Prognostic Relevance of Circulating Tumor Cells in the Peripheral Blood of Primary Breast Cancer Patients

B. Rack, C. Schindlbeck, U. Andergassen, R. Lorenz, T. Zwingers, A. Schneeweiss, W. Lichtenegger, M. W. Beckmann, H. Sommer, K. Pantel, K. Friese, W. Janni

for the SUCCESS study group

In collaboration with

Introduction

- Prognostic relevance of isolated tumor cells in bone marrow (ITCs) in EBC is confirmed
- Circulating tumor cells (CTCs) in blood are associated with reduced progression-free and overall survival in MBC
- Marker for treatment monitoring might inprove patient care
- Lack of data for relevance of CTCs in EBC

Braun et al, N Engl J Med 342: 525-533
Braun et al, N Engl J Med 2005; 353(8):793-802.
Janni et al, Breast Cancer Res Treat 2006; 100(Suppl 1)
Cristofanilli et al, N Engl J Med 2004; 351(8):781-791.
Hayes et al, Clin Cancer Res 2006; 12(14 Pt 1):4218-4224
Budd et al, Clin Cancer Res 2006; 12(21):6403-6409.
Hayes et al., Prog Mol Biol Transl Sci 2010; 95:95-112

Hypothesis

The presence of Circulating Tumor Cells (CTCs) in peripheral blood predicts reduced disease-free and overall survival in primary breast cancer.

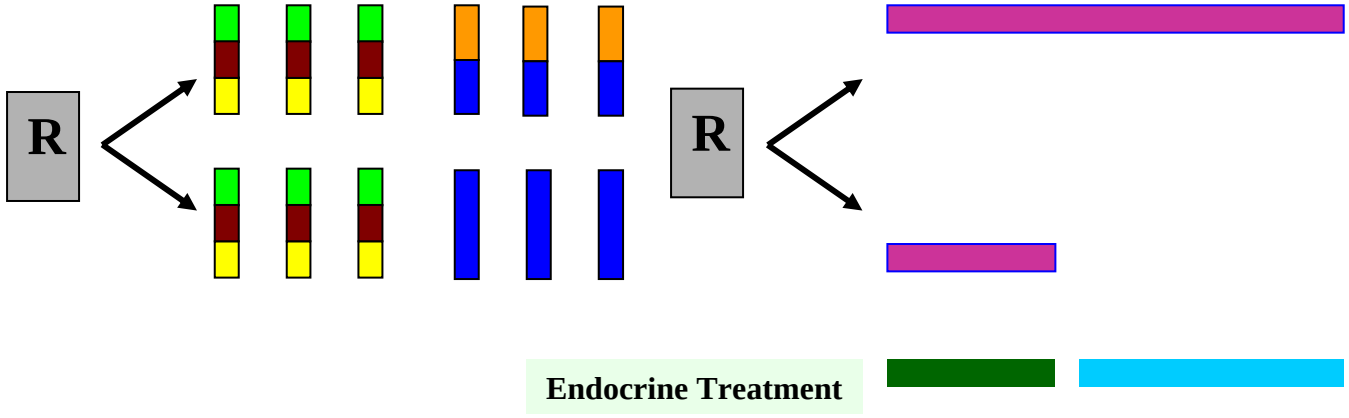
Inclusion Criteria

- Early breast cancer patients pT1-4 pN0-3 pM0
- Need for chemotherapy based on
  N+ or high risk N- (pT ≥ 2, G3, ≤ 35 years, HR-)
- Adjuvant chemotherapy treatment within the German multicenter SUCCESS trial
- Primary surgery with complete resection of the invasive breast cancer
- Detection of CTCs using the CellSearch system

SUCCESS Study Design

***S**imultaneous St**u**dy of Docetaxel-Gem**c**itabine **C**ombination adjuvant treatment, as well as **E**xtended Bi**s**phosphonate and **S**urveillance-Trial*

- Prospektive randomised controlled phase III study
- 2x2 faktorial design
- High risk N0 and N+ primary breast cancer pts
- n = 3.658



5- FU 500 mg/m², Epirubicin 100 mg/m², Cyclophosphamide 500 mg/m² q3w

Docetaxel 100 mg/m² q3w

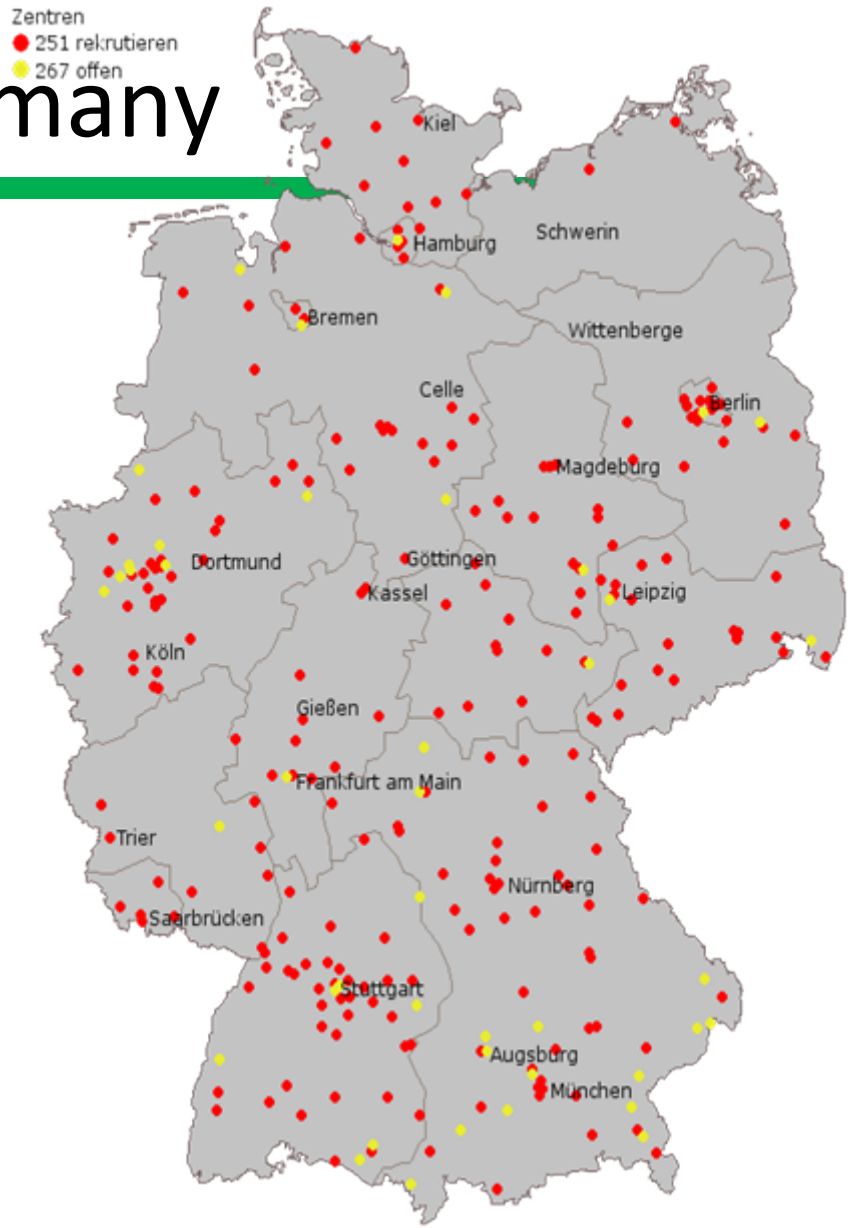
Docetaxel 75 mg/m², Gemcitabine 1000mg/m² D1,8 q3w

Zoledronate 4mg x 2a vs 5a (q3mx24m, vs. q3mx24m followed by q6mx36m)

Tamoxifen 20 mg qid p.o.x 2 a
(plus Goserelin 3.6 mg depot x 2 a in premenopausal women)

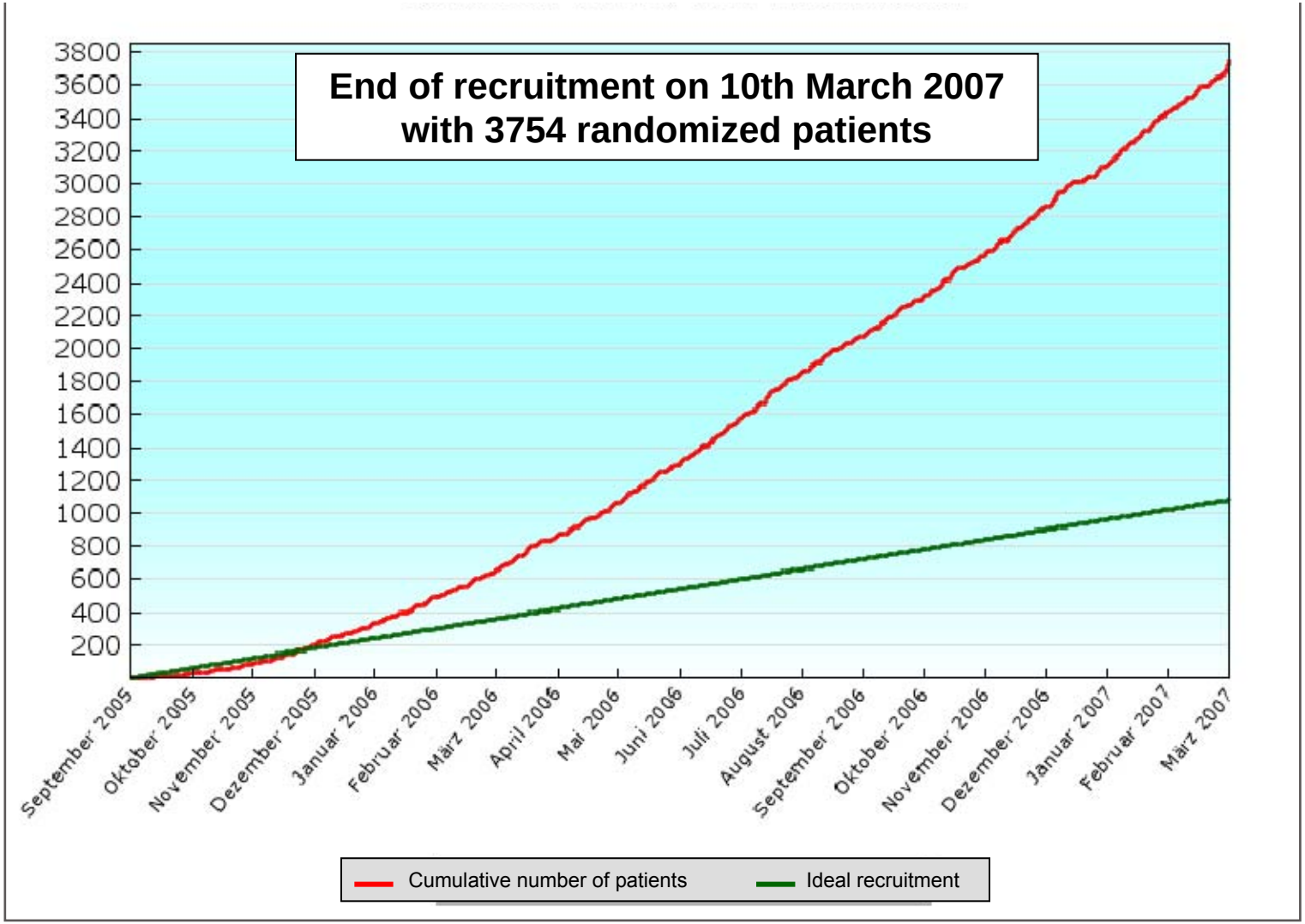
Anastrozole 1 mg qid p.o.x 3 a in postmenop. pts (Tam in premenop. pts)

Study Centers in Germany

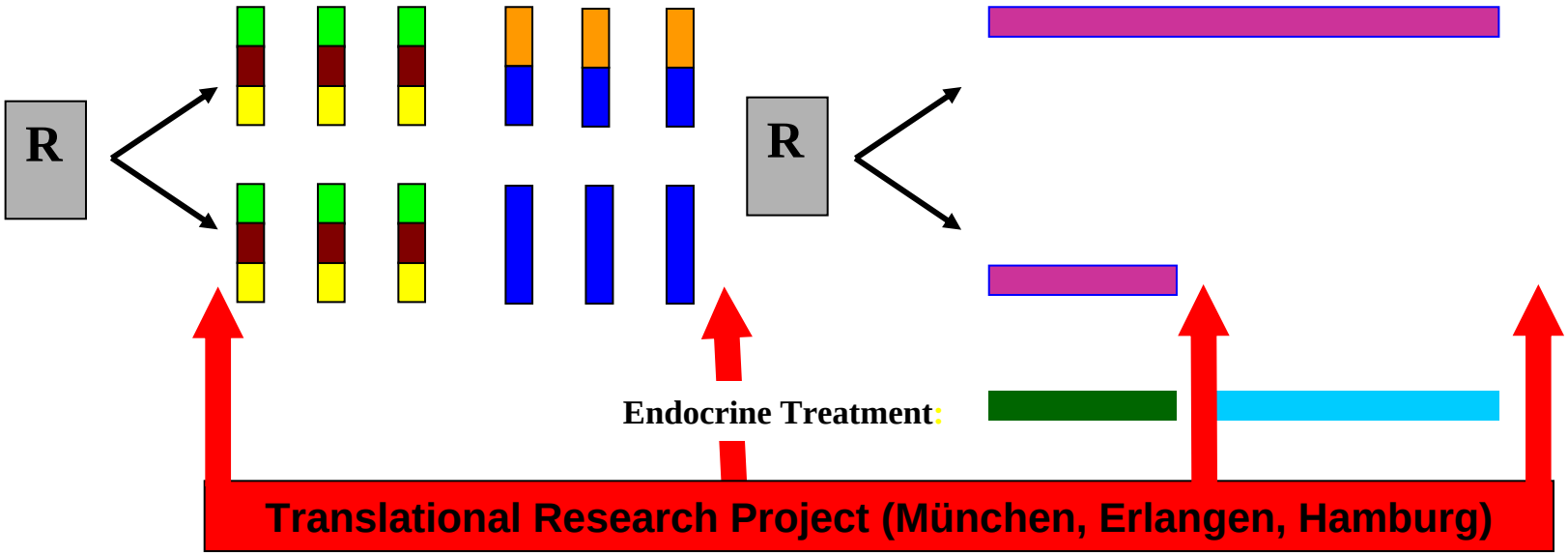


251 active study centers

Patient Recruitment within the SUCCESS-Study



SUCCESS Study Design



Blood sampling for CTC and tumor marker detection at 4 different time points during treatment

- before chemotherapy
- after chemotherapy
- after 2 years of endocrine/zoledronate treatment
- after 5 years of endocrine/zoledronate treatment

Detection of CTCs
By CellSearchSystem

- Analysis of 23 ml of peripheral blood
- Immunomagnetic enrichment using Anti-Epcam-Antibodies
- Immunocytochemical fluorescence staining for CD45 (Leukocytes) and Cytokeratine 8,18,19 (epithelial cell marker)
- Automated preparation and analysis by CellSearchSystem and CellSpotterAnalyzer (Veridex)
- Centralized blood preparation and CTC detection at the LMU Munich

Patients

- 2026 patients with primary breast cancer pT1-4 pN0-3 pM0
- CellSearch analysis available after complete tumor resection but before chemotherapy
- Patients with evidence of ≥ 1 CTC counted as positive
- Median follow-up 35 months
- 114 recurrences occurred
- 66 patients died of breast cancer

Prevalence of CTCs in peripheral blood in early breast cancer

	No CTCs in blood	CTCs in blood
Breast Cancer	**1591**	**435**
Patients Stage I – III	**78.5%**	**21.5%**

Prevalence of CTCs in peripheral blood in early breast cancer

Numbers of CTC	No. of patients	% of all patients
0	1591	78.5
1	245	12.1
2	86	4.2
3-5	56	2.7
6-10	21	1.0
>10	27	1.3
ALL	2026	100.0

Median 1.3 CTCs
Range 1 – 827 cells

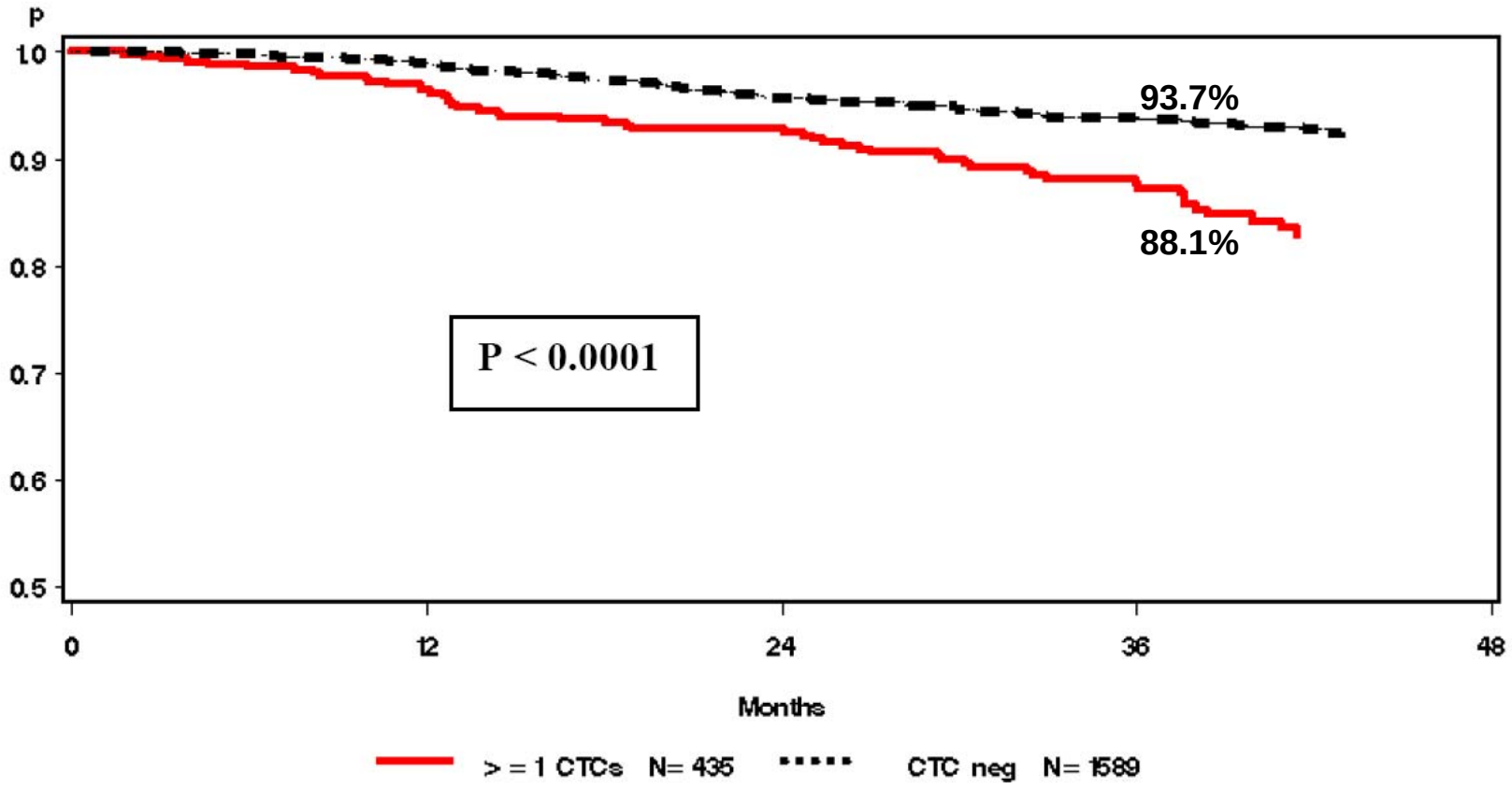
CTC Prevalence in Patient Subgroups I

Characteristic	CTC positive 435 (21.5%)	CTC negative 1591 (78.5%)	p-value
Age	**53.8**	**53.2**	**0.26**
Tumor size			**0.19**
pT1a	16 (1)	1 (0.2)	
pT1b	86 (5.4)	19 (4.4)	
pT1c	561 (35.3)	139 (32.0)	
pT2 – 4	906 (56.9)	268 (61.6)	
pTx	22 (1.4)	7 (1.6)	
Lymph Node Metastases			**<0.001**
pN0 / X	556 (35.0)	136 (31.3)	
pN1	747 (47.0)	178 (40.9)	
pN2	208 (13.0)	72 (16.5)	
pN3	80 (5.0)	49 (11.3)	
Grading			**0.19**
G1	85 (5.3)	14 (3.2)	
G2	740 (46.5)	206 (47.4)	
G3	753 (47.3)	212 (48.7)	

CTC Prevalence in Patient Subgroups II

Characteristic	CTC positive	CTC negative	p-value
Hormone Receptor Status			**0.64**
Negative	450 (8.3)	128 (9.4)	
Positive	1141(71.7)	307 (70.6)	
Histological Type			**0.15**
Ductal	1285 (80.8)	344 (79.1)	
Lobular	176 (11.1)	62 (14.3)	
Mixed ductal-lobular	118 (7.4)	27 (6.2)	
Menopausal Status			**0.2**
Premenopausal	672 (42.2)	169 (68.9)	
Postmenopausal	919 (57.8)	266 (61.1)	
Primary Operation			**0.27**
Breast Conserving	1119 (70.3)	295 (67.8)	
Mastectomy	460 (28.9)	138 (31.7)	
Radiotherapy			**0.11**
Performed	1211 (76.1)	212 (48.7)	
Not performed	460 (28.1)	138 (31.7)	
Systemic Therapy			**0.10**
Chemotherapy – FEC-D	820 (51.8)	205 (47.1)	
Chemotherapy – FEC-DG	771 (48.5)	230 (52.9)	

Disease-free Survival by CTCs before chemotherapy



	CTC+	CTC-
Recurrences	41 / 436	78 / 1589
Mean Survival Time	38.5 mon	41.4 mon

Multivariate Analysis for DFS

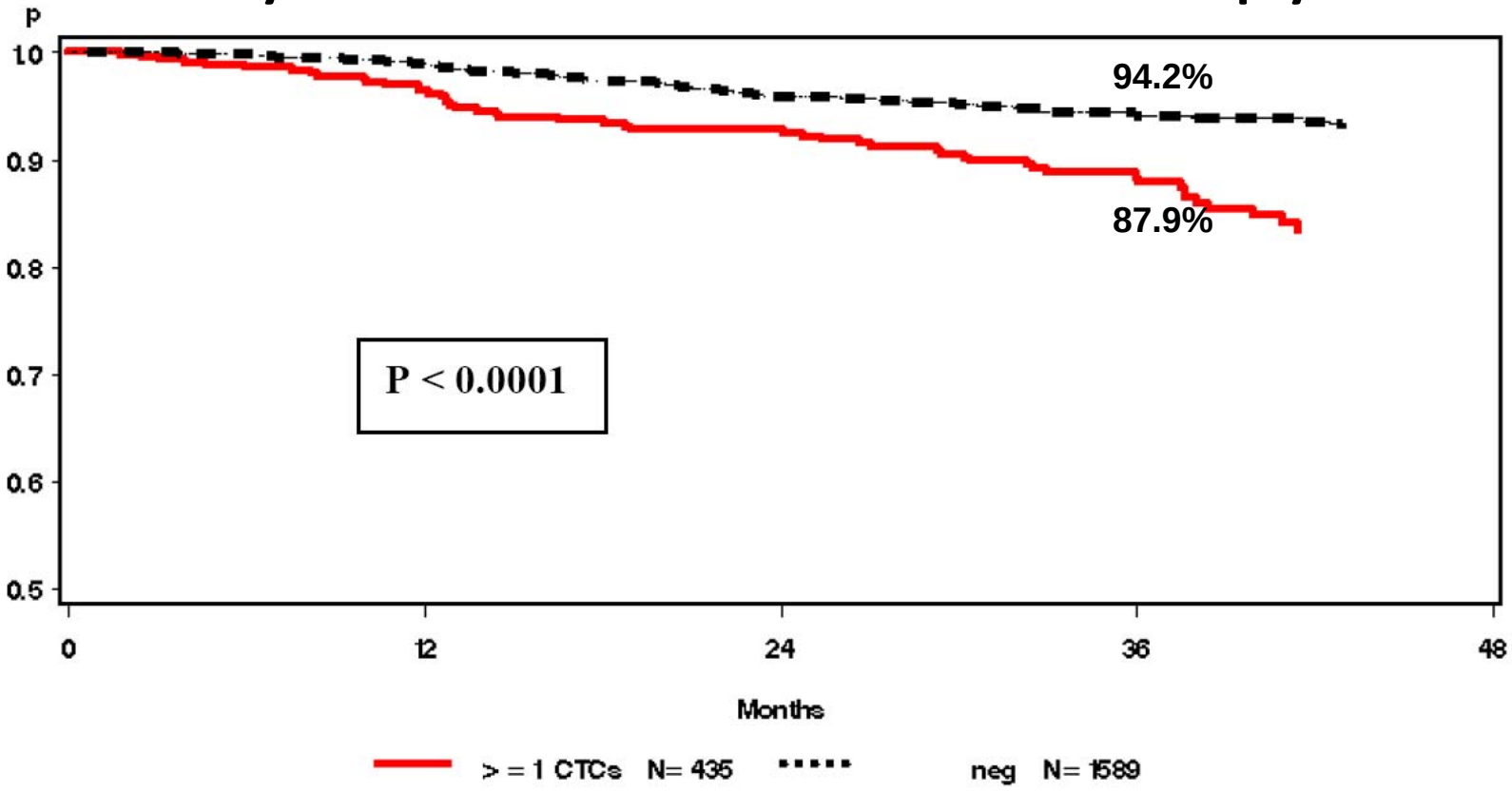
Variable	HR	95% CI	p-value
CTCs in blood			
pos/neg	**1.878**	**1.318 – 2.676**	**0.0005**
Hormone receptor status			
pos/neg	2.073	1.434 – 2.996	0.0001
Lymph Node Involvement			
pos/neg	1.698	1.434– 2.012	<.0001
Grading			
G1 vs. G2-3	2.961	2.004 – 4.375	<.0001
Tumor size			
T1 vs. T2-4	1.629	1.296 – 2.048	**<**.0001

Multivariate Analysis for DFS for different CTC cut-offs

Variable	Hazard Ratio adjusted for treatment		
	0 vs. ≥ 1	0, 1 vs. ≥ 2	0-4 vs. ≥ 5
CTCs in blood pos/neg	**1.878 ***	**2.825 ***	**4.035 ***
Hormone receptor status pos/neg	**2.073 ***	**2.020 ***	**3.273 ***
Lymph Node Involvement pos/neg	**1.698 ***	**1.664 ***	**1.574 ***
Grading G1 vs. G2-3	**2.961 ***	**3.182 ***	**3.245**
Tumor size T1 vs. T2-4	**1.629 ***	**1.655 ***	**2.573 ***

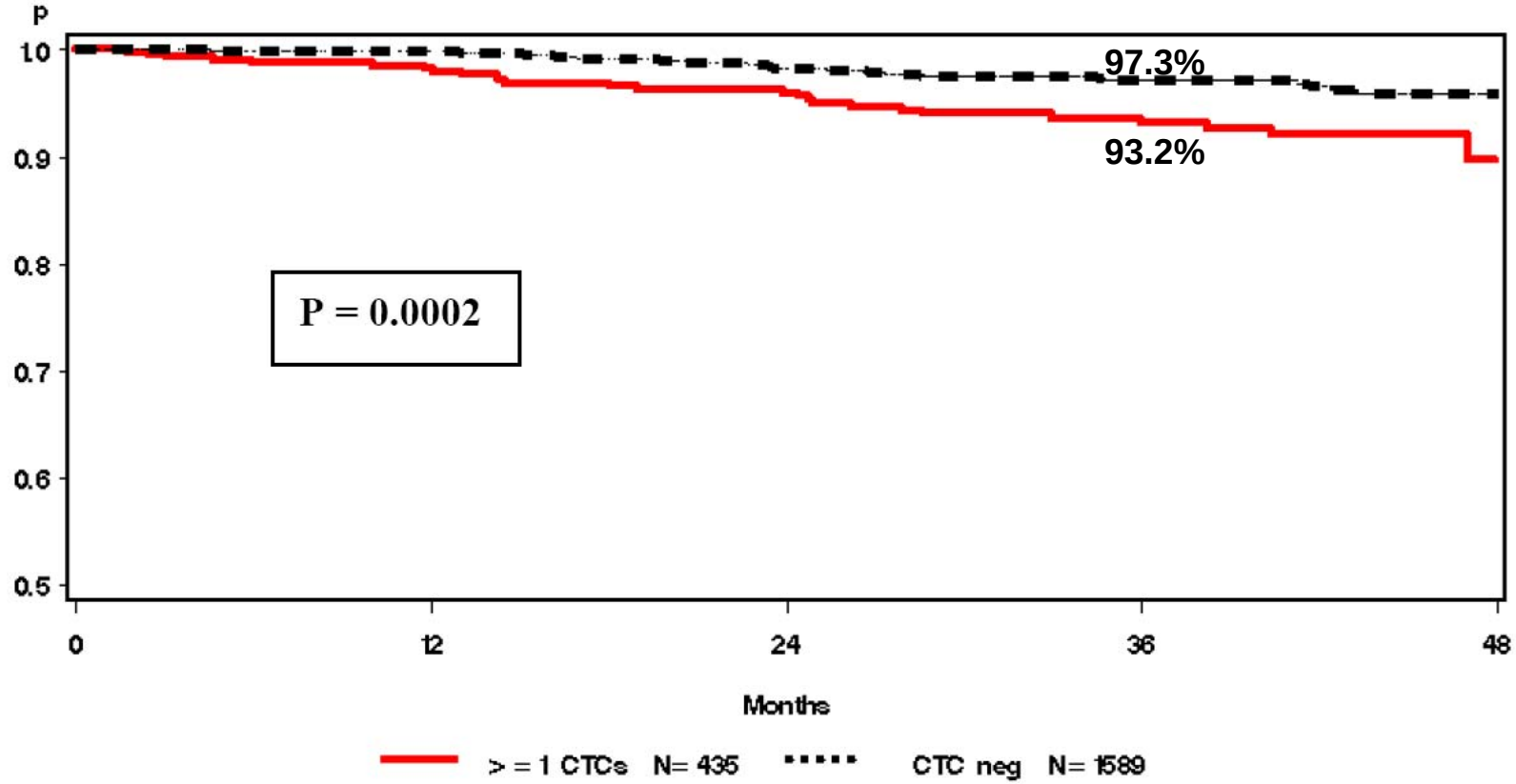
*** P < 0.05**

Distant Disease-free Survival by CTCs before chemotherapy



	CTC+	CTC-
Recurrences	41 / 436	72 / 1589
Mean Survival Time	38.6 mon	41.5 mon

Overall Survival by CTCs before chemotherapy



	CTC+	CTC-
Recurrences	23 / 436	33 / 1589

Multivariate Analysis for OAS

Variable	HR	95% CI	p-value
CTCs in blood			
pos/neg	**1.907**	**1.142 – 3.183**	**0.0136**
Hormone receptor status			
pos/neg	3.326	1.948 – 5.678	<.0001
Lymph Node Involvement			
pos/neg	1.835	1.448 – 2.327	<.0001
Grading			
G1 vs. G2-3	3.287	1.782 – 6.064	0.0001
Tumor size			
T1 vs. T2-4	1.879	1.363 – 2.590	0.0001

Multivariate Analysis for OAS for different CTC cut-offs

Variable	Hazard Ratio adjusted for treatment		
	0 vs. ≥ 1	0, 1 vs. ≥ 2	0-4 vs. ≥ 5
CTCs in blood pos/neg	**1.907 ***	**2.242 ***	**3.051 ***
Hormone receptor status pos/neg	**3.326 ***	**3.287 ***	**7.858 ***
Lymph Node Involvement pos/neg	**1.835 ***	**1.825 ***	**1.805 ***
Grading G1 vs. G2-3	**3.287 ***	**3.476 ***	**3.001**
Tumor size T1 vs. T2-4	**1.879 ***	**1.863 ***	**3.914 ***

SUCCESS

*** P < 0.05**

LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN

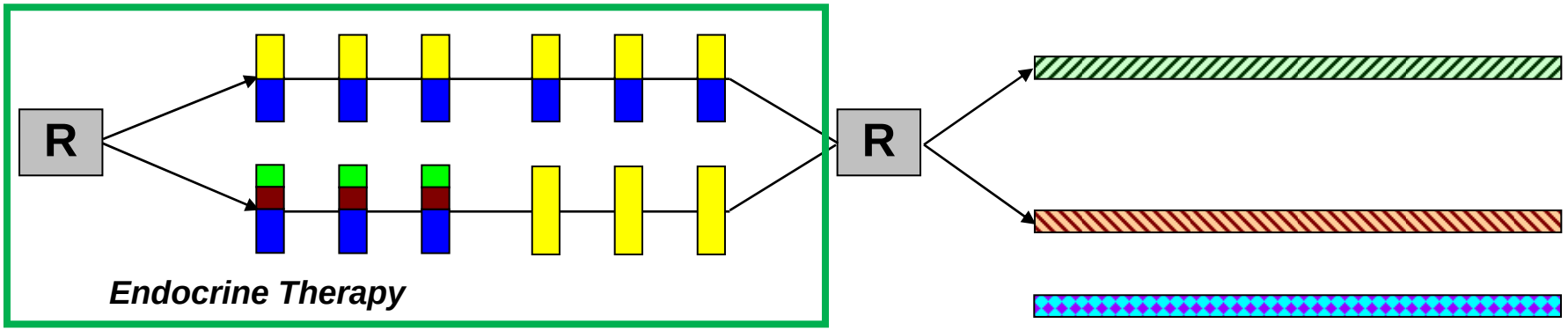
Limitations

- Interims analysis
- Short follow-up
- Low Prevalence of CTCs in the adjuvant setting

Conclusions

- CTCs were detected in 21.5% of early breast cancer patients before the start of adjuvant chemotherapy.
- Presence of CTCs predicted poor disease-free ($p < 0.0001$), distant disease-free ($p < 0.001$) and overall survival ($p = 0.0002$).
- The SUCCESS trial confirms independent prognostic relevance of CTCs in early breast cancer in a large patient cohort.
- Ongoing trials evaluate
  - CTCs as marker for early prediction of treatment efficacy
  - Efficacy of more individualized treatment approaches based on phenotyping of minimal residual disease

Study Design



MRD-Überwachung im peripheren Blut

Legende

- Docetaxel 75 mg/m² + Cyclophosphamid 600 mg/m² — q3w
- 5- FU 500 mg/m² + Epirubicin 100 mg/m² + Cyclophosphamid 500 mg/m² — q3w
- Docetaxel 100 mg/m² — q3w

- Telefone based lifestyle intervention: Reduction of BMI, increase of physical activity (2 year program)
- Controll-Arm (no lifestyle intervention)

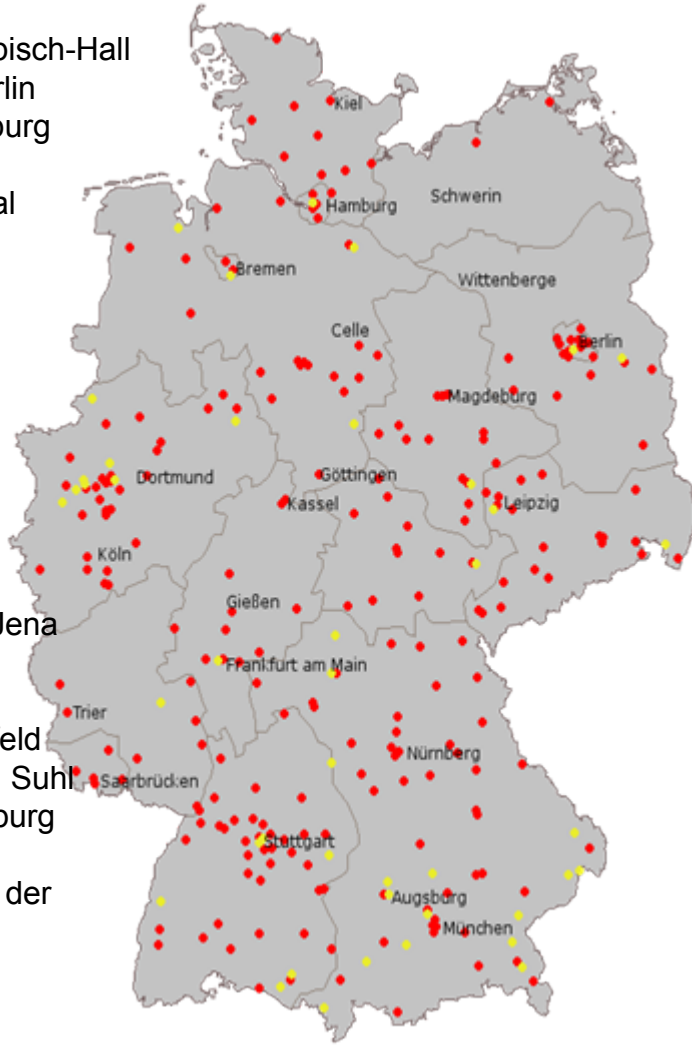
Acknowledgements

3754 breast cancer patients participating in the SUCCESS trial

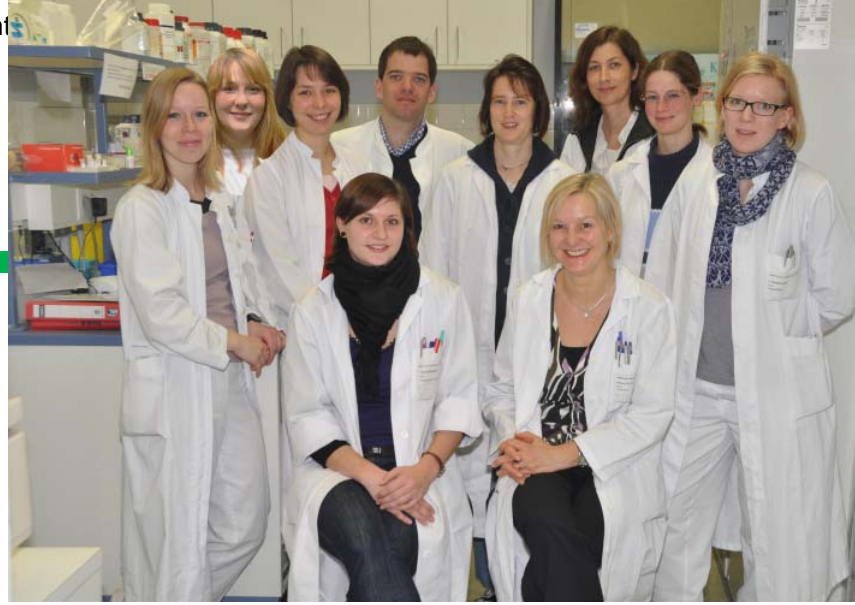
All 251 participating study centers throughout Germany:

Universitätsfrauenklinik Heidelberg
Universitätsfrauenklinik Erlangen
Henriettenstiftung Krankenhaus, Hannover
Onk.Praxis Dr. R. Lorenz / N. Hecker, Braunschweig
Onk. Praxis Prof. Tesch, Frankfurt
Onk.Praxis Dr. Forstbauer / Dr. Ziske, Troisdorf
Luisenkrankenhaus, Düsseldorf
Städt. Klinikum Karlsruhe
Onk. Praxis Drs. Siehl / Söling, Kassel
Städtisches Klinikum Rosenheim
Klinikum Hannover Nordstadt
Onk.Praxis Dr. Heinrich, Fürstenwalde
SRH Wald-Klinikum Gera gGmbH
St. Antonius-Hospital, Eschweiler
I. Universitätsfrauenklinik LMU, München
Klinikum Chemnitz gGmbH
Diakoniekrankenhaus Schwäbisch-Hall
DRK - Kliniken Köpenick , Berlin
Onk.Praxis Dr. Schlag, Würzburg
Stadtklinik Baden Baden
Onk.Praxis Dr. Fett, Wuppertal
Klinikum Meiningen GmbH
Krankenhaus Böblingen
Onk.Praxis Dr. Müller, Leer
Universitätsklinikum Lübeck
Onk. Praxis Dr. Göhler / Dipl. med. Dörfel, Dresden

Diakoniekrankenhaus Schwäbisch-Hall
DRK - Kliniken Köpenick , Berlin
Onk.Praxis Dr. Schlag, Würzburg
Stadtklinik Baden Baden
Onk.Praxis Dr. Fett, Wuppertal
Klinikum Meiningen GmbH
Krankenhaus Böblingen
Onk.Praxis Dr. Müller, Leer
Universitätsklinikum Lübeck
Onk. Praxis Dr. Göhler / Dipl. med. Dörfel, Dresden
Klinikum Bremerhaven Reinkenheide
Universitätsklinikum des Saarlands, Homburg/Saar
Friedrich-Schiller-Universität Jena
Hochwald Krankenhaus, Bad Nauheim
Onk.Praxis Dr. Glados, Coesfeld
Zentralklinikum Südthüringen, Suhl
Universitätsfrauenklinik Würzburg
Klinikum Südstadt, Rostock
Krankenanstalten Mutterhaus der Borromäerinnen, Trier
Onk.Praxis Dr. Deertz, Essen
Kath.Klinikum Mainz



We also thank...



Laboratory:

Dr. Schindlbeck, Dr. U. Andergassen,
Dr. J. Jückstock, Dr. Neugebauer,
S. Hofmann, V. Rengel, B. Zill,
L. Majunke, A. Kölbl

Collaborations:

Prof. Dr. W. Janni, Prof. Dr. M.-W. Beckmann, Prof. Dr. K. Pantel,
Prof. Fasching, Prof. Dr. W. Lichtenegger, Dr. H.-J. Hindenburg,
Prof. Dr. A. Schneeweiss, Prof. Dr. A. Schneider, Prof. Dr. K. Friese

Success Study Office:

Dr. J. Neugebauer, S. Kambylis, S. Döring, F. Stahl

Unrestricted grants by:

AstraZeneca, Chugai, Lilly, Novartis, Sanofi-Aventis, Tosoh, Veridex