Predictors of Breast Cancer Recurrence *(ProBeCaRe)* study: Tamoksifenresistens hos præmenopausale kvinder med brystkræft

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Overview of presentation

- Background to the Danish ProBeCaRe Premenopausal Study
- The ProBeCaRe dataset
- Project progress:
  1. Tumor block collection
  2. Validation study findings
  3. Drug-drug interaction results
- Ongoing work

TAM competes with other drugs for CYP enzymatic activity

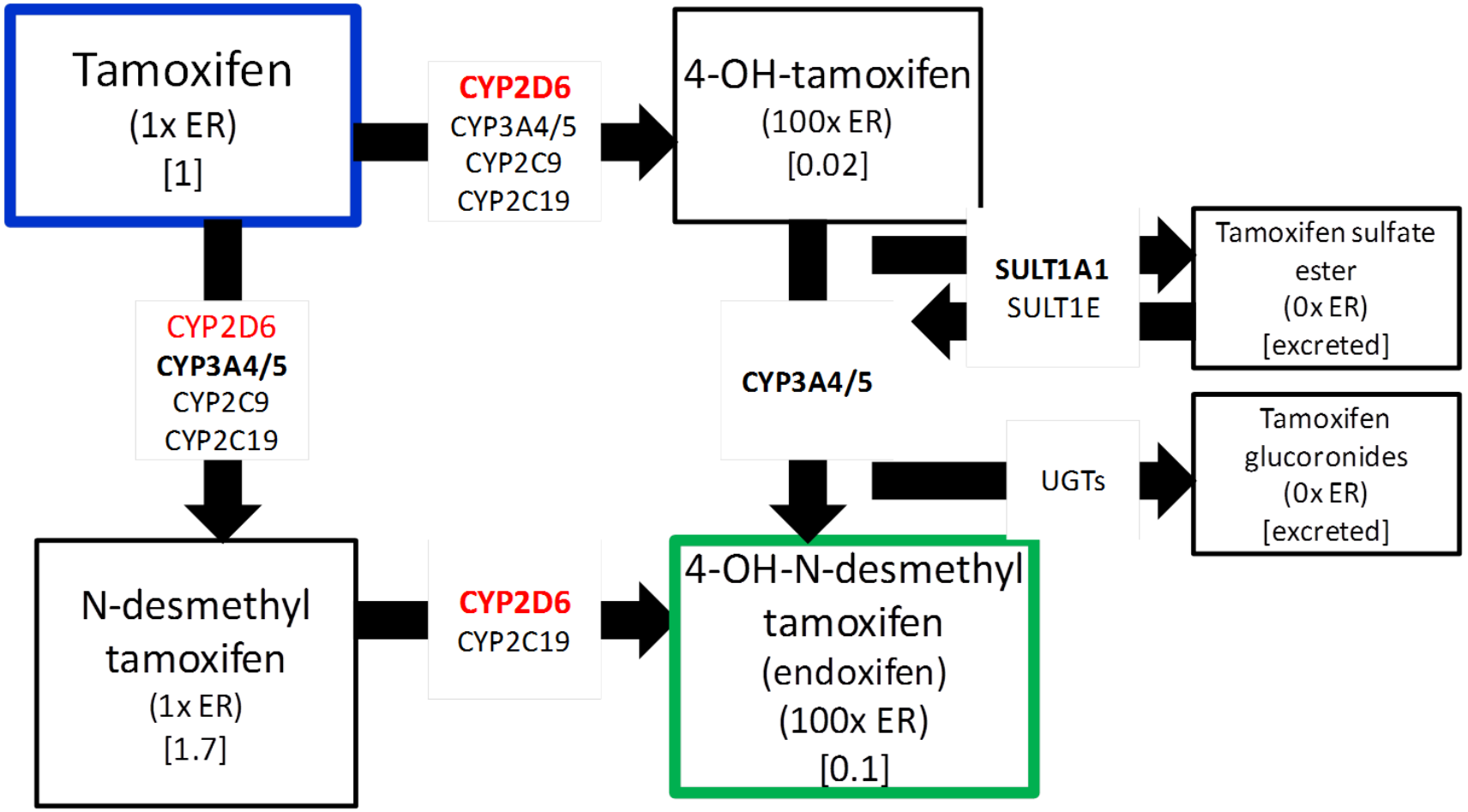

Figure 1: Major metabolic pathways for tamoxifen. Bold type denotes the enzyme(s) primarily involved in each step. (Nx ER) = binding affinity to estrogen receptor relative to tamoxifen itself. [C] = plasma concentration of the metabolite, relative to tamoxifen's concentration, after four months of tamoxifen therapy at 20 mg per day.

Cronin-Fenton et al., Future Oncology, 2014

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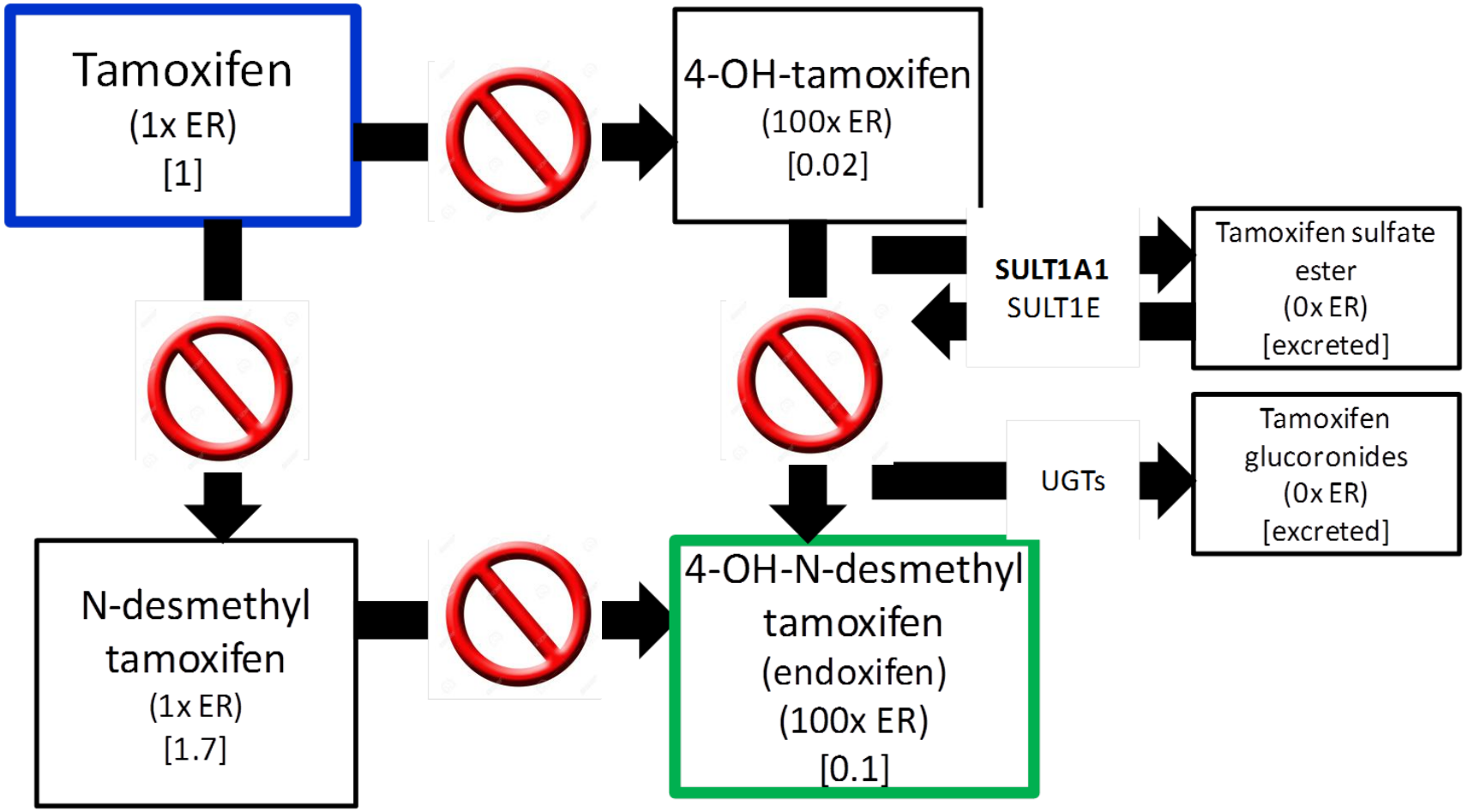

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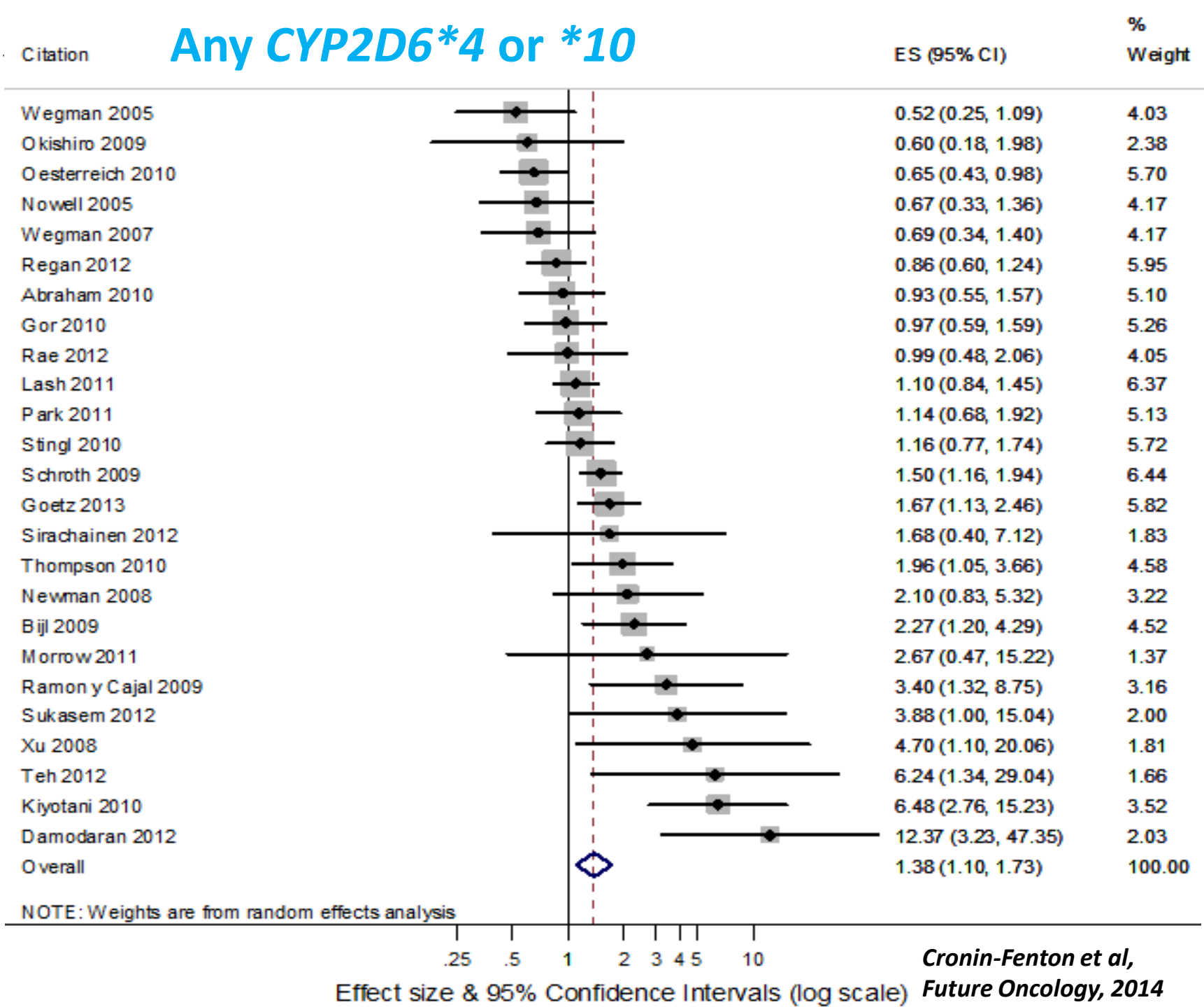
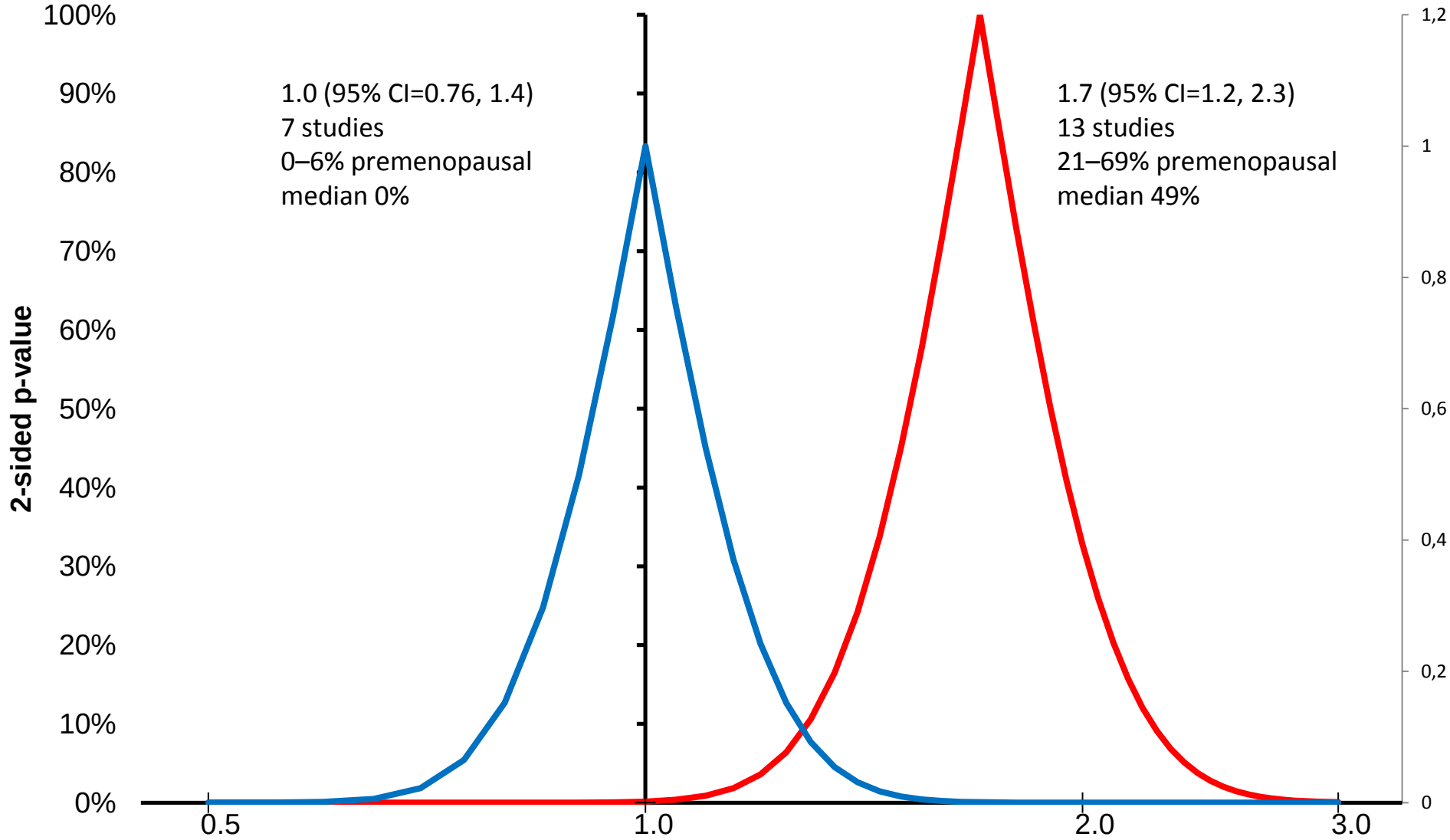
Any *CYP2D6*4* or *10*



Cronin-Fenton et al, Future Oncology, 2014

New perspectives 3: Pre-menopausal women

Cronin-Fenton et al., Future Oncology, 2014



Study aims

- Assess inhibition of tamoxifen metabolism via comprehensive genotyping & concurrent drug use, and risk of breast cancer recurrence
  - Examine genetic variants in 13 enzymes that catalyze the biotransformation of tamoxifen

- Assess competitive inhibition of tamoxifen through assay of oestrogen regulating enzymes
  - 17βHSD1 & 17βHSD2

- Assess interaction between inhibition of tamoxifen metabolism and ERβ expression

=> This is the first and largest study of premenopausal women

Network of Danish Registries

ProBeCaRe Cohort (DBCG data)
High quality clinical database
Cancer-directed treatment
Clinical characteristics
Patient characteristics
Follow-up

Danish Breast Cancer Group

FFPE tumor & normal tissue

Danish Pathology Registry

Danish National Registry of Patients

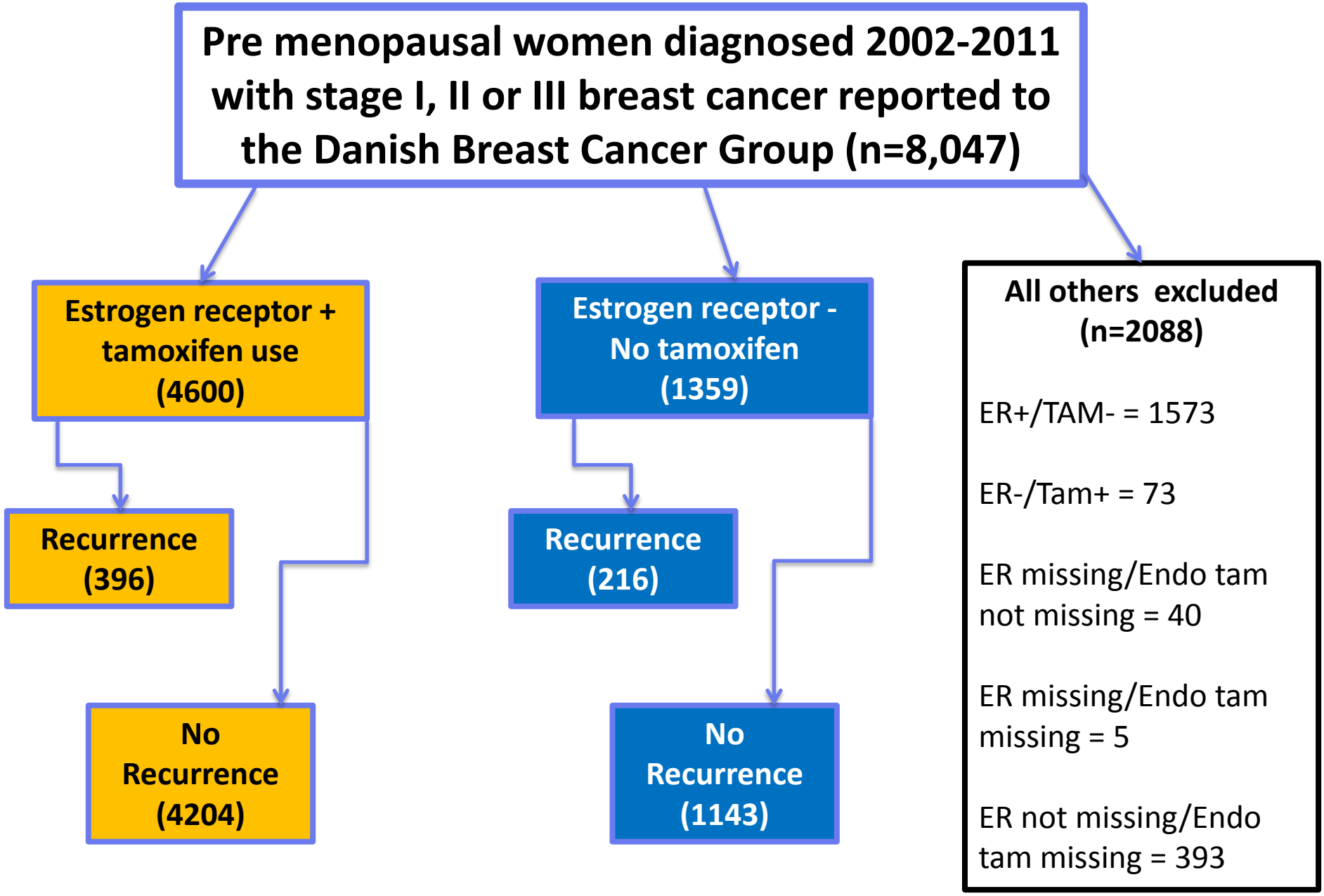
Comorbid diseases

National Prescription Registry:
Prospectively collected prescription data:
CYP2D6, CYP3A4, CYP2C19 inhibiting medications

National Prescription Registry

Danish Civil Registry

1968+
Emigration
Vital status


Pre menopausal women diagnosed 2002-2011 with stage I, II or III breast cancer reported to the Danish Breast Cancer Group (n=8,047)
Estrogen receptor + tamoxifen use (4600)
Estrogen receptor - No tamoxifen (1359)
All others excluded (n=2088)
ER+/TAM- = 1573
ER-/Tam+ = 73
ER missing/Endo tam not missing = 40
ER missing/Endo tam missing = 5
ER not missing/Endo tam missing = 393
Recurrence (396)
Recurrence (216)
No Recurrence (4204)
No Recurrence (1143)

Patient and tumour characteristics	ER+/TAM+		ER-/TAM-	
	N	%	N	%
Age at diagnosis				
<35	222	4.8	182	13.4
35-49	487	10.6	229	16.9
40-44	1123	24.4	321	23.6
45-49	1668	36.3	385	28.3
50+	1100	23.9	242	17.8
Stage at diagnosis				
Stage I	1184	25.7	402	29.6
Stage II	2476	53.8	702	51.7
Stage III	917	19.9	246	18.1

1. Cooperation from Danish pathology departments

- First letter requesting blocks sent **April 2014**
- End of block collection **August 2015** for majority of pathology departments
- N=5,500 FFPE blocks received

Thank you!

2. ProbeCaRe Validation Study Aims

Compare DBCG data used in the ProBeCaRe study with medical records as a gold standard

Aims:

To validate:

- Changes in menopausal status during follow-up
- Changes in endocrine therapy during follow-up
- Breast cancer recurrence

Study sampling criteria

50 patients each hospital:

Aarhus, Aalborg, Odense University Hospitals

- **2002-2006 & 2007-2011**
- **ER/TAM status**
- **Stage I, II, III**

- 36 strata
- random numbers to each patient within each stratum
- selected 4-5 patients from each stratum

=> 151 patients in total

Results

~100% agreement between registry and medical records for clinical, demographic and treatment characteristics

Results: Menopausal transition

DBCG Registry Menopausal transition (n=151 patients)	Medical Record Menopausal transition		
Frequency	No	Yes	Total
No	78	18	96
Yes	11	17	28
Total	89	35	124
PPV= 61% (42%, 77%)			

Results: Changes in endocrine therapy

Registry Change in endocrine therapy among ER+ patients only (n=77 patients)	Medical Record Change in endocrine therapy		
Frequency	No	Yes	Total
No change from tamoxifen	48	3	51
Change from tamoxifen to aromatase inhibitor	1	25	26
Total	49	28	77
PPV= 96% (83%, 100%)			

Results: Breast cancer recurrence

Registry Recurrence (n=151 patients)	Medical Record Recurrence		
Frequency	No recurrence	Yes recurrence	Total
No recurrence	131	6	137
Yes recurrence	0	14	14
Total	131	20	151
PPV= 100%			

Implications of the validation study

- Changes in endocrine therapy can be incorporated as time-varying covariates

- Changes in menopausal status were difficult to validate
  - Other variables (e.g., prescription drugs) may be more robust as as time-varying variables
  - The medical record may be a poor gold standard for menopausal transition

- Recurrence may be missing for some patients but this will not bias most ratio measures of association

3. Drug-interaction study

Tamoxifen biotransformation chiefly catalyzed by CYP2D6, CYP2C19, and CYP3A4

Aim:

- To evaluate whether tamoxifen-treated premenopausal breast cancer patients have a higher recurrence rate if concomitantly exposed to a metabolism-impairing drug

Study Population

- Stage I-III premenopausal breast cancer patients in Denmark (n=5,959)
- Diagnosed 2002-2011, registered in DBCG
- Follow-up for breast cancer recurrence in the DBCG registry
- 10 years of follow-up or through 01/07/2013

Prescription drugs

Exposure drugs:

- >=1 prescription each year, updated daily & lagged by one year

Statistical analyses:

- Crude and adjusted Cox proportional hazards regression models with time-varying drug exposure updated yearly & lagged by one year
- Sensitivity analyses altering the definition of drug exposure

	Adjusted HR	95% CI
CYP2D6 weak inhibitors		
ER-	1.71	(1.17, 2.50)
ER+	1.00	(0.74, 1.36)
CYP2D6 strong inhibitors		
ER-	0.52	(0.19, 1.41)
ER+	0.62	(0.34, 1.13)
CYP2D6 any inhibitors		
ER-	1.44	(0.99, 2.09)
ER+	0.98	(0.74, 1.30)
CYP3A4 inhibitors		
ER-	0.67	(0.25, 1.82)
ER+	1.82	(1.12, 2.96)
CYP2C19 inhibitors		
ER-	1.10	(0.67, 1.82)
ER+	0.99	(0.71, 1.38)

Conclusion: Drug-drug interaction study

- Positive association for CYP3A4 inhibition was specific to ER+/TAM+ women, as expected for a predictive marker
- The short-term use of CYP3A4-inhibiting drugs (antifungals and antibiotics) would not overlap much with five years of tamoxifen duration, so this association merits further investigation
- All associations warrant study with incorporation of functional variants in the genes encoding these enzymes

Ongoing work

- DNA extraction for comprehensive genotyping
- Optimising the analytic approach for statistical analyses
- Developing tissue microarrays for biomarker analyses

=> Collaborative projects?

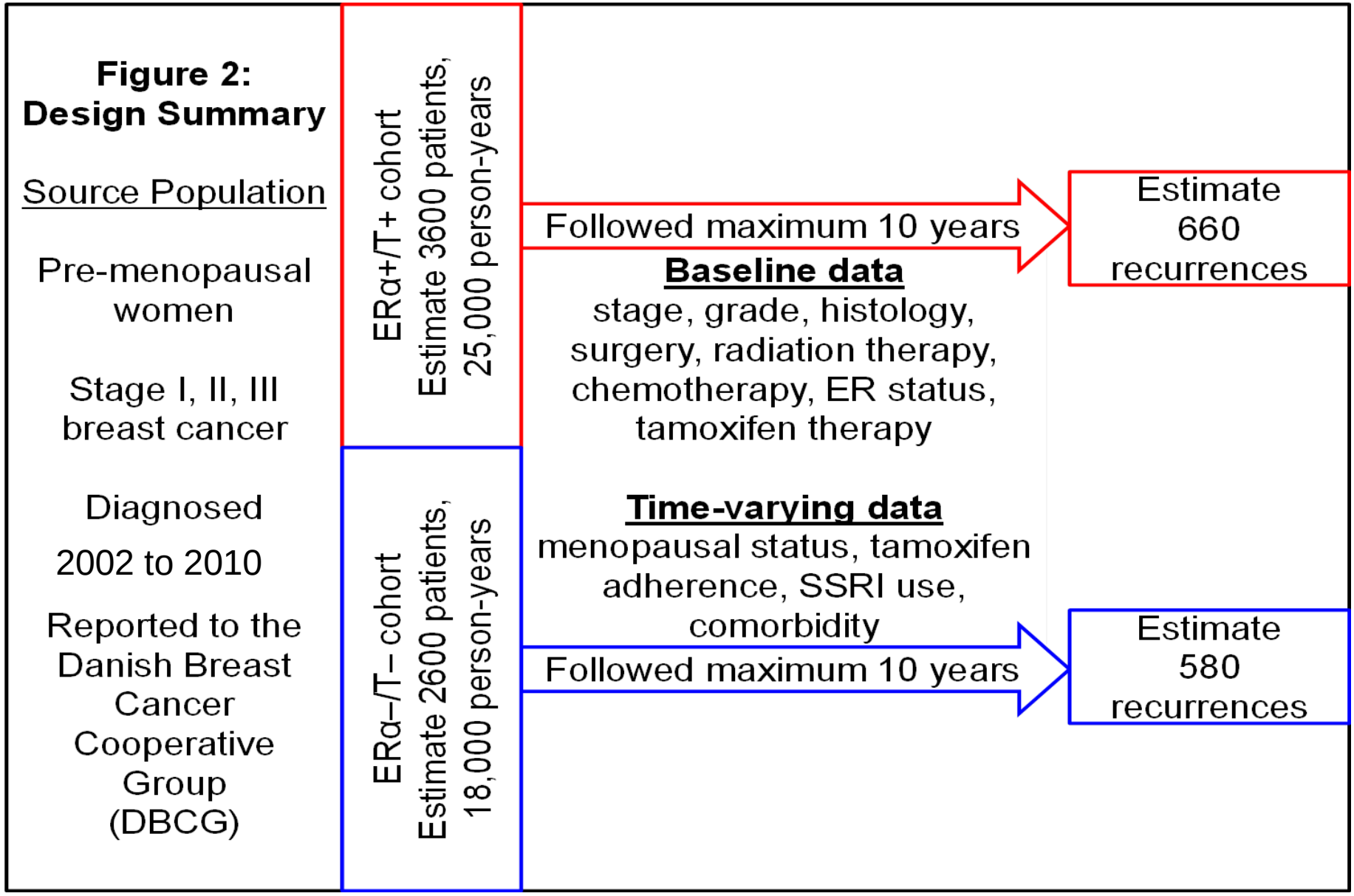
Acknowledgements & Funding

- DBCG
- Danish breast cancer pathologists , pathology departments & staff
- US National Cancer Institute, R01CA166825

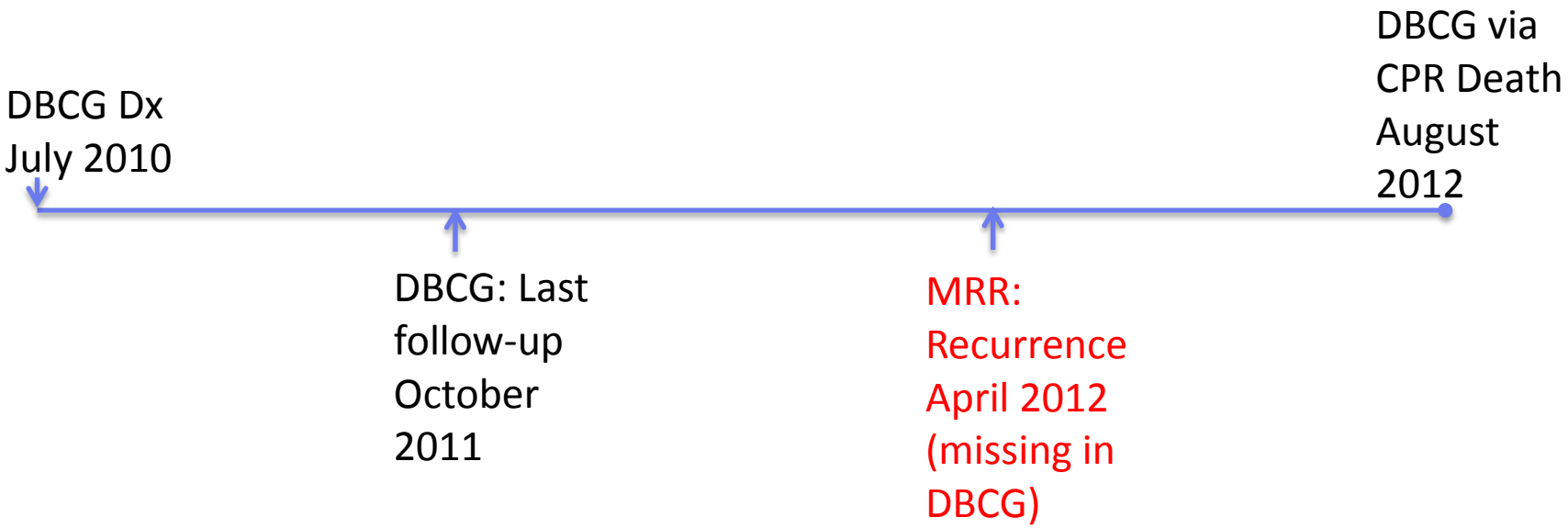

Thank you
for your
attention

Extra slides

ProBeCaRe:
Premenopausal Breast Cancer Cohort



The Few: Have no information on recurrence



Exposure drugs

CYP2D6:

- *Weak inhibitors:* mirtazapin, amitriptyline, propranolol, pindolol, zuclopenthixol, amiodarone, celecoxib, cimetidine, venlafaxine, diltiazem, diphenhydramine, citalopram, escitalopram, febuxostat, gefitinib, hydralazine, hydroxychloroquine, imatinib, methadone, propafenone, ranitidine, ritonavir, sertraline, verapamil, metoclopramide.
- *Strong/Moderate inhibitors:* fluoxetine, paroxetine, buproprion, quinidine, terbinafine, levomepromazine, duloxetine, moclobemide.

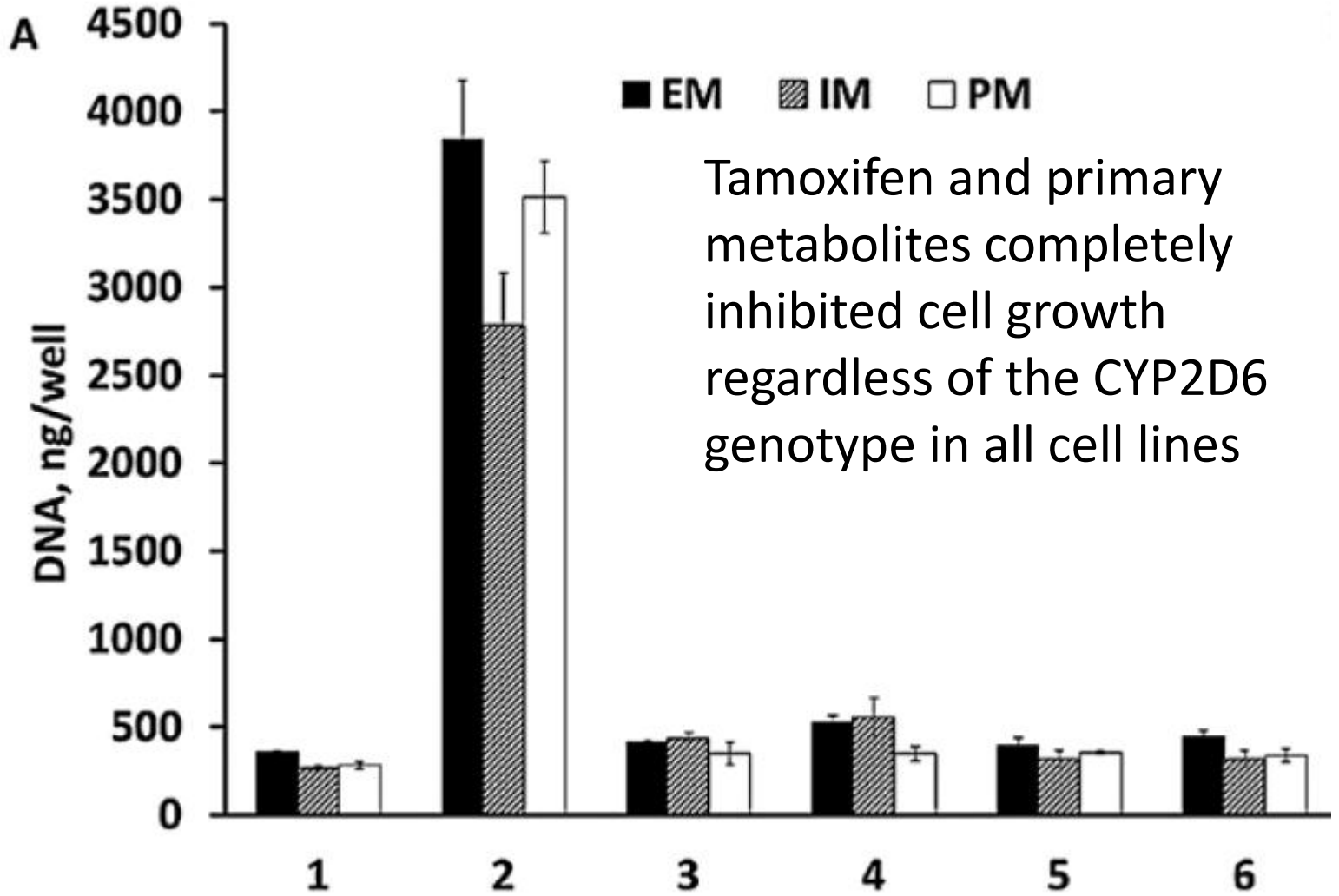
CYP2C19:

- *Strong/Moderate inhibitors:* Fluconazole, fluvoxamine (PPIs: omeprazole, esomeprazole are typically moderate inhibitors)

CYP3A4:

- *Strong inhibitors:* ketokonazole, itraconazole, posaconazole, voriconazole, clarithromycin, ritonavir, nelfinavir, saquinavir, telaprevir, indinavir, cobicistat

New perspectives: Comprehensive genotyping

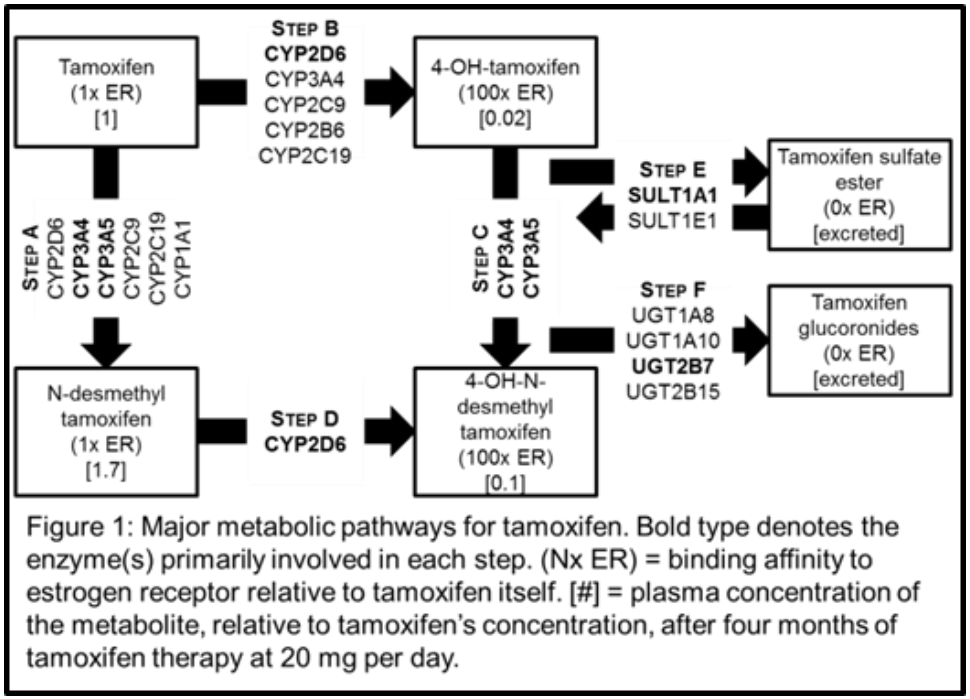


Tamoxifen and primary metabolites completely inhibited cell growth regardless of the CYP2D6 genotype in all cell lines

1 = vehicle control, 2 = postmenopausal concentration of E1/E2, 3 = tamoxifen plus primary metabolites (NDMTAM and 4OHT) (TPM), 4 = E1/E2 plus TPM, 5 = TPM plus endoxifen (EN), 6 = E1/E2 plus TPM plus EN.

Maximov et al., JNCI 2014; 106(10)

New perspectives 1: Comprehensive genotyping



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Gene	Step(s) (See Figure 1)	Number of selected functional variants	Inhibitor comedications
CYP2D6	A, B, D	19	■bupropion, cinacalcet, fluoxetine, paroxetine, quinidine, duloxetine, sertraline, terbinafine, amiodarone, cimetidine
CYP3A4	A, B, C	3	■indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin, aprepitant, erythromycin, fluconazole, verapamil, diltiazem, cimetidine, variconazole
CYP3A5	A, C	12	
CYP2C9	A, B	9	■fluconazole, amiodarone, variconazole
CYP2C19	A, B	9	
CYP1A1	A	3	
CYP2B6	B	1	
SULT1A1	E	2	
SULT1E1	E	3	
UGT1A8	F	2	
UGT1A10	F	1	
UGT2B7	F	1	
UGT2B15	F	1	

New perspectives 2: Comprehensive biomarkers

- Tamoxifen transport
  - TAM metabolites are substrates of ABC-transporters
  - Polymorphisms in transporter genes mediate TAM resistance?

- ER-beta
  - ERβ opposes ERα-mediated proliferation by heterodimerizing with it
  - This heterodimer does not stimulate proliferation equivalent to the ERα/ERα homodimer
  - Tumors that express both ERα and ERβ are therefore less aggressive than tumors that express only ERα

- Hydroxy-steroid dehydrogenase enzymes
  - Balance host estrogen concentration

Collaborators

- Aarhus University: Henrik T. Sørensen, Stephen Hamilton-Dutoit, Lars Pedersen, Sinna Ulrichsen, Anders Kjærsgaard, Anne Ording, Ylva Hellberg, Marco Mele, Deirdre Cronin Fenton
- DBCG: Peer Christiansen, Bent Ejlertsen
- Odense University: Per Damkier, Marianne Ewertz
- Emory University: Tim Lash, Mike Zwick
- Boston University: Rebecca Silliman
- University of Vermont: Thomas Ahern
- University of Louisville: Carolyn Klinge
- Stavanger University: Emiel Janssen, Kristin Jonsdottir, Nina Granlund, Håvard Søiland
- University of Bergen: Ernst Lien

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DBCG

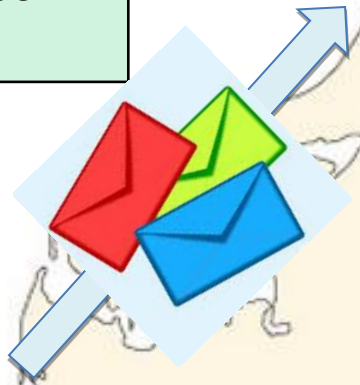
1. Dataset of premenopausal women with stage I-III breast cancer diagnosed 2002-2011

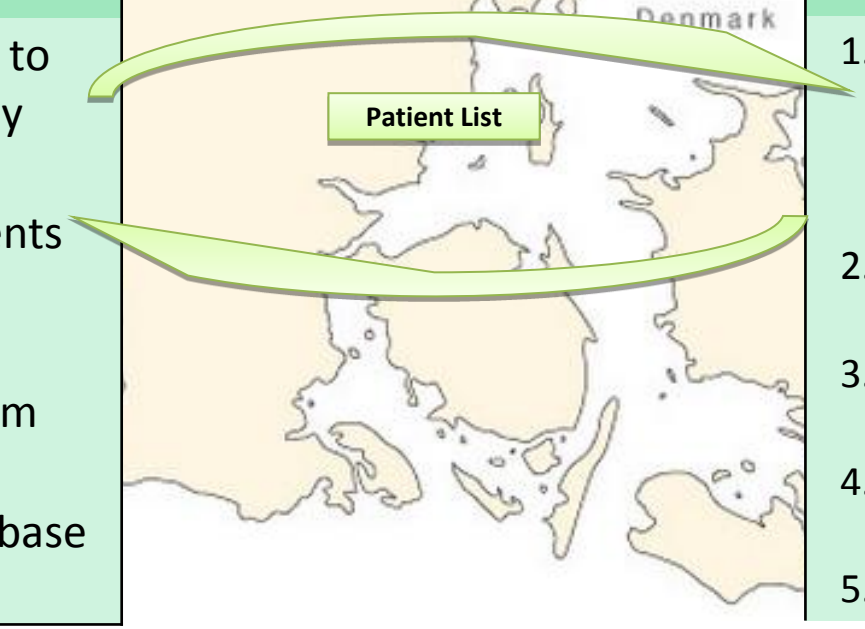
Danish Pathology Institutes

Breast cancer pathology departments

1. Collection of pathology blocks

FFPE blocks





KEA

1. Patient list and send to Institute of Pathology
2. Request blocks from pathology departments in Dk
3. Receive & register pathology blocks from departments in Dk
4. Create tracking database for blocks and slides

Aarhus University: Institute of Pathology

1. Select appropriate blocks based on tissue quantity and quality
2. Receive blocks from pathology departments in DK
3. Cut FFPE sections for DNA, RNA extraction
4. Mark slides for tissue microarray generation
5. Biomarker assays