UNC
INSTITUTE FOR
PHARMACOGENOMICS AND
INDIVIDUALIZED THERAPY

Interventional pharmacogenetics: moving the science into practice

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The clinical problem

- Multiple active regimens for the treatment of most diseases
- Variation in response to therapy
- Unpredictable toxicity

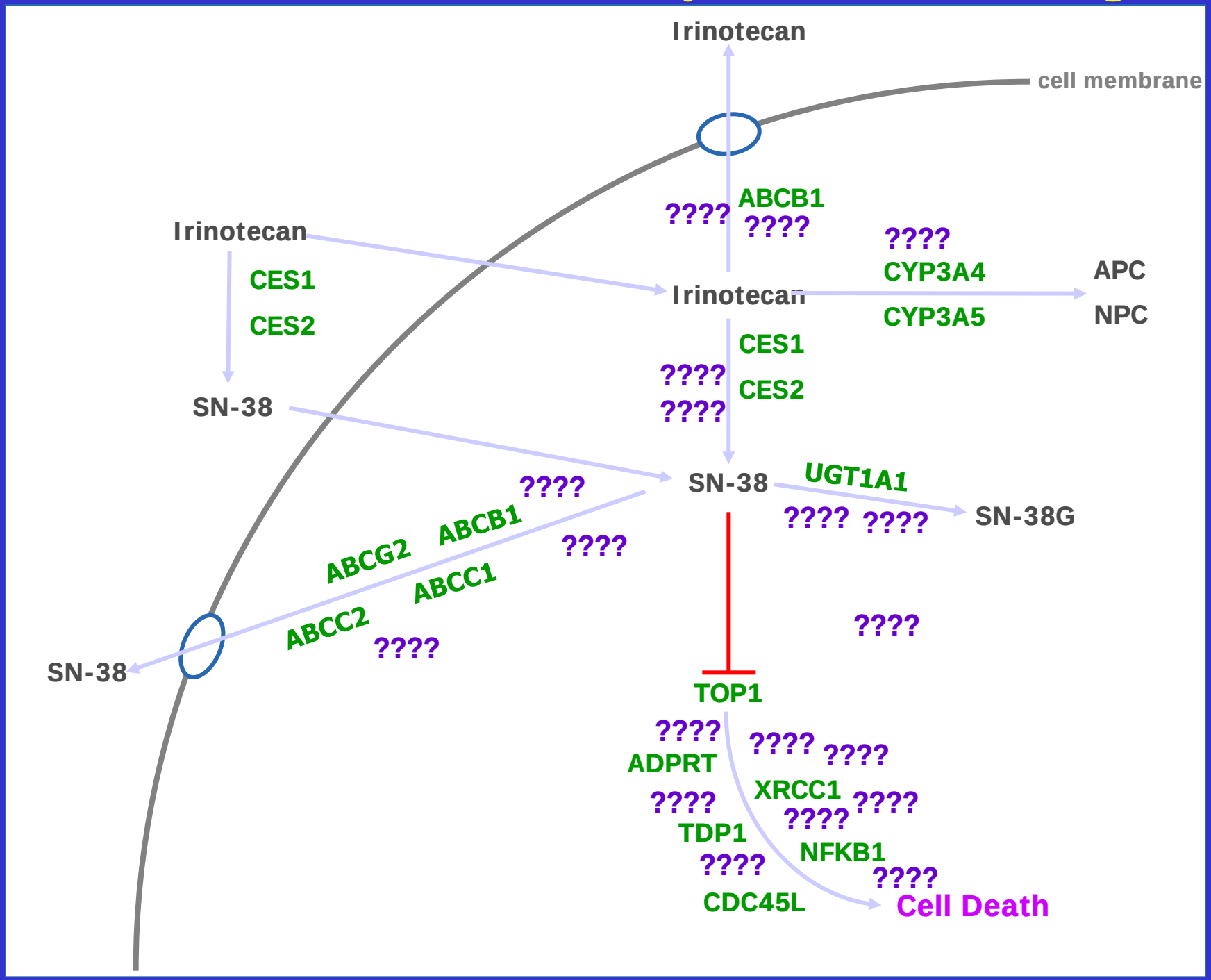
$$$$$$$$$$$$$

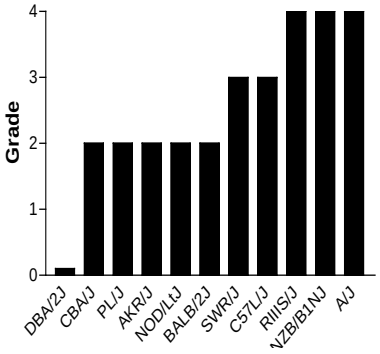
With choice comes decision

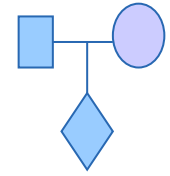
What needs to be done to determine hope vs hype?

- Find the 'right' biomarkers
- Validate in robust datasets
- Apply them!

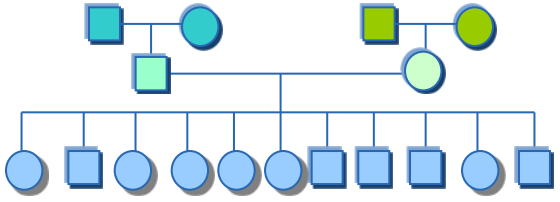
We do not know very much about drugs







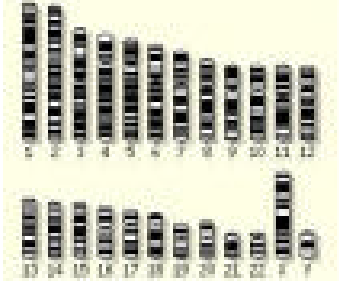
HapMap

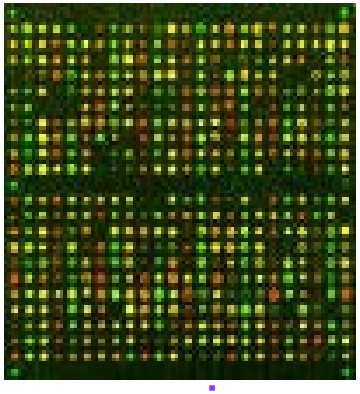


Linkage

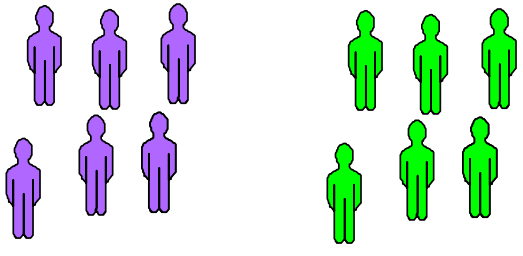
Model systems

Discovery Strategies





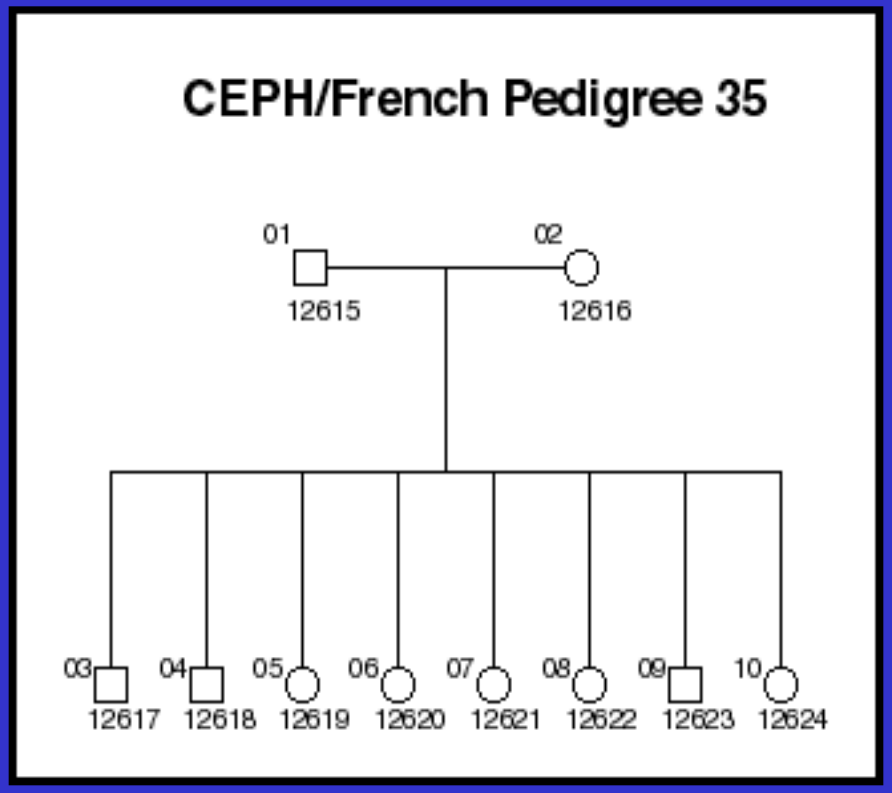
Expression array

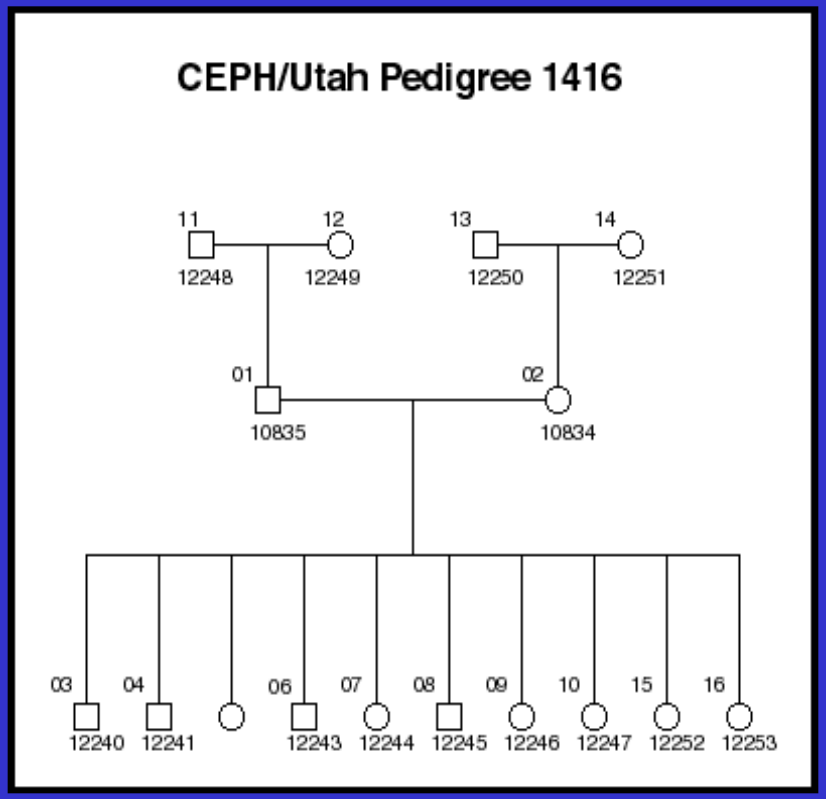


Association

Centre d' Etude du Polymorphisme Human (CEPH) Cell lines

- Large, multigeneration pedigrees widely studied
- Immortalized lymphoblastoid cell lines



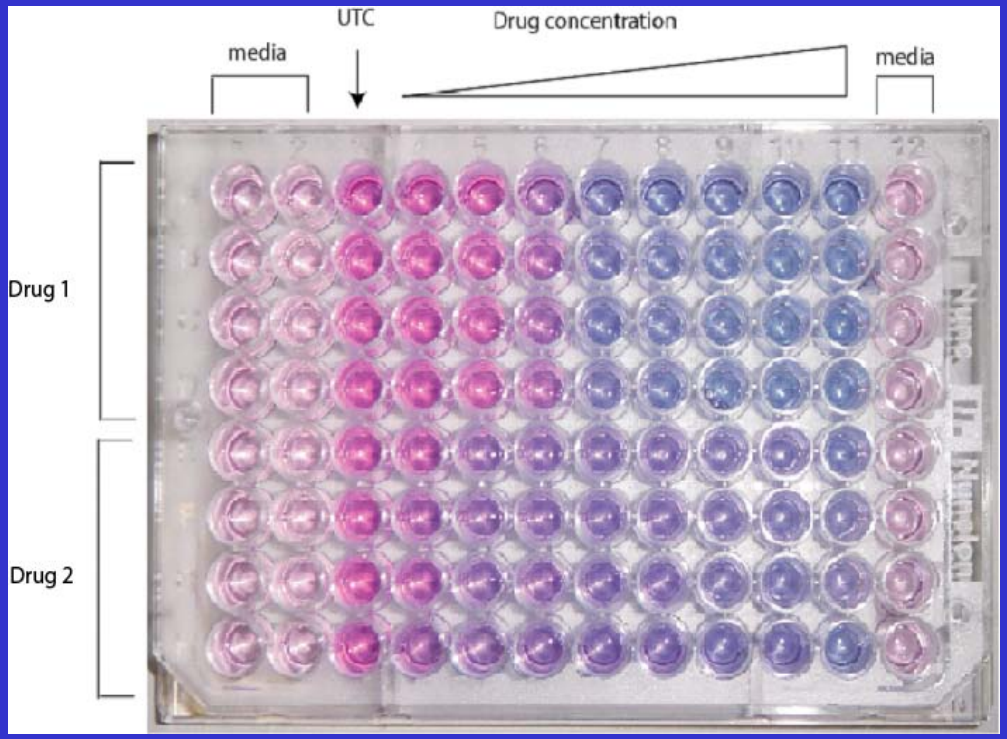


Methodology

Cells counted, plated at 1 x 10^4 / well

Cells incubated with increasing concentrations of drug

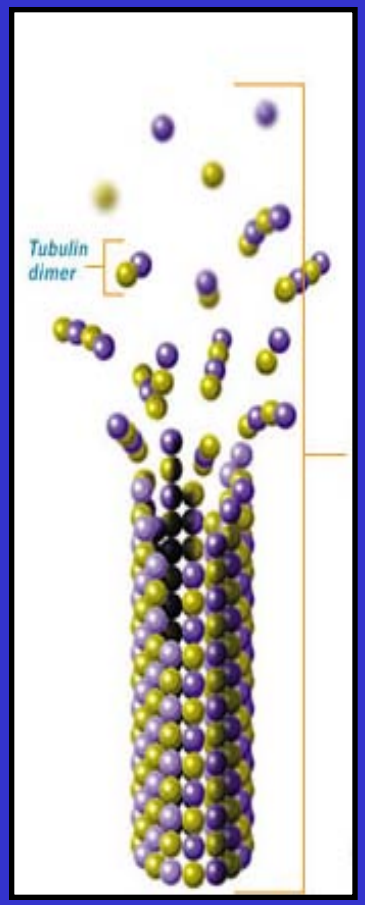
Alamar blue vital dye indicator added

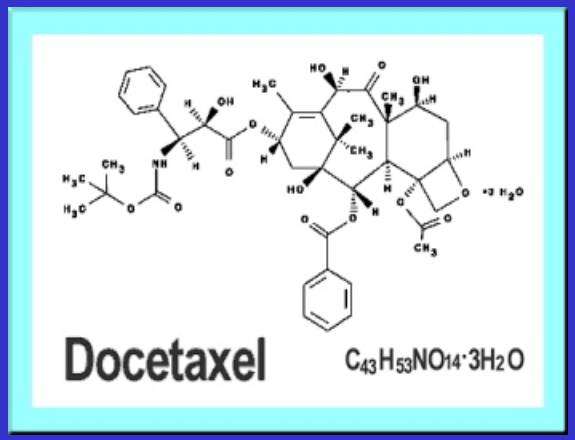


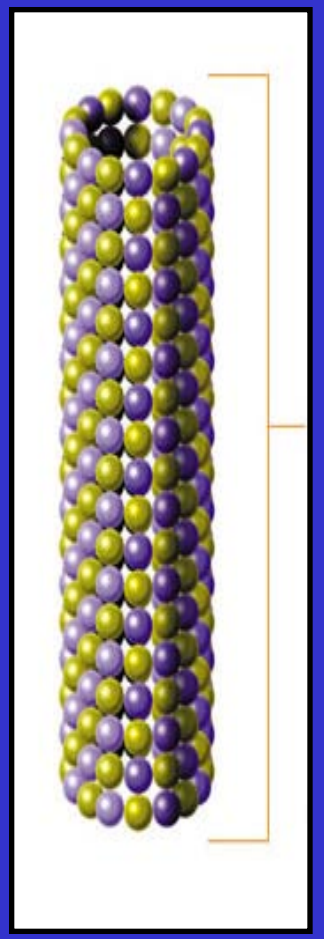
Viability relative to untreated control calculated by spectrophotometry

Docetaxel

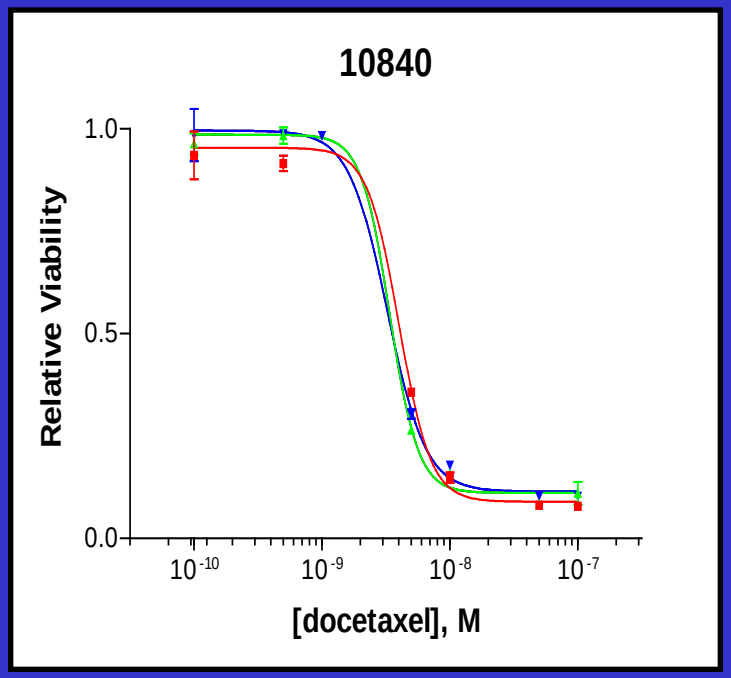
- Microtubule stabilizing agent used to treat lung, breast tumors

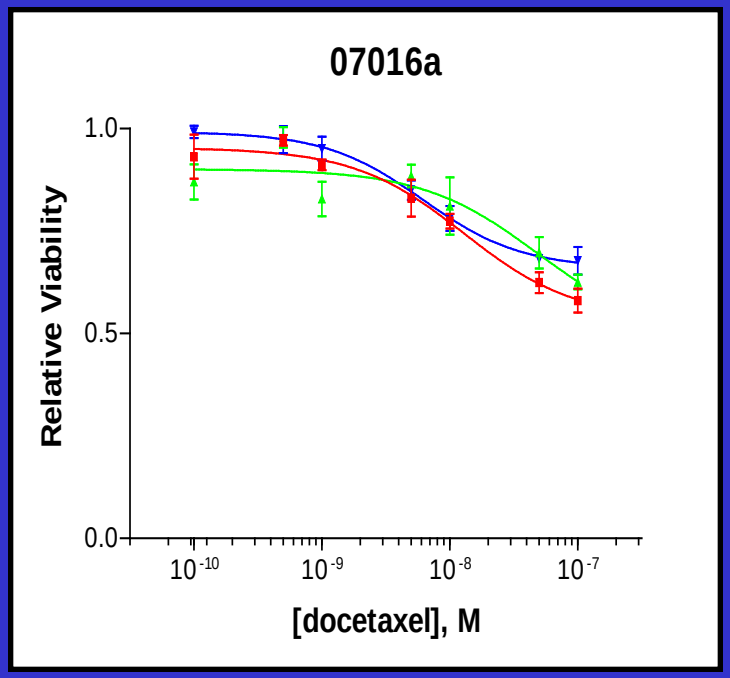






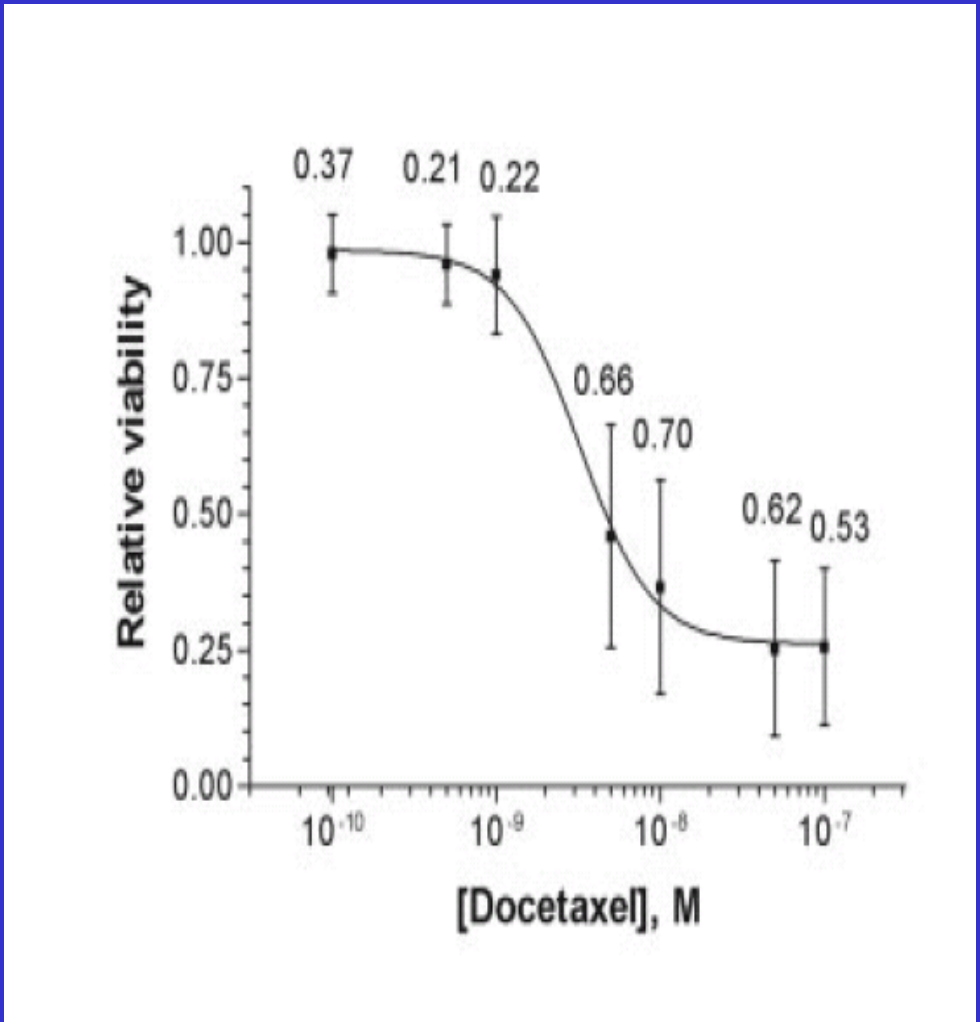
Significant Variation in Cellular Sensitivity to Docetaxel





427 cell lines analyzed, 38 CEPH reference pedigrees

Docetaxel cytotoxicity is a heritable trait!



Watters et al. *Proc Natl Acad Sci U S A*. 2004;101(32):11809-14. Copyright 2004 National Academy of Sciences, U.S.A.

'CE-PH/F-DA' project

- 126 CEPH cell lines from 14 nuclear families
- All FDA approved cytotoxic drugs + new kinase inhibitors/MTOR/demethylation
- No antiestrogen or vitamin A analogues
- Evaluate degree of heritability, presence of QTL(s), and evidence for correlations between drug sensitivity patterns.

Examples of Genes in Chromosome 9 Interval

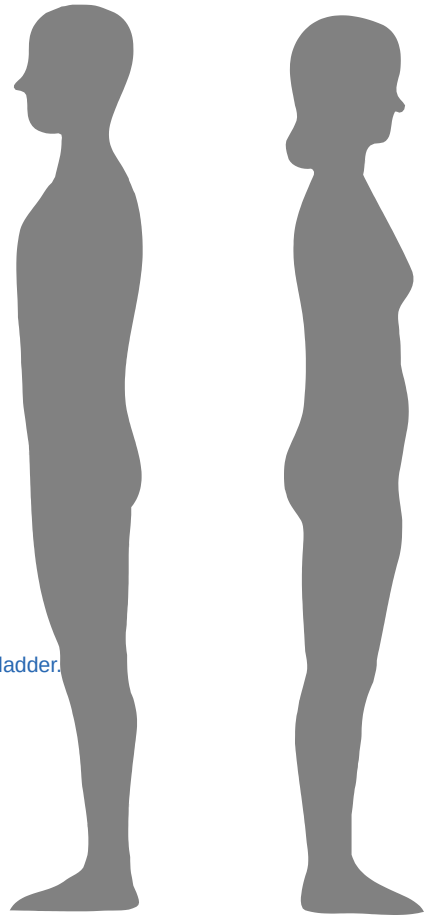
RAD23B	NM_002874	Hs.159087	RAD23 homolog B (S. cerevisiae)	105472558-105473090
FCMD	NM_006731	Hs.55777	Fukuyama type congenital muscular dystrophy (fukutin)	103782702-103783131
CDW92	AW165999	Hs.414728	CDw92 antigen	103525239-103527612
ABCA1	NM_005502	Hs.147259	ATP-binding cassette, sub-family A (ABC1), member 1	102923739-102924215
SMC2L1	AU154486	Hs.119023	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	102282761-102283241
RNF20	AK022532	Hs.168095	ring finger protein 20	99704963-99705433
ZNF189	NM_003452	Hs.50123	zinc finger protein 189	99552272-99552713
MRPL50	BG028213	Hs.288224	mitochondrial ribosomal protein L50	99532436-99532607
INVS	AF039217	Hs.150744	inversin	98439227-98443060
TXNDC4	BC005374	Hs.154023	thioredoxin domain containing 4 (endoplasmic reticulum)	98124676-98149788
SEC61B	NM_006808	Hs.191887	Sec61 beta subunit	97370032-97372556
ALG2	BE967331	Hs.40919	asparagine-linked glycosylation 2 homolog (yeast, alpha-1,3-mannosyltrans	97359553-97359942
TGFBR1	AA604375	Hs.28005	transforming growth factor, beta receptor I (activin A receptor type II-like kina	97295384-97295719
NANS	NM_018946	Hs.274424	N-acetylneuraminic acid synthase (sialic acid synthase)	96222949-96225118
ANP32B	NM_006401	Hs.459987	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	96157636-96158013
XPA	NM_000380	Hs.288867	xeroderma pigmentosum, complementation group A	95817102-95817607
NCBP1	BC001450	Hs.439203	nuclear cap binding protein subunit 1, 80kDa	95808875-95813344
TMOD1	NM_003275	Hs.374849	tropomodulin 1	95742775-95743279
PCTAIRE2BP	AW129593	Hs.416543	tudor repeat associator with PCTAIRE 2	95629328-95638103
CDC14B	AI921238	Hs.22116	CDC14 cell division cycle 14 homolog B (S. cerevisiae)	94656602-94657833
HABP4	AF241831	Hs.301839	hyaluronan binding protein 4	94630336-94632501
SLC35D2	AJ005866	Hs.386278	solute carrier family 35, member D2	94462851-94463372
FANCC	NM_000136	Hs.253236	Fanconi anemia, complementation group C	93202851-93203264
ZNF169	BC019228	Hs.387623	zinc finger protein 169	92382458-92396707
PHF2	AB014562	Hs.93868	PHD finger protein 2	91782703-91783231
C9orf10	AF214738	Hs.446534	chromosome 9 open reading frame 10	91634518-91635068
BICD2	AI934125	Hs.436939	coiled-coil protein BICD2	90815152-90815659
IARS	NM_013417	Hs.172801	isoleucine-tRNA synthetase	90314089-90326264
SPTLC1	BC007085	Hs.90458	serine palmitoyltransferase, long chain base subunit 1	90183077-90183244
NFIL3	NM_005384	Hs.79334	nuclear factor, interleukin 3 regulated	89512814-89513311
AUH	NM_001698	Hs.81886	AU RNA binding protein/enoyl-Coenzyme A hydratase	89317590-89318101
SYK	BF593625	Hs.192182	spleen tyrosine kinase	89001692-89002111
SBP2	BC001189	Hs.59804	SECIS binding protein 2	87430383-87439113
CKS2	NM_001827	Hs.83758	CDC28 protein kinase regulatory subunit 2	87415644-87421033
HFSE-1	AF072164	Hs.137570	HFSE-1 protein	87258704-87259146
SPIN	AL136719	Hs.439052	spindlin	86550195-86550700
DAPK1	NM_004938	Hs.244318	death-associated protein kinase 1	85780116-85780511

Correlative science: business as usual

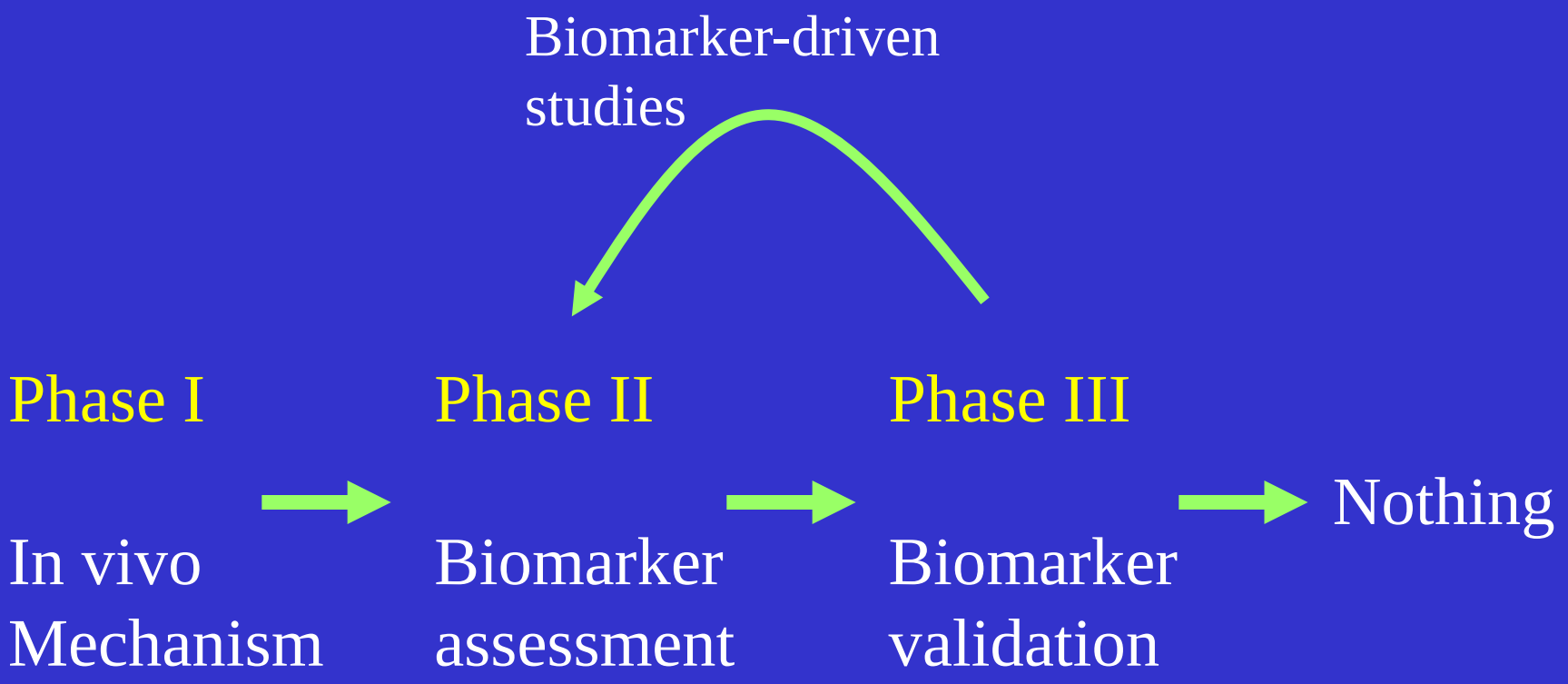
Phase I	Phase II	Phase III
In vivo Mechanism	Biomarker assessment	Biomarker validation

2009 Estimated US Cancer Cases*

Men 766,130		Women 713,220	
Prostate	25%	Breast	27%
Lung & bronchus	15%	Lung & bronchus	14%
Colon & rectum	10%	Colon & rectum	10%
Urinary bladder	7%	Uterine corpus	6%
Melanoma of skin	5%	Non-Hodgkin lymphoma	4%
Non-Hodgkin lymphoma	5%	Melanoma of skin	4%
Kidney & renal pelvis	5%	Thyroid	4%
Leukemia	3%	Kidney & renal pelvis	3%
Oral cavity	3%	Ovary	3%
Pancreas	3%	Pancreas	3%
All Other Sites	19%	All Other Sites	22%

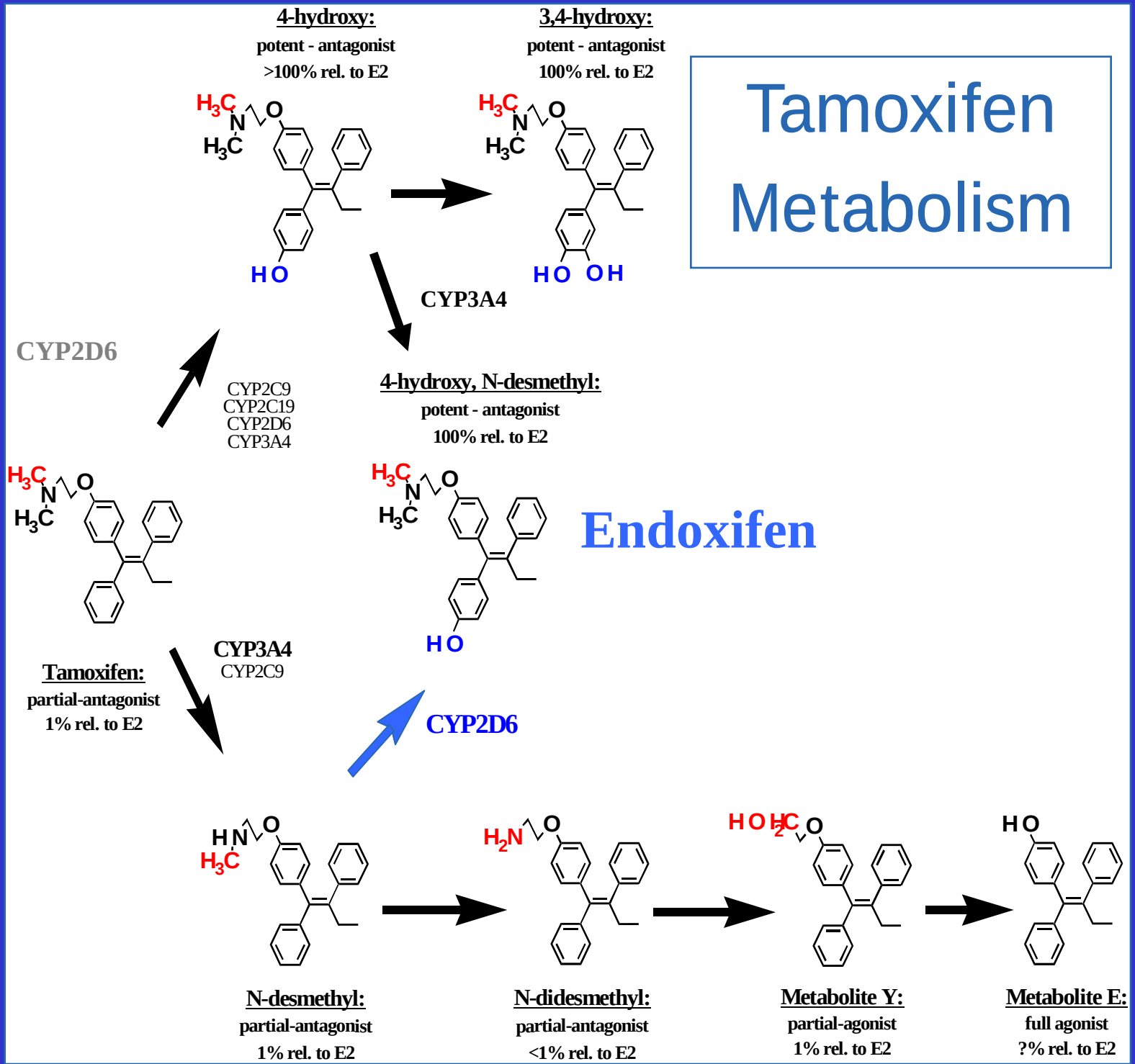


*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.


Biomarker-driven studies
Phase I
Phase II
Phase III
In vivo Mechanism
Biomarker assessment
Biomarker validation
Nothing

PGRN
PG Research Network

Tamoxifen Metabolism



PGRN
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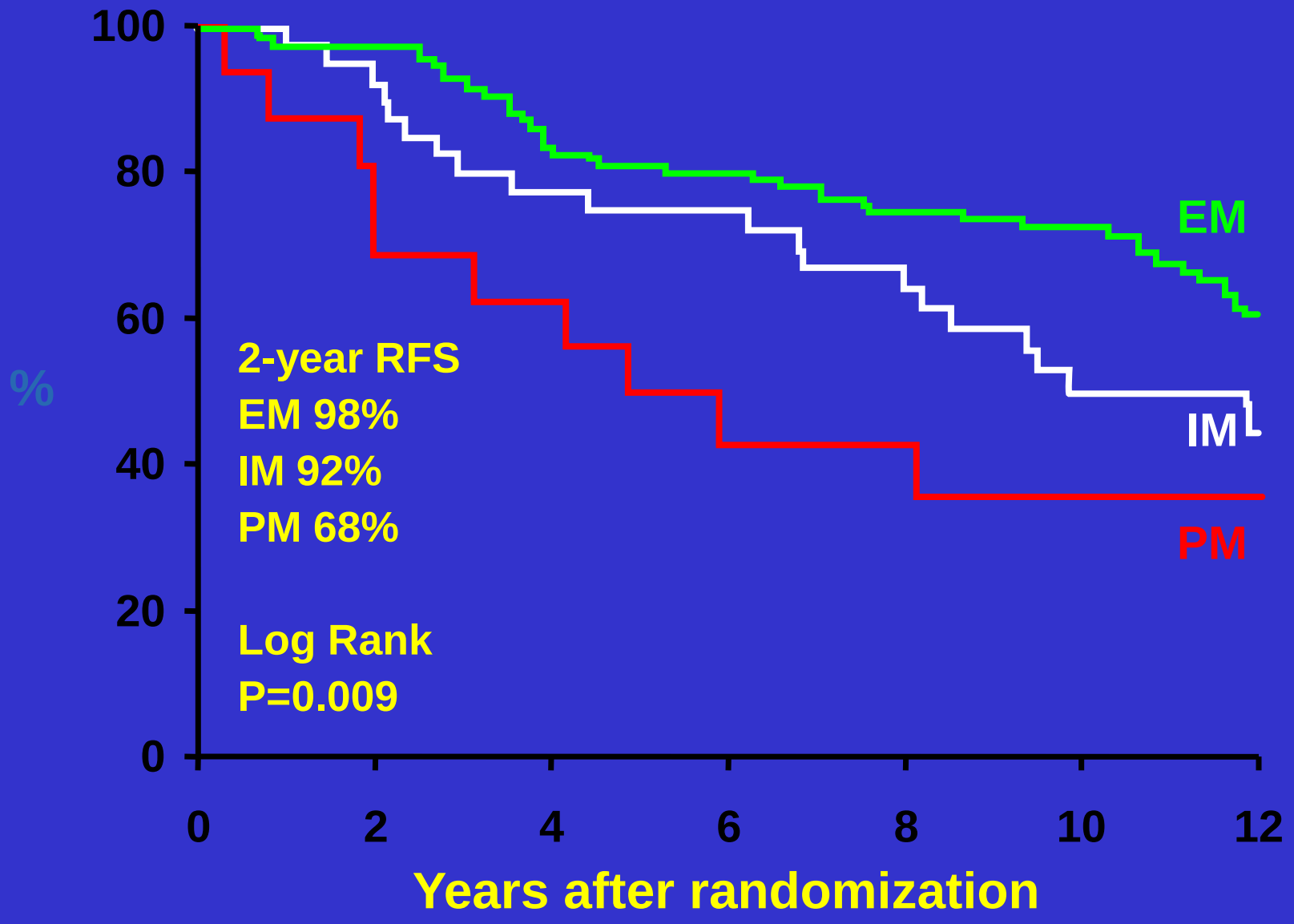
Relapse-free Survival



Goetz et al. *Breast Cancer Res Treat*. 2007; 101: 113-21. Reprinted by permission of Springer.

Adjuvant Tamoxifen and CYP2D6

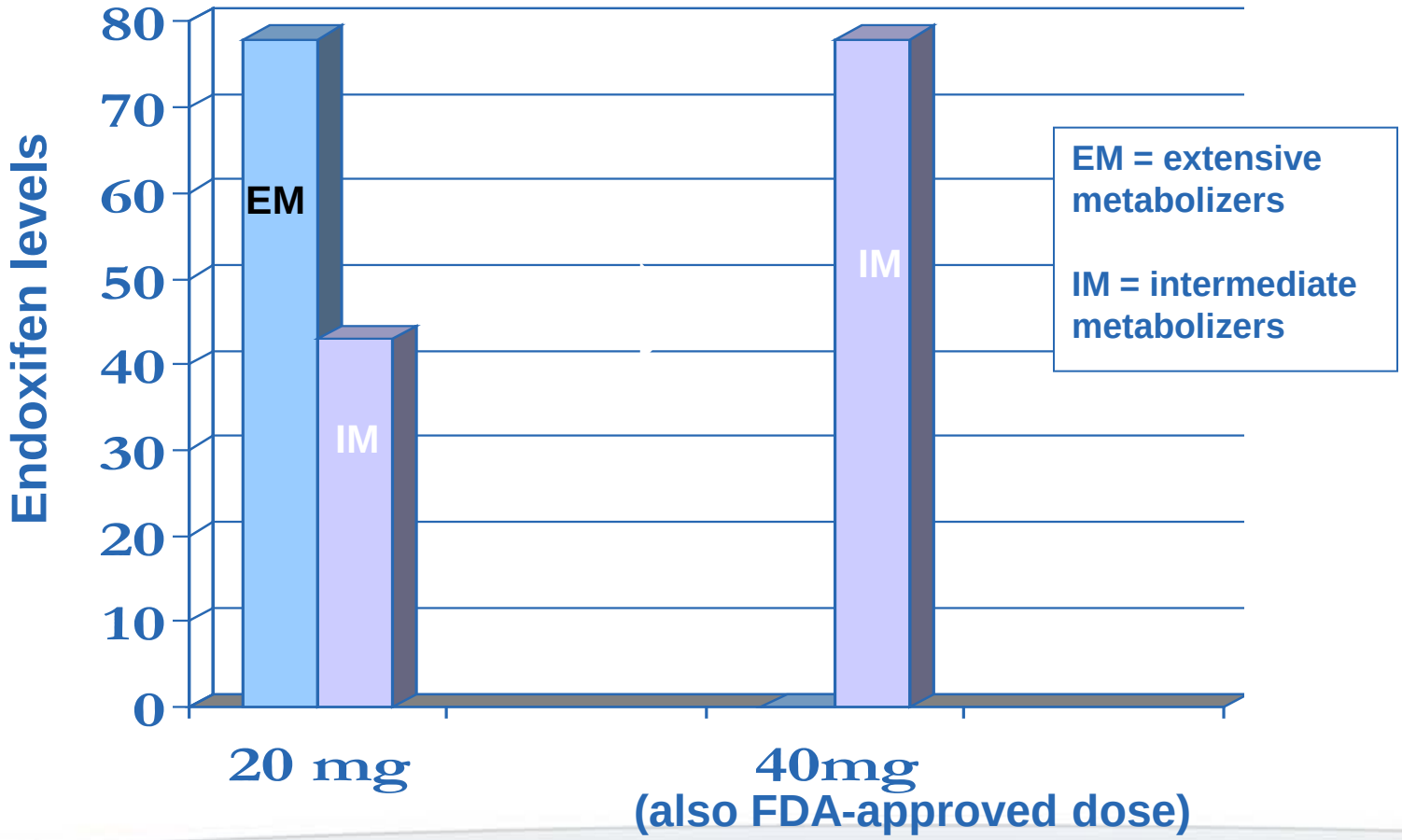
- **CYP2D6 associated with recurrence**
  - **Goetz et al. 2005, 2007 (USA)**
  - **Schroth et al. 2007 (Germany)**
  - **Kiyotani et al. 2008 (Japan)**
  - **Newman et al. 2008 (UK)**
  - **Xu et al. 2008 (China)**
  - **Okishiro et al. 2009 (Japan)**
  - **Ramon et al. 2009 (Spain)**
  - **Bijl et al. 2009 (Netherlands)**

- **CYP2D6 not associated with recurrence**
  - **Wegman et al. 2005, 2007 (Sweden)**
  - **Nowell et al. 2005 (USA)**

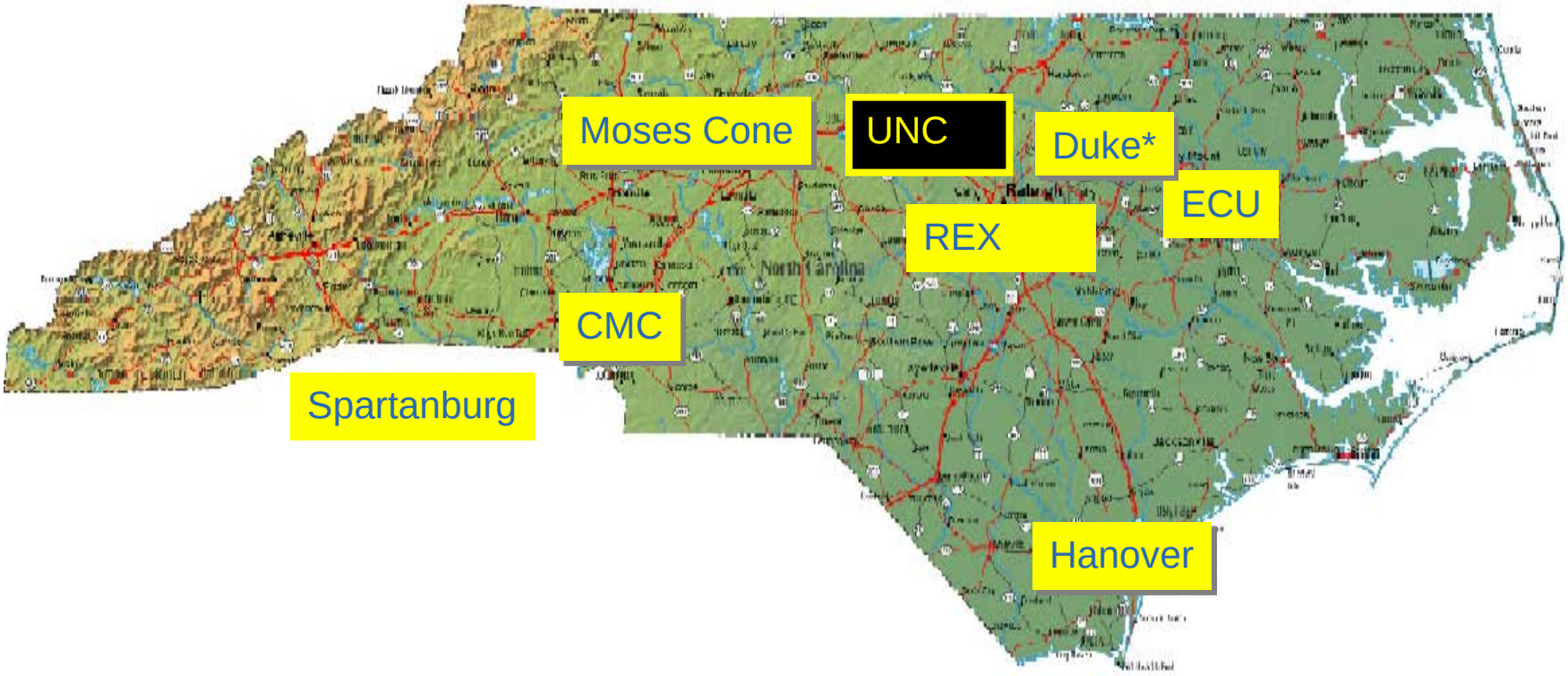
Objectives

- Primary objective
  - To evaluate the change in endoxifen levels following an increase in tamoxifen dose from 20 mg to 40 mg among patients with intermediate metabolizing CYP2D6 genotypes

- Hypothesis: We can compensate for CYP2D6 low activity by increasing tamoxifen dose to 40mg/d



LCCC 0801: Active community participation



Laboratory and clinical analysis

- Amplichip® P450 test used for genotyping, including the major EM (active) alleles (*1, *2, and *35), IM (reduced activity) alleles (*9, 10, *17, *29,*36, and *41), and PM (inactive) alleles (*3, *4, *5, *6, *7, *8, *11, *15, *19, *20, and *40)
- Tamoxifen and metabolite levels at baseline and 4 months
- Quality-of-life analysis performed using FACT-B, FACT-B (es), and BCPT Menopausal Symptom Scale at baseline and at 4 months

Results

- Trial open from 7/1/08-12/30/08; accruing in 6 months
- N=119, of whom 118 are evaluable for genotyping analysis (1 withdrew study consent)

Genotype (% of patients)

EM/EM	31 (26.3%)
EM/UM	1 (0.9%)
EM/IM	30 (25.4%)
EM/PM	25 (21.2%)
IM/IM	8 (6.8%)
IM/PM	11 (9.3%)
PM/PM	11 (9.3%)
unknown	1 (0.9%)

Conclusions

- **A high proportion of patients (72%) have at least one PM or IM allele which may result in lower levels of conversion of tamoxifen to endoxifen, the active metabolite.**
- **The frequency of PM alleles in Caucasians was similar to that seen in other studies.**

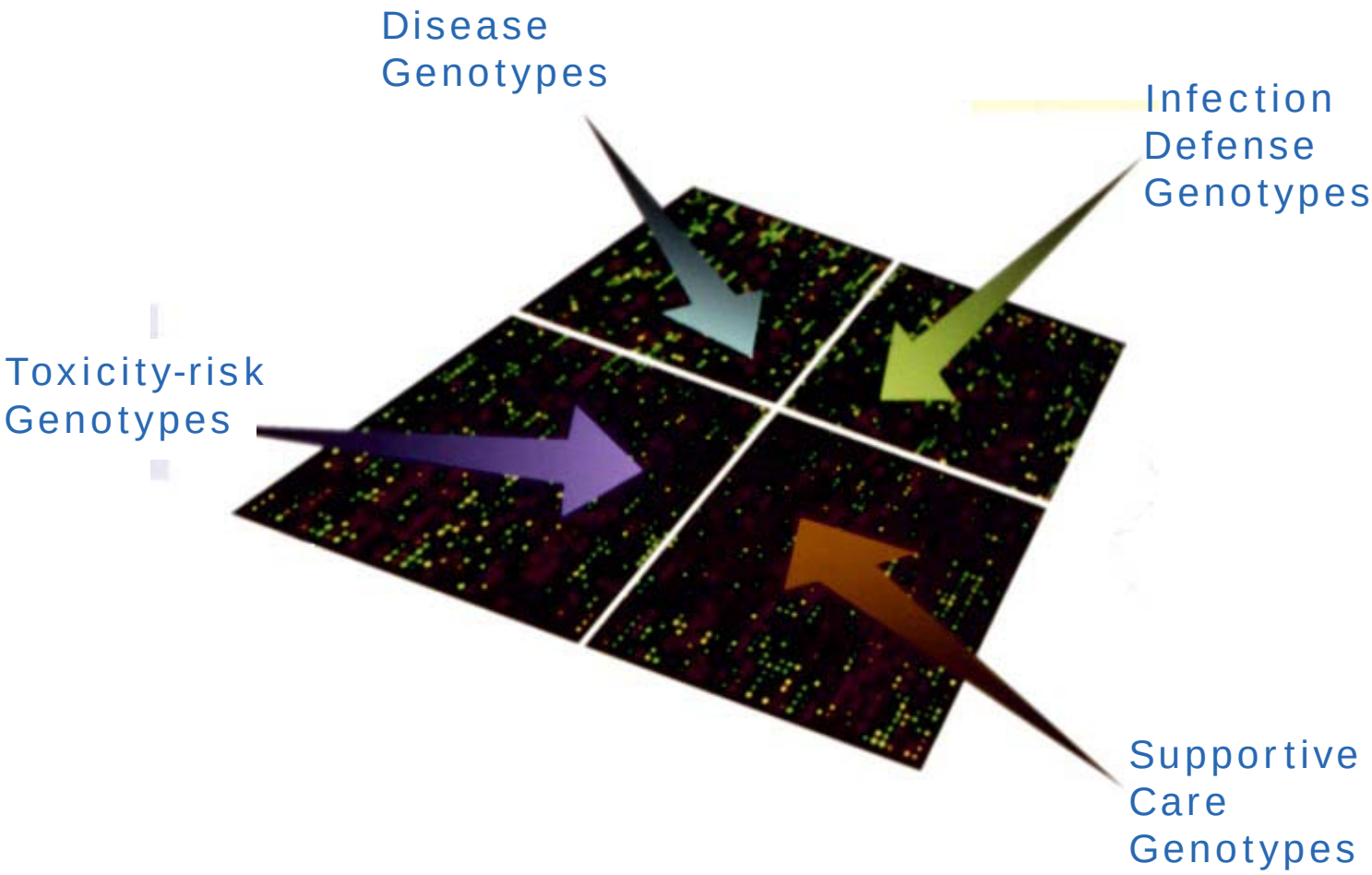
- **The allele frequency of reduced metabolism alleles was greater in African-Americans (62%) than in other patients (44%).**

- **The IM allele *17 is common in African-Americans, consistent with other reports. This raises the question of altered tamoxifen metabolism as one contributor to the disproportionately worse outcome suffered by African-American women with breast cancer.**

- **A significant portion of patients (25%) are heterozygous for an IM allele (EM/IM). The significance of EM/IM genotypes on tamoxifen metabolism requires further study.**

- **CYP2D6 genotyping studies that identify only the common null allele *4 would underestimate the incidence of reduced metabolism alleles, particularly in African-Americans.**

Comprehensive optimization of patient care



5 Stages of pharmacogenetics progress

Denial (and Isolation)
Anger
Bargaining
Depression
Acceptance

Apologies to Elizabeth KUBLER-ROSS, MD
On Death and Dying (1969)

What needs to be done to determine hope vs hype?

- Find the 'right' biomarkers
  - Support ‘anchored’ discovery
- Validate in robust datasets
  - Broaden the sampling in national trials
  - Build community cohorts for ‘real world’ evaluation
- Apply them!
  - Develop the teams required to draw a conclusion

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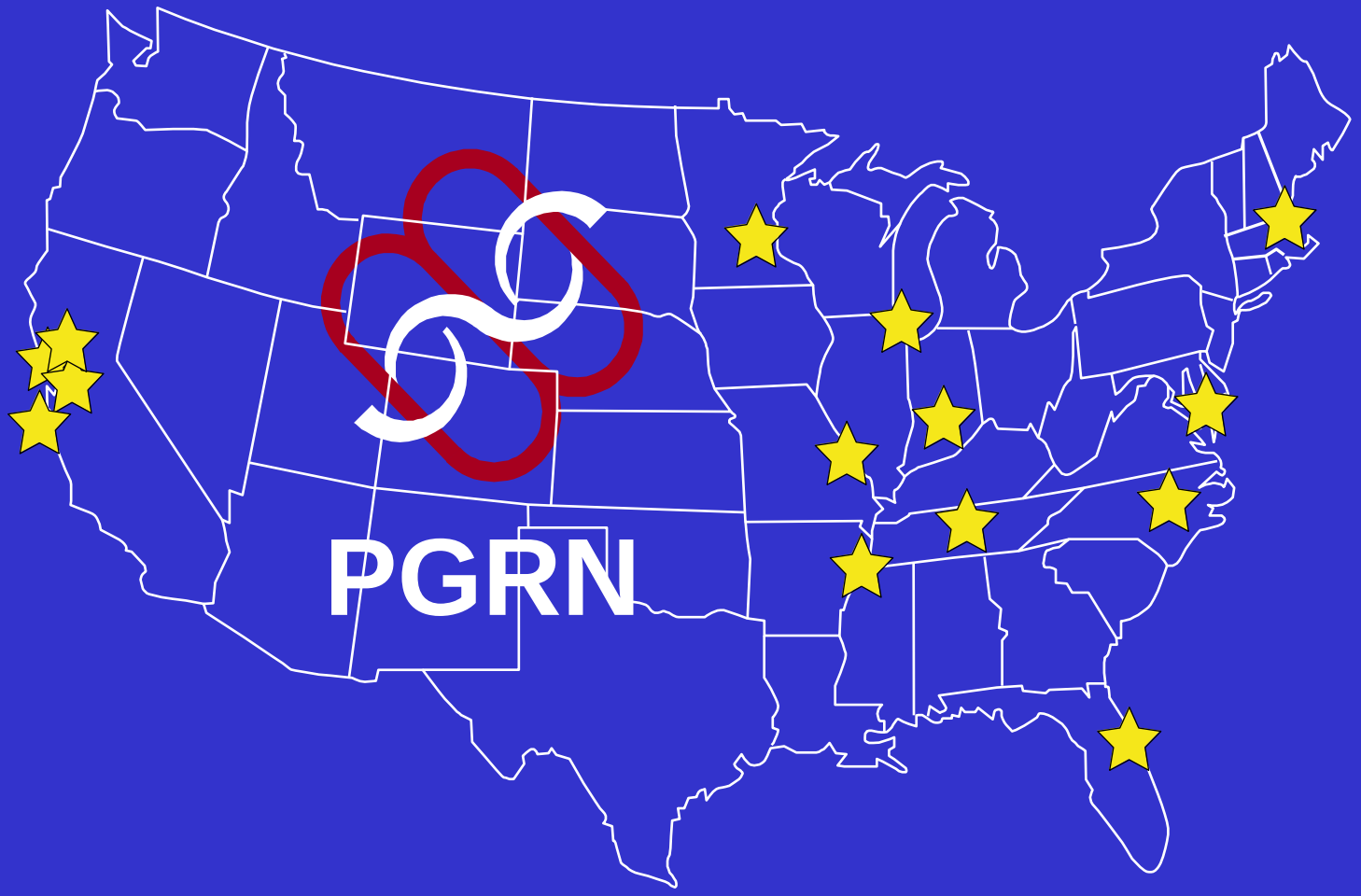

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