



# Biobanking incorporating digital pathology to accomplish large scale clinicopathological projects - the PARADIGM experience

Gwen MHE Dackus<sup>1,2</sup>, Natalie D ter Hoeve<sup>2</sup>, Mark Opdam<sup>1</sup>, Nikolas Stathonikos<sup>1</sup>, Jeroen AM Belien<sup>3</sup>, Jelle Wesseling<sup>4</sup>, Annegien Broeks<sup>4,5</sup>, Elsken van der Wall<sup>6</sup>, Jacob Rousseau<sup>4</sup>, Sabine Siesling<sup>7,8</sup>, Gabe S Sonke<sup>9</sup>, Sabine C Linn<sup>1,2,9\*</sup>, Paul J van Diest<sup>2\*</sup>



Universitair Medisch Centrum  
Utrecht



\*These authors contributed equally

1. Division of Molecular Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
2. Department of Pathology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands
3. Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
4. Department of Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
5. Core Facility Molecular Pathology and Biobanking, Division of Molecular Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands
6. Division of Internal Medicine and Dermatology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands
7. Department of Research, Netherlands Comprehensive Cancer Organization (IKNL), Utrecht, The Netherlands
8. Department of Health Technology & Services Research, MIRA, Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands
9. Department of Medical Oncology, The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands

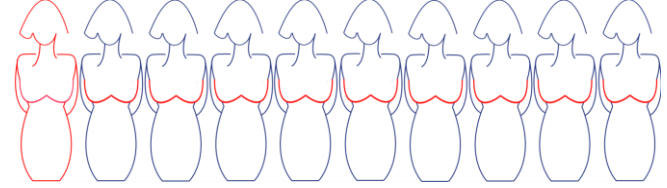




# Background - PARADIGM

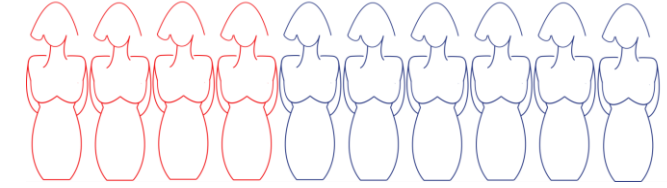
## All breast cancer patients

- 6-7% ≤40 years



## All young cancer patients

- 40% breast cancer



- Breast cancers in young women behave more aggressively
  - Mortality is higher
  - Proper tools for prognostication are lacking
  - Most young women receive adjuvant systemic treatment
- Several women would have been cured by locoregional treatment alone



# Aim & ethical approval - PARADIGM

## PARADIGM

**P**Atients with **bReAst** cancer **D**ia**G**nosd pre**M**enopausally

## Aim

To reduce the overtreatment of women diagnosed with breast cancer aged  $\leq 40$  years

## Ethical approval

PARADIGM uses observational data and left over archival patient material - secondary use (opt-out). The study was reviewed and approved by the Institutional Review Board (IRB) January 24<sup>th</sup>, 2013





## PARADIGM workflow

### 1. Identify cases



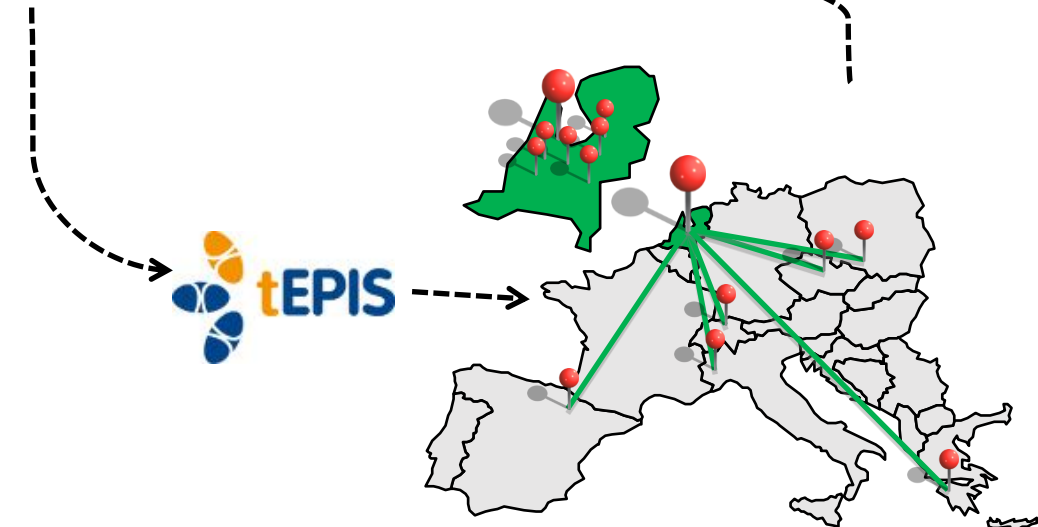
### 2. Linkage & tissue acquisition



### 3. Digital pathology

- Cutting slides
- Staining slides
- Scanning slides
- Quality control
- Online revision
- eCRFs
- Data-management
- Data storage

### 4. PARADIGM biobank & early results





## PARADIGM workflow – Case selection (1)

### 1. Identify cases



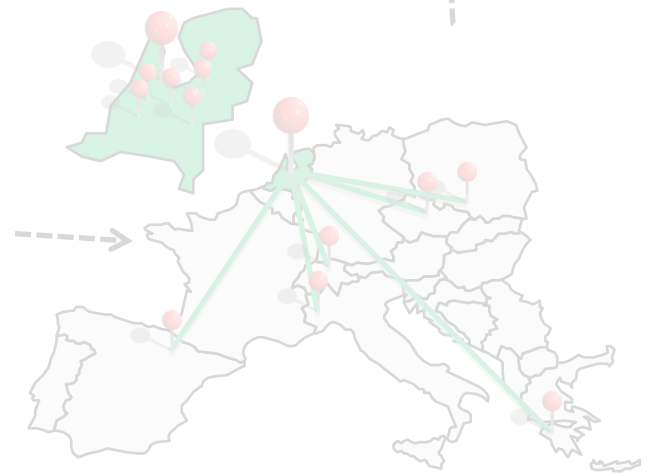
### 2. Linkage & tissue acquisition



### 3. Digital pathology

- Cutting slides
- Staining slides
- Scanning slides
- Quality control
- Online revision
- eCRFs
- Data-management
- Data storage

### 4. PARADIGM biobank & early results





## PARADIGM workflow – Case selection (2)

### 1. Identify cases



#### Inclusion criteria NCR (n=3524):

- Diagnosed between 1989 and 2000
- Females  $\leq 40$  years
- Primary invasive breast cancer
- Histologically proven disease
- No history of prior malignancies
- No (distant) metastasis at diagnosis
- No (neo-)adjuvant systemic treatment



## PARADIGM workflow – Linkage & tissue acquisition (1)

### 1. Identify cases



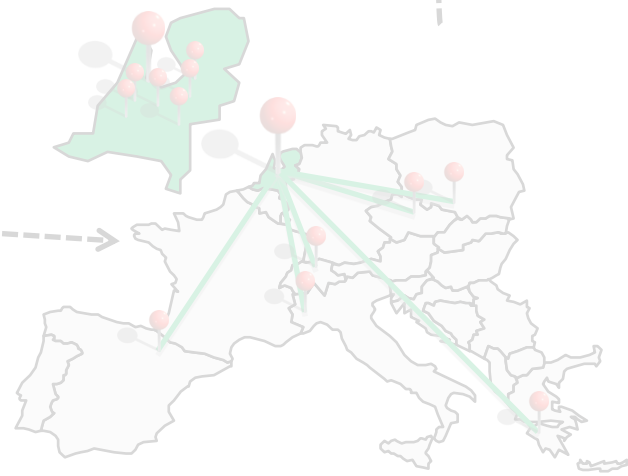
### 2. Linkage & tissue acquisition



### 3. Digital pathology

- Cutting slides
- Staining slides
- Scanning slides
- Quality control
- Online revision
- eCRFs
- Data-management
- Data storage

### 4. PARADIGM biobank & early results







# PARADIGM workflow – Linkage & tissue acquisition (2)

1. Identify cases



2. Linkage & tissue acquisition



- Anonymous linkage NCR-PALGA by Zorg – TTP (trusted third party)
- >15,000 FFPE blocks on 2,777/3,524 patients were retrieved (78.8%)
- 1214 of 3524 patients were excluded
  - Database mismatch (n = 148)
  - Not meeting inclusion criteria (n = 454)
  - No tissue available (n = 612)
- PARADIGM cohort currently consists of 2310 patients





## PARADIGM workflow – Digital pathology (1)

### 1. Identify cases



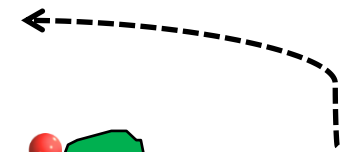
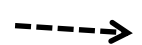
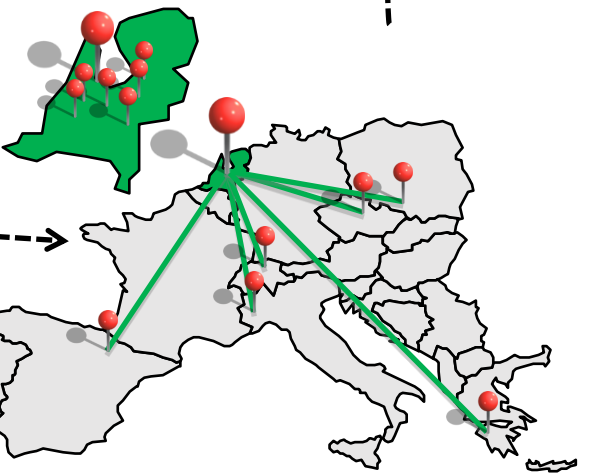
### 2. Linkage & tissue acquisition



### 3. Digital pathology

- Cutting slides
- Staining slides
- Scanning slides
- Quality control
- Online revision
- eCRFs
- Data-management
- Data storage

### 4. PARADIGM biobank & early results





# PARADIGM workflow – Digital pathology (2)



Philips ultrafast scanner 1.6.1.3 RA (Amsterdam, The Netherlands)

Slide capacity: 320 slides  
Imaging time: 45 sec  
Pixel size: 0.25µm

- Cut new H&E slides for all >15,000 FFPE blocks
- Scanned slides onto tEPIS
  - Quality control
- Size: 50MB - 8GB for 15mm<sup>2</sup> tissue (depends on tissue size and file compression)



Nanozoomer XR C12000-21/-22 (Hamamatsu photonics, Hamamatsu, Shizuoka, Japan)

Slide capacity: 300 slides  
Imaging time: 60 sec  
Pixel size: 0.23µm



# PARADIGM workflow – Digital pathology – tEPIS (3)



- Slides were scanned onto the TraIT Enhanced Pathology Image Sharing system (tEPIS)
- Scanning performed 2014 - 2016
- Image storage (~25TB)
  - tEPIS platform (redundant storage)
  - Back-up on the UMCU Utrecht network share, hosted by the central IT department (redundant storage)







## PARADIGM workflow – Digital pathology – OpenClinica (4)

**REVISION (0/21)**


Title: Revision

Page:  Mark CRF Complete   


**Tumor**

Tumor present for revision?  Yes  No \* 


**In-situ carcinoma**

In-situ carcinoma?  Yes  No 


**Normal tissue**


Normal tissue present?  Yes  No \* 

**Lymphnodes**

Lymphnodes present?  Yes  No \* 

**Remarks**

Remarks?  

Mark CRF Complete   

- 16 dedicated European breast pathologists
- Performed revisions using tEPIS - OpenClinica
- OpenClinica
  - Web based data capturing tool
  - Linked to tEPIS
  - Four tissue types
  - Four study specific eCRFs
  - 19 parameters





## PARADIGM workflow – Digital pathology – Reflection (5)

### Advantages

- It is not necessary to send around slides
- More (remote) pathologists can be reached
- Pathologists can work from every physical location
- More economic use of patient tissues
- Digitalized images remain stable over time
- Linkage of viewer with database in research limits errors
- Images can be used for other purposes

### Challenges

- Time consuming workflow
- Need for specialized equipment and IT infrastructure
- Digital revisions may take more time
- Counting mitoses is more challenging
- More expensive
- Need for both physical and online storage space



## PARADIGM workflow – PARADIGM biobank & early results (1)

### 1. Identify cases



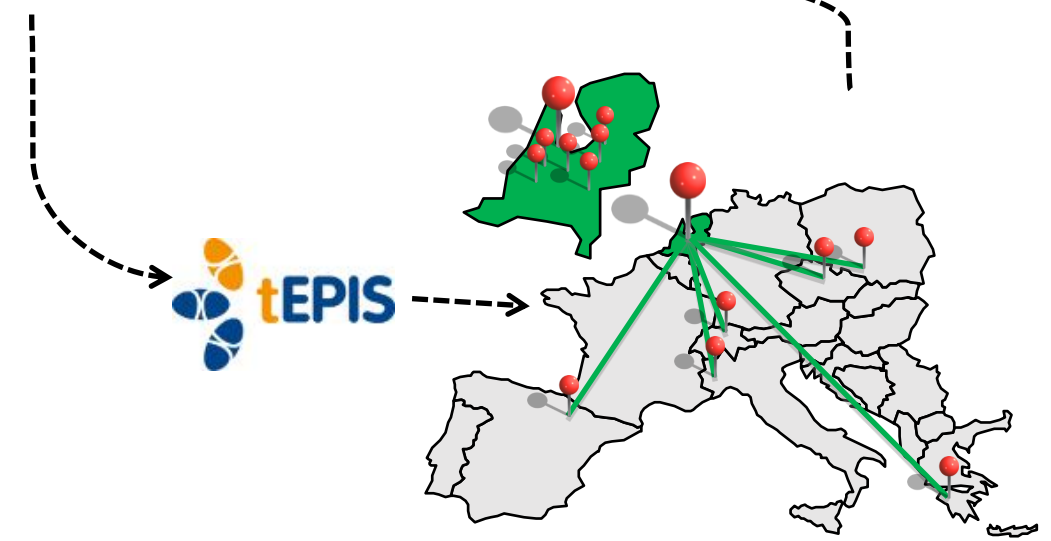
### 2. Linkage & tissue acquisition



### 3. Digital pathology

- Cutting slides
- Staining slides
- Scanning slides
- Quality control
- Online revision
- eCRFs
- Data-management
- Data storage

### 4. PARADIGM biobank & early results





## PARADIGM workflow – PARADIGM biobank & early results (2)

	HR+HER2-	HR+HER2+	HR-HER2+	TN
<b>Number of patients</b>	1144	233	98	490
<b>Mean age (range), years</b>	35 (20-40)	35 (23-39)	35 (24-39)	35 (22-39)
<b>Mean follow-up (range), years</b>	15.8 (0.3-24.9)	13.4 (0.8-24.9)	14.5 (0.7-25.0)	15.1 (0.1-25.0)
<b>Histological subtype</b>				
Ductal Carcinoma	70.5%	78.5%	82.7%	80.4%
Lobular Carcinoma	8.3%	6.0%	-	0.4%
Other	21.2%	15.5%	17.3%	19.2%
<b>Grade</b>				
I	28.6%	6.9%	3.1%	0.6%
II	47.8%	48.1%	30.6%	13.9%
III	23.5%	44.6%	66.3%	85.5%
Unknown	0.1%	0.4%	-	-
<b>Pathological T stage</b>				
pT1	71.9%	69.0%	61.2%	55.7%
pT2	23.3%	27.5%	27.6%	39.0%
pT3	0.7%	1.3%	-	2.0%
pT4	0.3%	0.9%	-	0.2%
Unknown	3.8%	1.3%	11.2%	3.1%

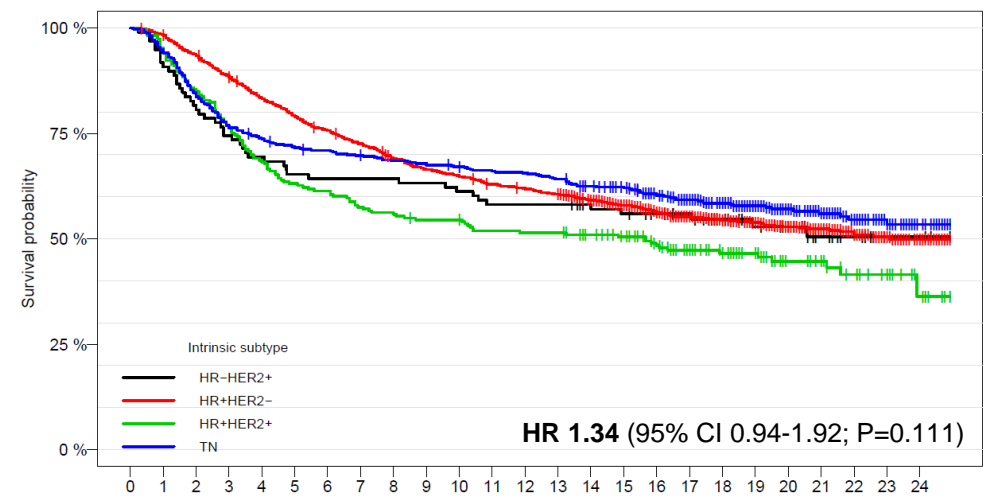
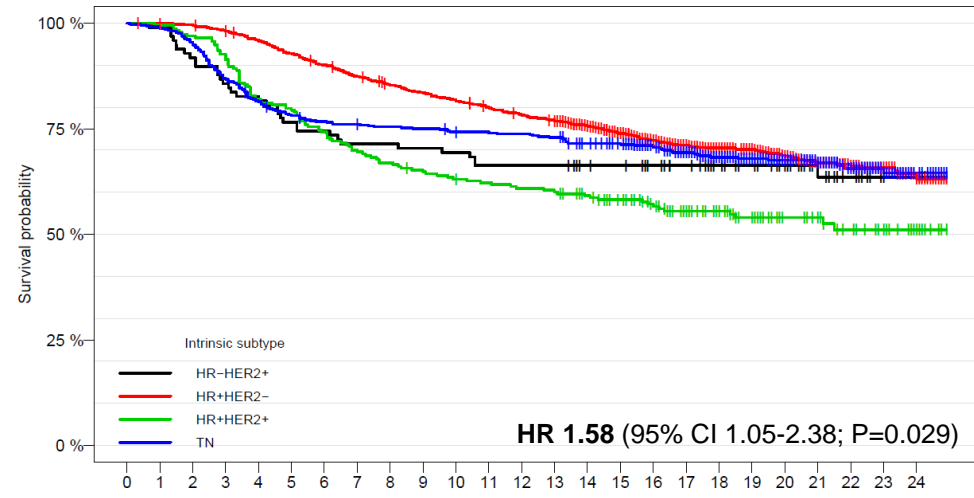




# PARADIGM workflow – PARADIGM biobank & early results (3)

## Overall Survival (OS)

## Recurrence Free Survival (RFS)



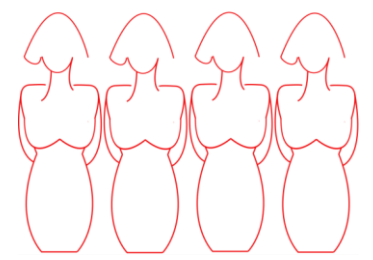
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Number at risk	98	88	75	70	68	65	59	49	35	12	1															
HR-HER2+	98	88	75	70	68	65	59	49	35	12	1															
HR+HER2-	1144	1130	1058	981	927	875	722	507	306	144	1															
HR+HER2+	233	225	186	159	146	140	120	86	47	24	1															
TN	490	447	382	367	359	354	329	249	157	70	1															

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Number at risk	98	77	64	63	60	57	50	40	27	8	1														
HR-HER2+	98	77	64	63	60	57	50	40	27	8	1														
HR+HER2-	1144	1040	903	811	736	690	562	393	233	109	1														
HR+HER2+	233	192	147	131	126	118	104	71	38	18	1														
TN	490	397	350	336	324	313	286	211	130	57	1														





# PARADIGM workflow – PARADIGM biobank & early results (4)



- Clinical data n= 3,525
- >15,000 on n=2,777
  - FFPE blocks
  - H&E slides
  - Digitalized H&E slides
- 39 TMAs
  - 34 automated
  - 5 manual
- IHC/SISH on n=1,965
  - ER
  - PR
  - HER2
- Triple negative patients n=490
  - DNA & RNA n=467
  - RNAseq data n=467



## Future plans - PARADIGM

- To develop:
  - a combined clinico-molecular prognosticator for the triple negative (TN) patient subset
  - prognostic models directly derived from the image data using deep learning approaches
  
- To estimate:
  - the prognostic performance of established clinical and molecular prognosticators on breast cancer patients  $\leq 40$  years
  - the prognosis of women with breast cancer during pregnancy in the absence of adjuvant systemic treatment



# Acknowledgements

## NKI

Sabine Linn  
Mark Opdam  
Michael Hauptmann  
Katarzyna Jozwiak  
Marjanka Schmidt  
Petra Nederlof  
Frans Hogervorst  
Efraim Rosenberg  
Lodewyk Wessels  
Marlous Hoogstraat  
Gabe Sonke  
Sophie Bosma

Ewa Chmielik  
Ales Ryska  
Jelle Wesseling  
Emilie Groen  
Vincent Smit  
Antien Mooyaart  
Jos Bart  
Stefan Willems  
Carolien van Deurzen  
Pieter Westenend  
Willem Vreuls  
Esther Koop

## Pathologists

Paul van Diest  
Zsuzsanna Varga  
Alicia Cordoba  
Jan van den Tweel  
Vicky Zolota  
Anna Sapino



## CFMPB

Annegien Broeks  
Esther Holman  
Jose Overwater  
Sanne Broersen  
Sten Cornelissen  
Chantall Curial  
Dennis Peters  
Donnee Majoor  
Ingrid Hofland

## VU

Ellen Engelhardt

## University of Cambridge

Paul Pharoah

## TU Eindhoven

Mitko Veta



## Netherlands Cancer Registry:

Sabine Siesling  
Otto Visser  
Marjorie de Kok  
Margery Duin  
Irma van Beuningen  
Jittie Streurman  
Rianne Hoekman  
Sonja van der Mije  
Connie Spijkers  
Annemarie Eeltink  
Erica Masseling  
Gitty Jaanen  
Adri Voogd  
Marissa van Maaren

## TraIT

Jeroen AM Belien  
Jacob Rousseau



Universitair Medisch Centrum Utrecht



A SISTERS HOPE

