced Neutropenia: Dose Matters

Janelle M. Hoskins, Richard M. Goldberg, Pingping Qu, Joseph G. Ibrahim, Howard L. McLeod

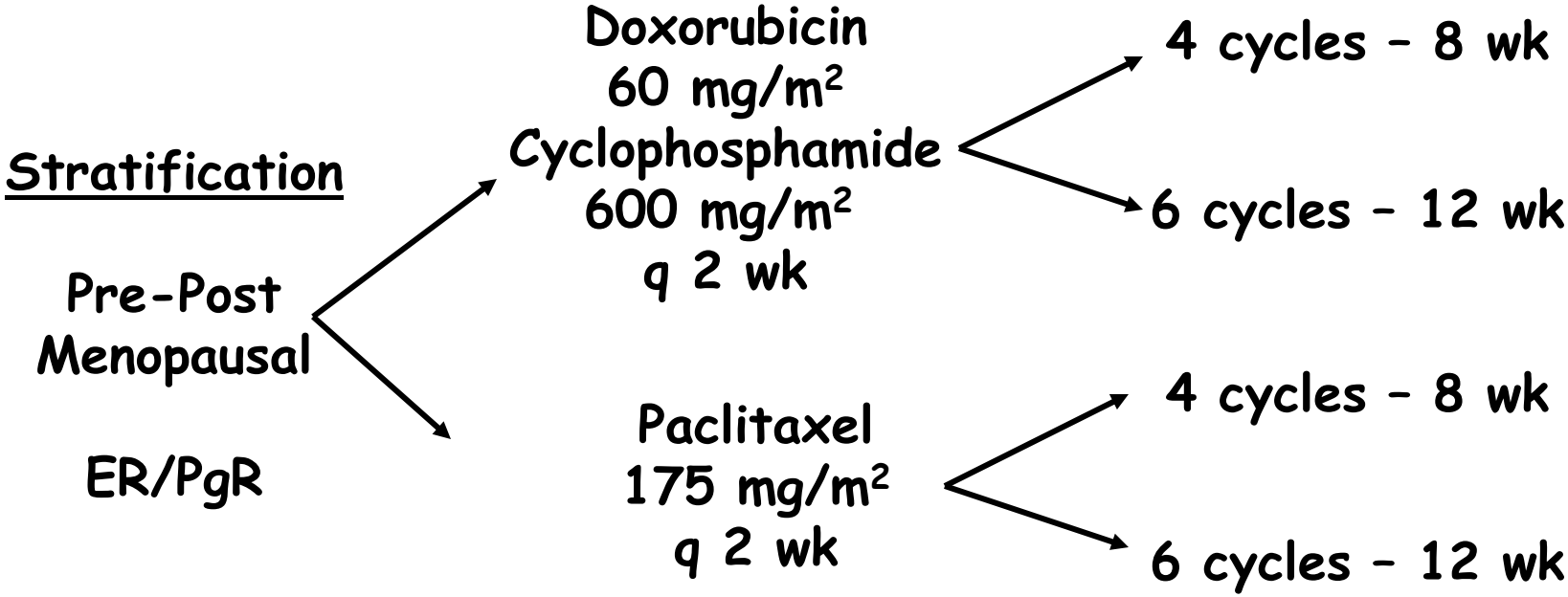


Hoskins et al, *Journal of the National Cancer Institute*, 2007; 99(17):1290-5. Reprinted by permission of Oxford University Press.

Considerations in study design – sample size

- Phase III studies will always have sufficient sample size to test pharmacogenomic hypotheses of clinical relevance
- Discovery studies incorporating genome-wide typing may be fruitful even with relatively small sample sets (e.g., 300 patients)
- For replication studies, the effect size is likely to be smaller than in the discovery study

CALGB 40101 – 2 X 2 Factorial Design Adjuvant Therapy for Women with Breast Cancer with 0-3 Positive Nodes



AC = doxorubicin/cyclophosphamide

Target Accrual 4,646 pts

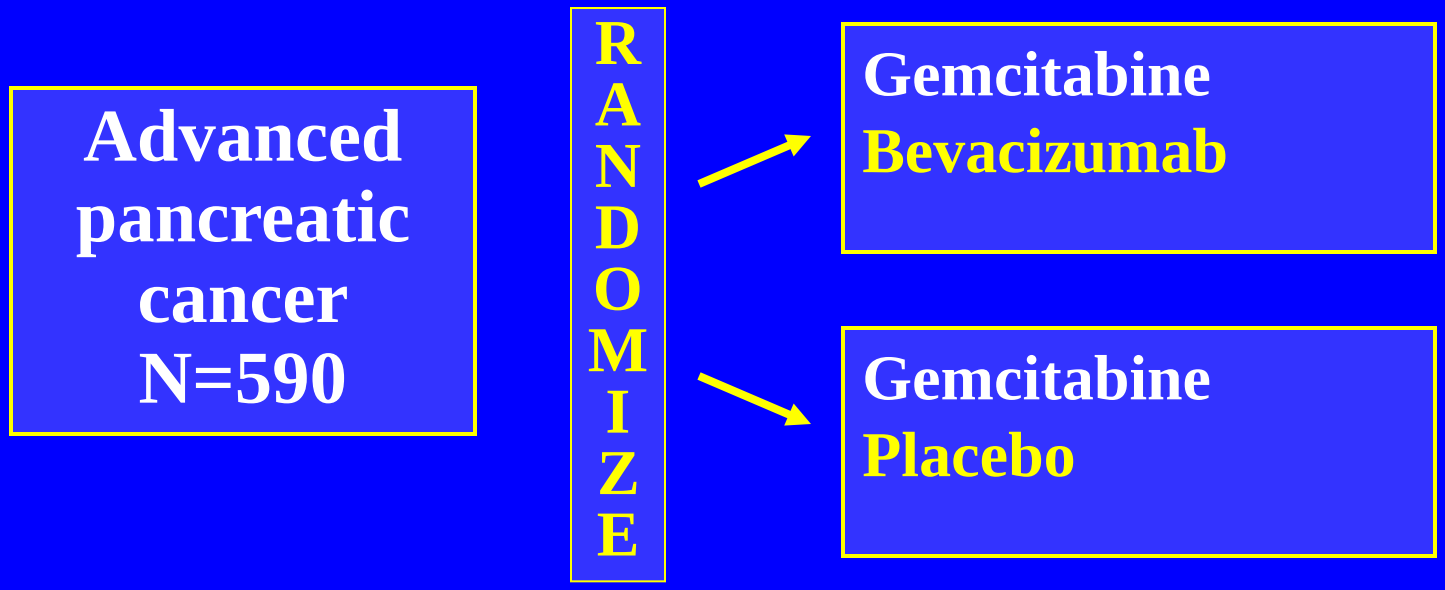
Pharmacogenetic analysis of predictors of paclitaxel and doxorubicin/cyclophosphamide toxicity

CALGB 40101 Accrual as of 9/9/08

	Parent Protocol*	Pharmacogenetics Protocol	Pharmacogenetics w/sample
AC x4	730 (140)	619 (85%)	513 (83%)
AC x6	645 (145)	533 (83%)	459 (86%)
Paclitaxel x4	720 (141)	598 (83%)	507 (85%)
Paclitaxel x6	648 (141)	549 (85%)	474 (86%)
Total	2743 (577)	2299 (84%)	1953 (85%)

*Number of patients enrolled prior to opening the pharmacogenetics study is in parentheses

CALGB 80303 Trial design



Stratification Factors:

- **Performance status: 0/1 vs. 2**
- **Extent of disease: metastatic vs. locally advanced**
- **Prior radiation: yes/no**

Kindler et al. *2007 ASCO Annual Meeting Proceedings* (post-meeting edition) . 2007; 25(18S):4508. Reprinted by permission of author.

GWAS in pancreatic cancer patients treated with chemotherapy

CALGB #80303

Federico Innocenti, MD, PhD
University of Chicago

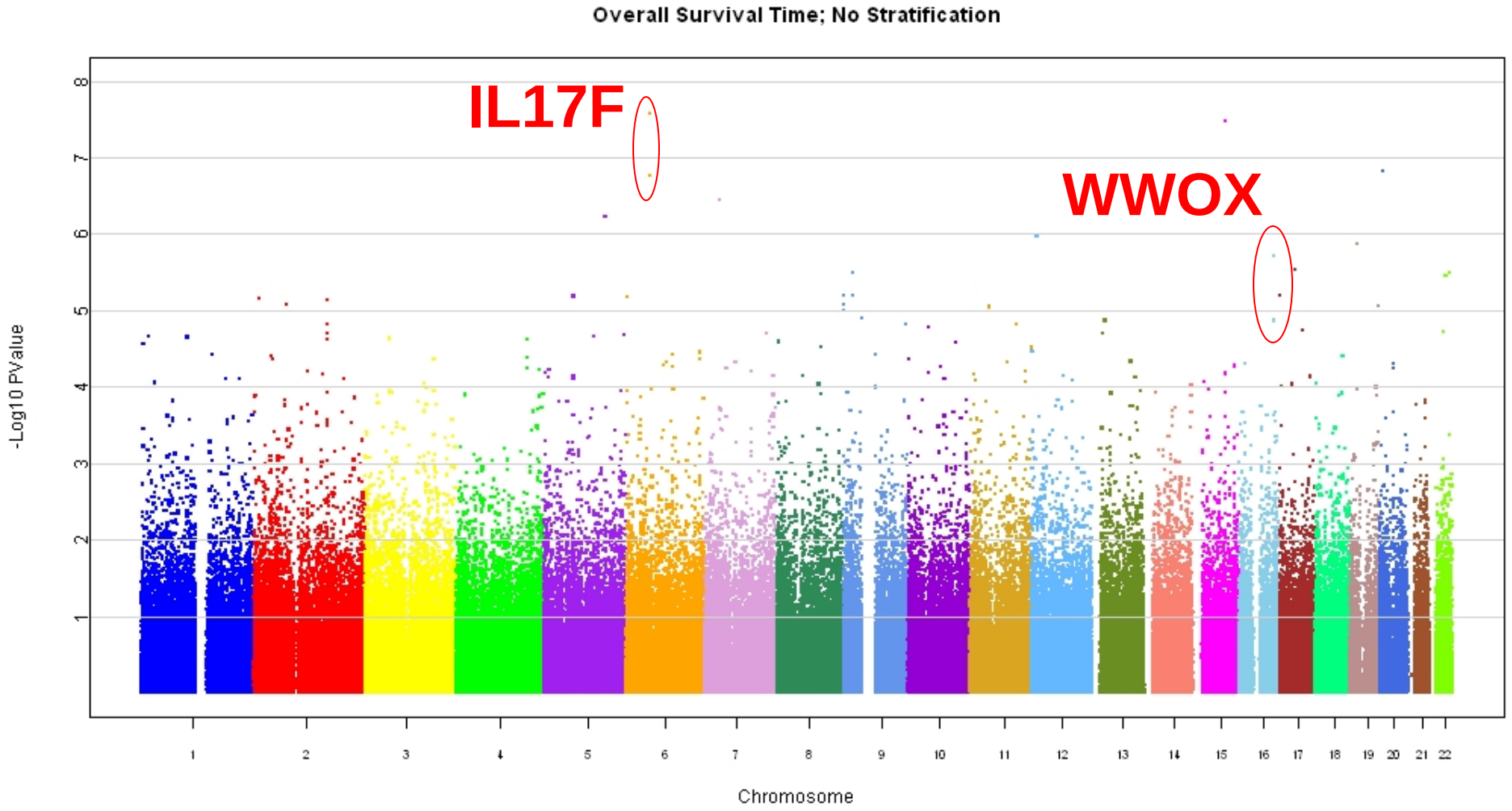
CALGB
Tomorrow's Cancer Treatments Today

RIKEN

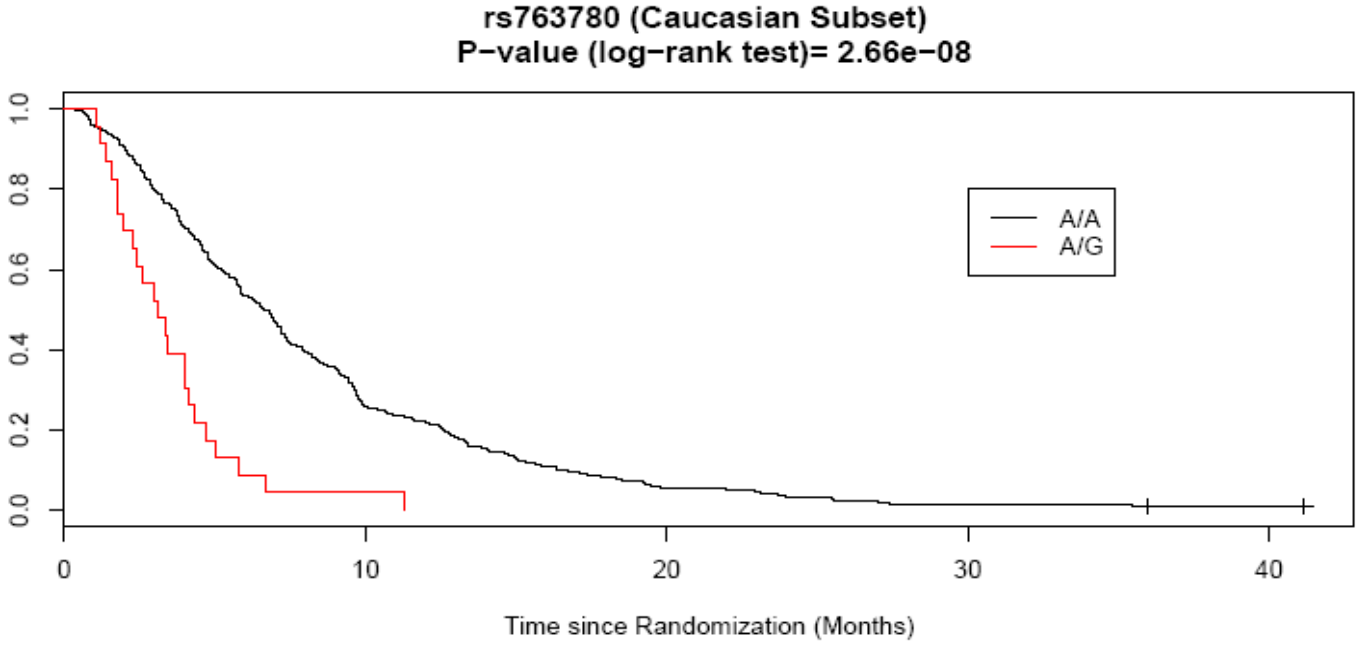
PGRN

PHARMACOGENETICS OF ANTICANCER AGENTS RESEARCH GROUP
PAAR

Overall Survival



IL17F, ch 1, 10^{-8}, q 0.04, HR 3.27 coding nonsynonymous



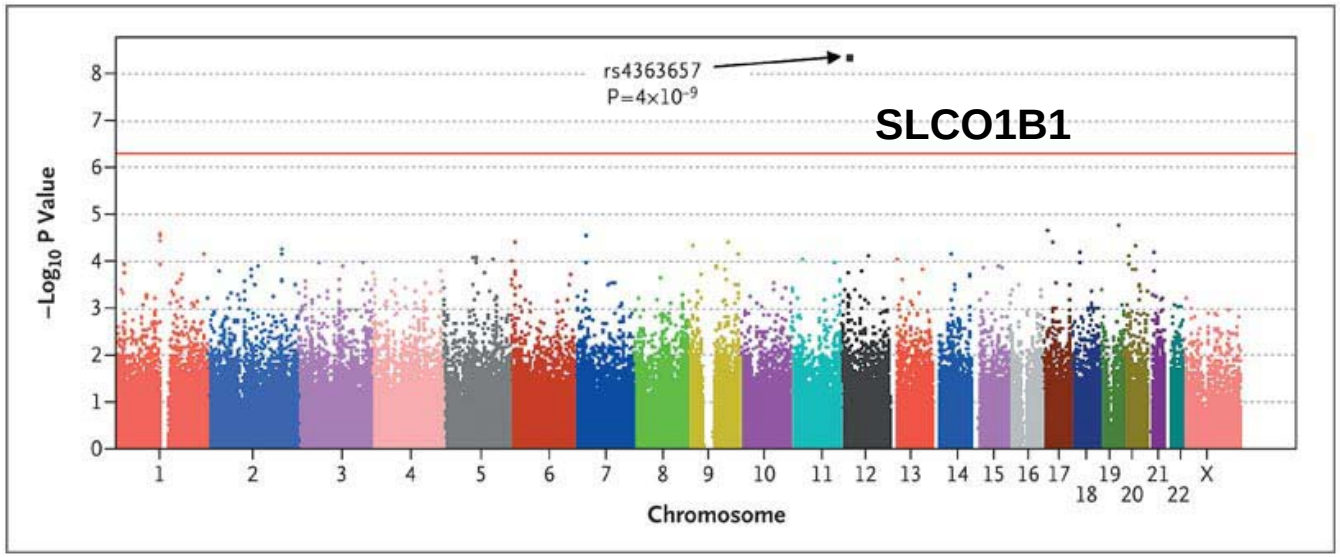

```
Call: survfit(formula = SURV[CAUC] ~ snpi[CAUC])

                      n events median 0.95LCL 0.95UCL
snpi[CAUC]=C/A   22      22   3.24    2.43    4.34
snpi[CAUC]=C/C 272     269   6.64    5.82    7.23
```

The NEW ENGLAND JOURNAL of MEDICINE

SLCO1B1 Variants and Statin-Induced Myopathy — A Genomewide Study

The SEARCH Collaborative Group*



85 cases, 90 controls

The SEARCH Collaborative Group. *New England Journal of Medicine,* 2008; 359(8): 789-799.

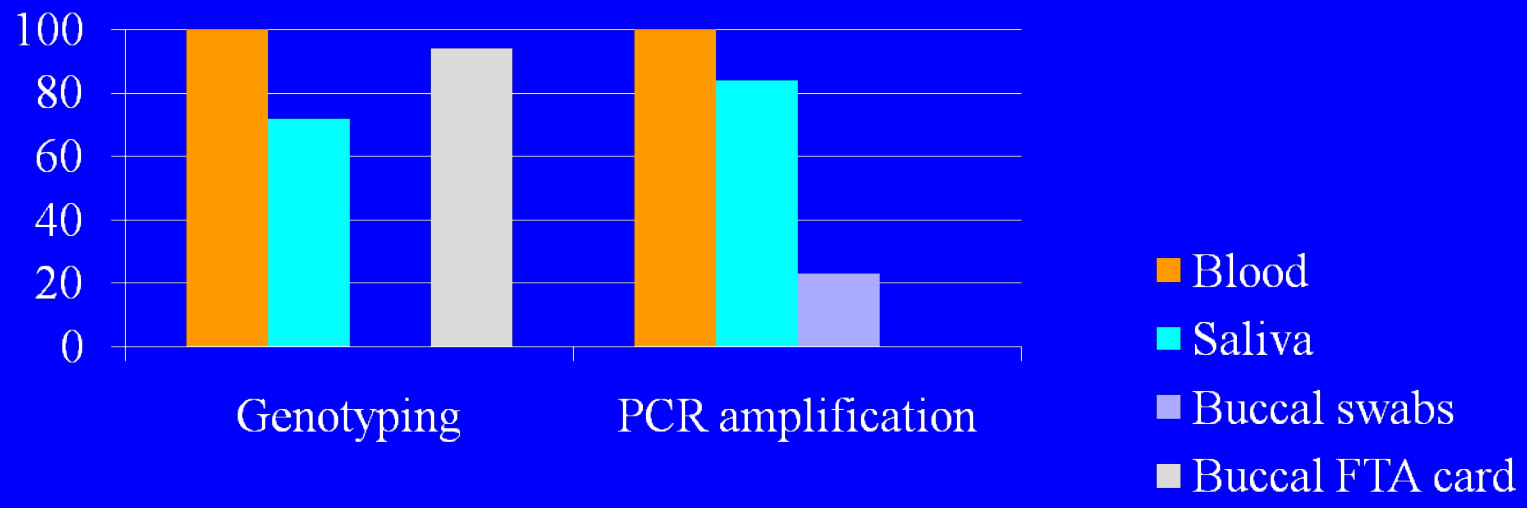
Considerations in study design – DNA source

- Blood samples are preferable to other sources of germline DNA
- Tissue samples can be utilized, but are complicated by the potential for somatic mutations
  - Genome-wide typing is difficult from paraffin-embedded samples

Collection of Blood, Saliva, and Buccal Cell Samples in a Pilot Study on the Danish Nurse Cohort: Comparison of the Response Rate and Quality of Genomic DNA

Thomas v. O. Hansen,[1] Mette K. Simonsen,[2] Finn C. Nielsen,[1] and Yrsa Andersen Hundrup[2,3]

[1]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark; [2]The Danish Nurse Cohort Study, National Institute of Public Health, Copenhagen, Denmark; and [3]The Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark



Hansen et al. *Cancer Epidemiology Biomarkers and Prevention.* 2007; 16(10): 2072-6.

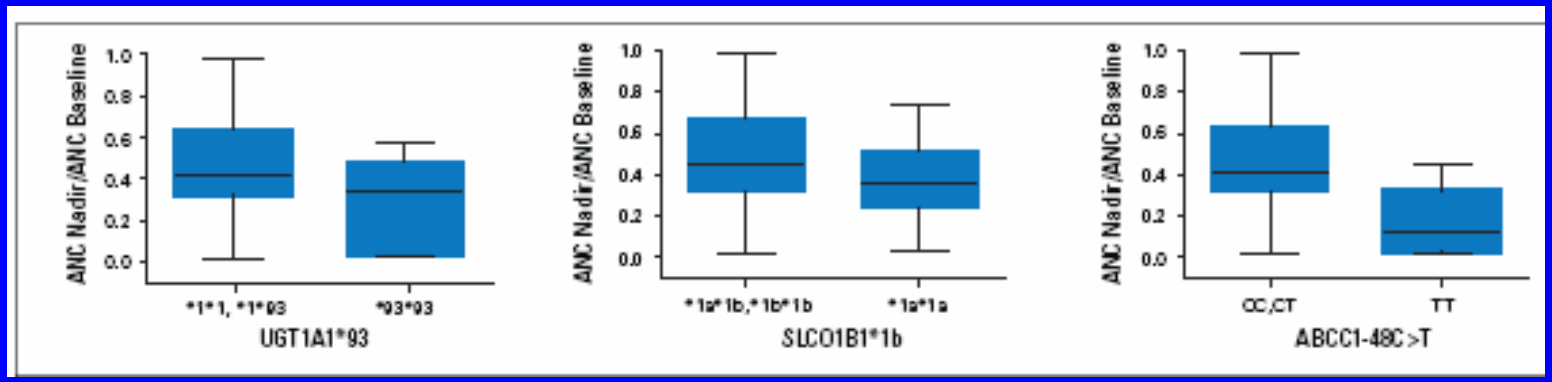
Considerations in study design – gene selection

- Candidate genes should be prioritized
- Endophenotypes increase the value of candidate and pathway gene studies
- Genome-wide discovery studies should always be considered as a secondary objective

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Comprehensive Pharmacogenetic Analysis of Irinotecan Neutropenia and Pharmacokinetics

Federico Innocenti, Deanna L. Kroetz, Erin Schuetz, M. Eileen Dolan, Jacqueline Ramírez, Mary Relling, Peixian Chen, Soma Das, Gary L. Rosner, and Mark J. Ratain

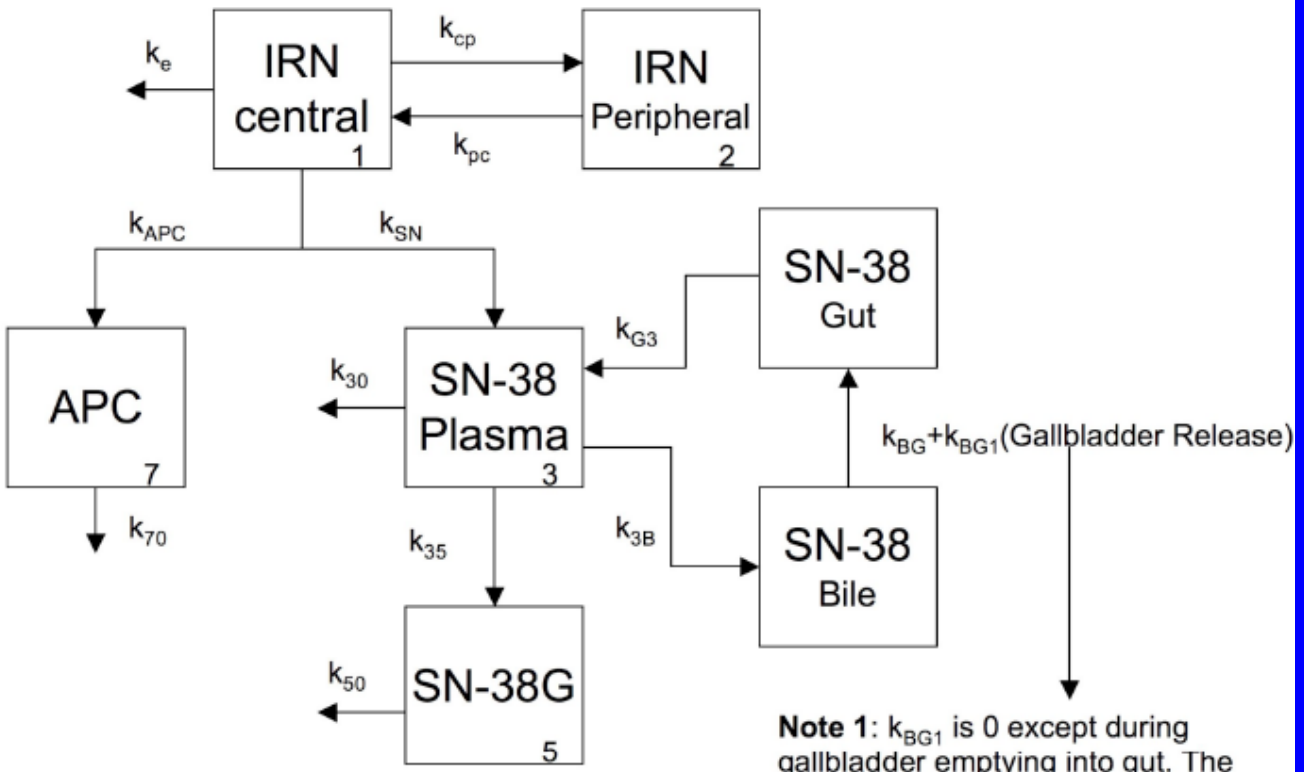


Log ANC nadir, log cells/μL				
ABCC1 IVS11 −48C>T	TT, **CC, CT**	−0.401	0.148	.009
*UGT1A1*93*	*93*93, ***93*****1**, ***1*****1**	−0.373	0.111	.001
*SLCO1B1*1b*	*1a*1a, ***1a*****1b**, ***1b*****1b**	−0.211	0.071	.004

Innocenti F, et al*: J Clin Oncol.* 2009;(16):2604-14. Reprinted with permission.

Rosner et al, CPT, 2008

Figure 1: Pharmacokinetic model with enterohepatic recirculation (EHRT).



Association of ABCC1 SNPs with principal components (p value)

1. IRN to SN-38 & SN-38 recirculation (0.001)
2. IRN compartments (0.004)
3. SN-38 to SN-38G & SN-38G elimination
4. IRN to APC
5. APC elimination
6. Enterohepatic recirculation (EHR)
7. SN-38 recirculation without EHR
8. SN-38 elimination
9. IRN elimination

Considerations in study design – variant selection

- Linkage disequilibrium must always be considered
- Haplotype tag SNPS should be included whenever feasible
- Genome-wide platforms do not have complete coverage of all genes, particularly for individuals of African descent

Considerations in study design – control of type I error

- Even exploratory studies should attempt to correct for multiple testing!
  - Avoid testing of associations that lack plausibility
- Fundamental principles of statistics still apply to clinical pharmacogenomic studies!!
- Sources of false discovery include multiple testing of
  - Genes
  - Phenotypes
    - Continuous, subsets
  - Genetic models
  - Multivariate analyses

A comprehensive review of genetic association studies

- **"most reported associations are not robust"**
- **603 putative gene-disease associations**
  - **166 putative associations studied 3+ times**
    - **6/166 (3.6%) consistently replicated**

Hirschhorn J, et al. *Genetic Medicine*. 2002; 4(2): 45-61.

What is TheraGuide 5-FU™?

TheraGuide 5-FU™ is the most comprehensive clinically available test for assessing the risk of toxicity due to 5-FU–based chemotherapy. It detects variations in 2 genes, dihydropyrimidine dehydrogenase (*DPYD*) and thymidylate synthetase (*TYMS*), that are responsible for a significant portion of serious adverse reactions to 5-FU–based therapy.[1-4] One in 4 individuals carries variations in either of these genes. These variations are associated with up to a 60% risk of dose-limiting toxicity that is largely avoidable. TheraGuide 5-FU™ is a simple blood test that provides comprehensive analysis of *DPYD* and *TYMS* gene variations to predict and help prevent 5-FU–related adverse events.

References: 1. Morel A, Boisdron-Celle M, Fey L, et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Mol Cancer Ther.* 2006;5:2895-2904. **2.** Lecomte T, Ferraz J-M, Zinzindohoué F, et al. Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. *Clin Cancer Res.* 2004;10:5880-5888. **3.** Pullarkat ST, Stoehlmacher J, Ghaderi V, et al. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics J.* 2001;1:65-70. **4.** Ichikawa W, Takahashi T, Suto K, et al. Orotate phosphoribosyltransferase gene polymorphism predicts toxicity in patients treated with bolus 5-fluorouracil regimen. *Clin Cancer Res.* 2006;12:3928-3934. **5.** Schwab M, et al. Role of genetic and non-genetic factors for fluorouracil treatment related severe toxicity; a prospective clinical trial by the German 5-FU toxicity study group. J Clin Oncol, 2008.

TheraGuide 5-FU™

References cited by Myriad Genetics regarding TheraGuide 5-FU

- Morel, 2006 (DPYD)
- LeComte, 2004 (TYMS)
- Pullarkat, 2001 (TYMS)
- Ichikawa, 2006 (OPRT)
- Schwab, 2008 (DPYD, TYMS, MTHFR)

Thymidylate Synthase Gene Polymorphism Predicts Toxicity in Colorectal Cancer Patients Receiving 5-Fluorouracil-based Chemotherapy

Thierry Lecomte, Jean-Marc Ferraz, Franck Zinzindohoué, Marie-Anne Loriot, David-Alexandre Tregouet, Bruno Landi, Anne Berger, Paul-Henri Cugnenc, Raymond Jian, Phillipe Beaune, and Pierre Laurent-Puig

Clinical Cancer Research, 2004; 10: 5880-8.

CONFIDENTIAL

MMM447

TheraGuide 5-FU™
Analysis Result

PHYSICIAN
John Doe MD Myriad Genetics, Inc. 320 Wakara Way Salt Lake City, UT 84108

SPECIMEN	
Specimen Type:	Blood
Draw Date:	Sep 1, 2007
Accession Date:	Sep 5, 2007
Report Date:	Sep 25, 2007

PATIENT	
Name:	Doe, Jane
Date of Birth:	
Patient ID:	
Gender:	Female
Accession #:	00254463-BLD
Requisition #:	00254463

Test Results and Interpretation

HIGH RISK

Genes Analyzed	Results	Interpretation
DPYD	No Variant Detected	No Variant Detected
TYMS	2R/2R	High Risk

LeComte, CCR, 2004 - Methods

- 90 patients over 6 years (1995-2001)
- 8 different fluoropyrimidine regimens
  - Included combinations with CPT-11 and OHP
- Retrospective phenotyping
  - Toxicities, response, PFS, survival, etc.
- Tumor samples (frozen/FFPE) used for genotyping
- 1 gene (TYMS)
  - 3 variants

LeComte, CCR, 2004 – Results

- 25 reported tests of association of genotypes/haplotypes and phenotypes
  - 0.28 p values/subject
- “Patients with a 2R/2R, a 2R/3R, or a 3R/3R genotype had a grade 3 or 4 toxicity rate of 43, 18, and 3% respectively ($P < 0.01$).”

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ORIGINAL REPORT

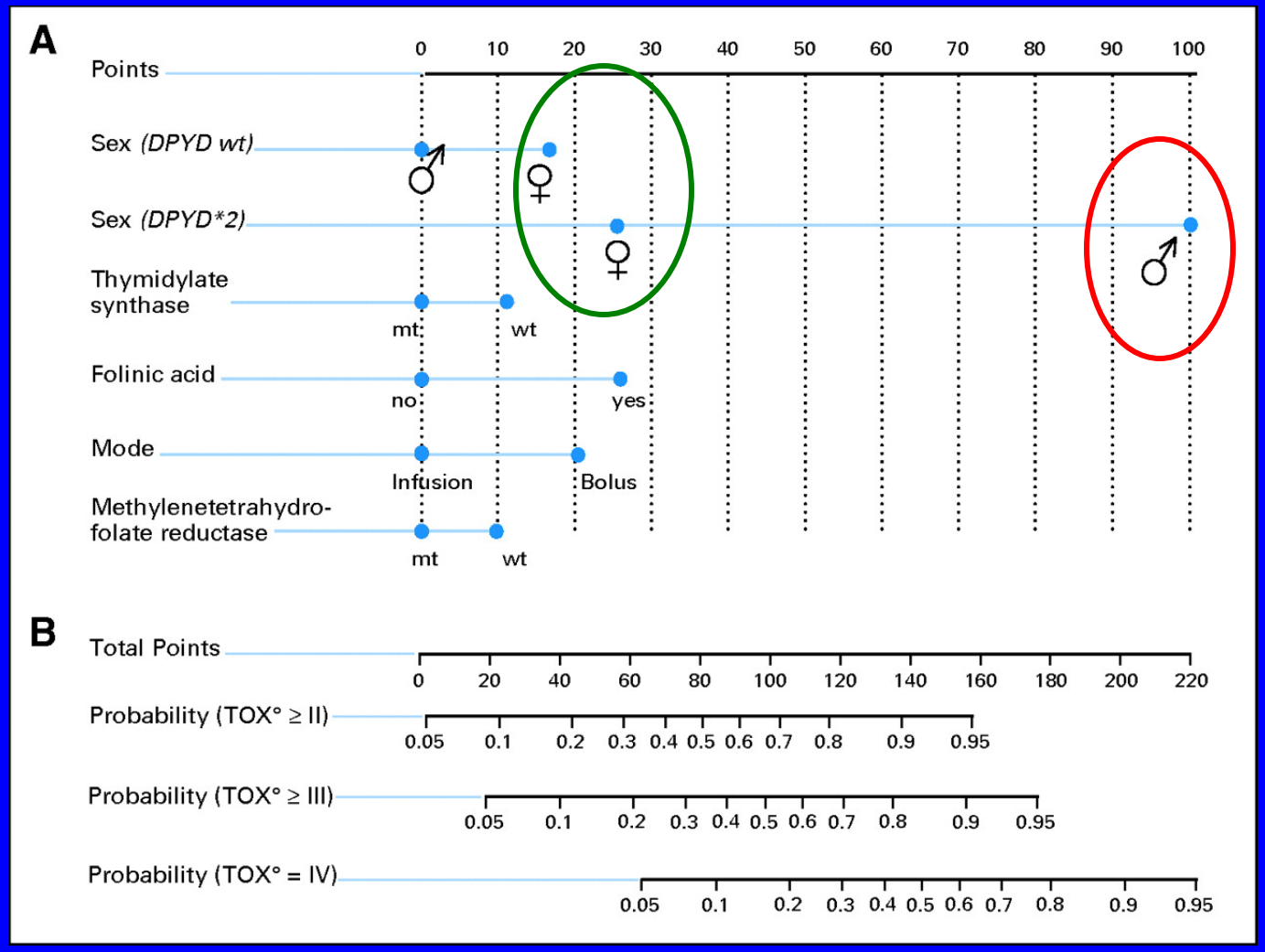
Role of Genetic and Nongenetic Factors for Fluorouracil Treatment-Related Severe Toxicity: A Prospective Clinical Trial by the German 5-FU Toxicity Study Group

Matthias Schwab, Ulrich M. Zanger, Claudia Marx, Elke Schaeffeler, Kathrin Klein, Jürgen Dippon, Reinhold Kerb, Julia Blievernicht, Joachim Fischer, Ute Hofmann, Carsten Bokemeyer, and Michel Eichelbaum

- **N=683**
- **Phenotyping prospective**
- **Treatment uncontrolled (no other cytotoxics)**

Schwab M, et al: *J Clin Oncol*. 2008; 26(13): 2131-8. Reprinted with permission.

Fig 2. Nomogram for estimating individual fluorouracil (FU) toxicity risk based on the multifactorial model



Schwab M, et al*: J Clin Oncol.* 2008; 26(13):2131-8. Reprinted with permission.

In conclusion…

- **NCI should mandate (and fund) germline DNA collection on all NCI-funded phase III trials, unless acceptable justification is provided by the PI.**
- **NCI should interact more closely with the PGRN to ensure that its studies are of the highest possible quality.**
- **NCI also needs to consider how results of its studies will be appropriately replicated and translated into clinical practice.**