

Diagnosis and Treatment of Patients with early and advanced Breast Cancer

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Prognostic and Predictive Factors

Prognostic and Predictive Factors

- **Versions 2002–2022:**

**Costa / Fasching / Fersis / Friedrichs / Gerber / Göhring / Harbeck /
Jackisch / Janni / Kolberg-Liedtke / Kreipe / Loibl / Lück / Mundhenke /
Nitz / Rody / Schaller / Schmidt / Schmutzler / Schneeweiss / Simon /
Solomayer / Thill / Thomssen / Untch / Witzel / Wöckel**

- **Version 2023:**

Gluz / Witzel

Definition

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A **Prognostic Factors** is associated with the probability of the course of the disease (e.g. disease-free or progression-free survival, overall survival). The probability can be influenced by therapy.

A **Predictive Factor** is associated with the probability of the effect of a given therapy.

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**“Low absolute risk implies
low absolute benefit”**

Quality Criteria

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- **Biological hypothesis**
- **Simple and standardized assessment method, quality assurance (QA) of the test**
- **Prospectively planned statistical evaluation (primary goal)**
- **Validation of clinical significance according to**
 - „Oxford Level of Evidence (LoEOx2001)“ criteria and „Grades of Recommendation (GR)“
 - „Grades of Recommendation (GR)“ as well as modified LoE criteria for the use in archived specimen (LoE2009) and category of tumor marker study (CTS)
- **Clinical relevance for treatment decisions**

Early Breast Cancer (M0) – eBC

Prognostic Factors I

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Factor	Oxford		
	LoE _{Ox2001}	GR	AGO
▪ Tumor size - pT	1a	A	++
▪ Axillary lymph node status - pN	1a	A	++
▪ Histological tumor type (mucinous, tubular etc.)	2b	B	++
▪ Grade (Elston & Ellis) - G	2a	B	++
▪ Age	2a	B	++
▪ Histologically proven peritumoral lymphatic vessel and vascular invasion (L1 V1)	1b	B	++
▪ pCR after NACT* in (luminal-B-like, HER2+, TN)	1a	A	++
▪ Increased risk of recurrence in invasive-lobular BC, cT3/4, N+	2a	B	+/-
▪ Obesity (BMI > 30 kg/m ²)	1b	B	+
▪ Margins (resection status) - R0 / R1	1a	A	+

* NACT = Neoadjuvant Chemotherapy

Early Breast Cancer (M0) - eBC

Prognostic Factors II

Oxford

Factor

	LoE	GR	AGO
■ ER / PR	1a	A	++
■ HER2 (IHC, ISH)	1a	A	++
■ ER / PR / HER2/ Ki-67 to assess the intrinsic type with regards to tumor histology and biology	2b	B	++
■ Proliferation markers			
■ Ki-67 before, during, or after treatment	1a	B	+
■ Ki-67 Re-Evaluation after short term preoperative endocrine therapy (2-4 weeks) (ypT and ypN)*	1a	B	+

* Biomarker and Multi Gene Expression test should be evaluated on core needle biopsy prior endocrine therapy

Reproducibility – Quality Assurance is Key for Clinical Decision Making

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- **ER / PR: concordance central vs local is high (97%; Plan B, SABCS 2014)**
- **Grade: concordance central vs local is 68% (PlanB, JCO 2016)**
- **HER2: frequency of false-positive test results 6% (ASCO /CAP JCO 2013)**
- **Impact of routine pathologic review in N0 BC: 20% changes: grade 40%, LVI 26%, N 15%, margin 12% (JCO 2012)**
- **pN0 from MIRROR study: pN0 was upstaged in 22%, in central pathology review (Ann Oncol 2012)**
- **Ki67:**
 - **Inter- and intraobserver variability in measurement of Ki-67 is high (J Nat. Cancer Institute 2011)**
 - **High reproducibility for low and high Ki67 levels (J Pathol 2002)**
 - **Standardized methodology improves analytical validity (JNCI 2020)**

Early Breast Cancer (M0) - eBC

Prognostic Factors III

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Factor	Oxford		
	LoE	GR	AGO
<ul style="list-style-type: none"> ▪ Gene expression profiles (GEP, multigene assays, gene signatures) <ul style="list-style-type: none"> ▪ MammaPrint® (N0-1) ▪ Oncotype DX® (N0-1, HR+ HER2-) ▪ EndoPredict® (N0-1, HR+, HER2 -) ▪ Prosigna® (N0-1, HR+, HER2 -) ▪ Breast Cancer IndexSM (N0-1, HR+ HER2-)** ▪ IHC4 (ER / PR / HER2 / Ki-67) (validated for central testing) ▪ PREDICT® algorithm (https://breast.predict.nhs.uk/) ▪ Clinical-pathological score for lobular breast cancer (nodal status, tumor size, lymphovascular invasion LVI) ▪ CTS5 Clinical Treatment Score** ▪ CPS-EG Score 	 1b 1b 2b 2b 2b 2b 1b 2b 2b 2b	 A A B B B B A B B B	 +* +* +* +* +/-* +/- + +/- + +

* Should only be used in the context of clinical-pathological criteria (tumor size, nodal involvement, grade, Ki-67, ER, PR, HER2)

** Estimation of late recurrence

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Prognostic Factors IV

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Factor	Oxford		
	LoE	GR	AGO
▪ Disseminated tumor cells (DTC, in bone marrow)	1a	A	+/-
▪ Circulating tumor cells (CTC, in blood, Cell Search®)*	1b	A	+/-
▪ CTC before NACT (regarding OS, DDFS, LRFI)	1b	B	+/-
▪ Therapy decisions based on CTC phenotypes	3a	C	-
▪ Cell-free DNA (cfDNA, in blood, for DFS, PFS, OS)	2a	B	+/-

Commercially Available Molecular Tests

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	70 gene signature (MammaPrint®) §	21 gene Recurrence score (Oncotype DX®) §	8 gene signature (Endopredict®) §	PAM 50 (Prosigna®) §	Breast Cancer Index® (BCI) §
Provider	Agendia	Genomic Health	Sividon (Myrirads)	NanoString	Biotheranostics
Type of assay	70-gene assay	21-gene recurrence score	11-gene assay	50-gene assay	5 + 2 (MGI+H/I)
Type of tissue	fresh frozen (technical validation for FFPE available)	FFPE	FFPE	FFPE	FFPE
Technique	Microarrays for RNA	qRT-PCR	q-RT-PCR	Direct hybridization (nCounter®)	q-RT-PCR
Central lab	yes	yes	no	no	yes
Indication and population studied	prognostic N-/+, < 70 Jahre	prognostic N-/+, ER+ endocrine treated	prognostic (pre-) postmenopausal N-/+, ER+ HER2- endocrine treated	prognostic postmenopausal N-/+, ER+ HER2- endocrine treated	Prognostic pT1-3pNo – pN1 ER+ / HER2– Endocrine treated
Risk classes	Low – high	RS (Low – intermediate – high)	Low – high	ROR (Low – inter- mediate – high), molecular types	Low - high
Clinical Validation	Yes	yes	yes	yes	Yes
Registration	FDA clearance as “In Vitro Diagnostic Multivariate Index Assay (IVDMIA)» CE-Mark (fresh tissue and FFPE)	Clinical Laboratory Improvement Amendments (CLIA) + College of American Pathologists (CAP)- accredited ref lab	CE-Mark	CE-Mark FDA 510(k) Clearance	Service Mark (SM)

§ Validated clinical data only available for this assay

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	70 gene signature (MammaPrint®) §	21 gene Recurrence score (Oncotype DX®) §	8 gene signature (Endopredict®) §	PAM 50 (Prosigna®) §	Breast Cancer Index® (BCI)
Prognosis after 5 yrs (late recurrences)	not separately shown	yes	yes	yes	yes
Predictive impact (chemotherapy benefit)	poorly validated	yes	not shown	not shown	EAT after 5 yrs
Prospective-retrospective evidence (% of recruited patients)	Multicenter validation	NSABP B-14 (14%) NSABP B-20 (28%) ECOG 9127 SWOG 8814 (40%) ATAC (30%)	ABCSG 6 (19%) ABCSG 8 (36%) GEICAM-9906 (45%) ATAC (10%)	MA.12 (59%) MA.5 (66%) ABCSG 8 (44%) ATAC (16%)	TransATTOM (11%)
Prospective evidence	MINDACT (N0, N1) (8y DFS, OS)	TAILORx (12 y DFS, OS), N0, RS ≤ 25 vs. ≥ 26 PlanB (N0 highrisk/N+) (5 y DFS, OS) RxPONDER (5 y DFS, OS), N1, RS ≤ 25 vs. ≥ 26) ADAPT (5 y DFS, OS), N0-1, RS 0-11; RS 12-25 / Ki67 response	–	–	--

§ Validated clinical data only available for this assay

Prospective Clinical Trials (Oncotype DX® [TAILORx, PlanB, RxPONDER, ADAPT], MammaPrint® [MINDACT])

Prognosis in low-risk groups excellent for both tests: ~ 94% 5 J. DFS with only adjuvant endocrine therapy (ET)

	TailorX	RxPONDER	PlanB	ADAPT	MINDACT
Follow-up	median 7.5 years	median 5.1 years	5-year-DFS	median 60 months	median 8.7 years
Trial design (biomarker question)	pN0; Randomization RS 11-25 (+/- CTX)	pN1; Randomization RS0-25 (+/- CTX)	Prospective ODX testing: ET alone in RS 0-11 pN0-1	Non-inferiority (iDFS) ET alone: RS 0-11 vs RS12-25/ET response	Prospectively defined 5y-DMFS threshold for ET alone
Percentage clinically defined low-risk group	6615/9427 (70.2%, adj-online)	all 1-3 involved lymph nodes	all clinical CTX indication (pN0-1)	all clinical chemotherapy (CTX) indication (c/pN0-1)	3336/ 6693 (49.8%, adj-online)
Percentage high clinical risk and low genomic risk (clinical CTX indication)	16.7% (RS 0–10)	42.8% (RS 0-13)	15.3% (RS 0–11)	ET-trial (pN0-1): all RS 0-25, i.e. low genomic risk with ET alone	23.2% (high clinical/low genomic risk)
Test failure rate	n.r.	n.r.	2.9%	n.r.	26% (fresh frozen)
Percentage genomically intermediate-risk group (only for Oncotype DX, ODX)	69.1% (RS 11–25)	57.2% (RS 14-24)	60.4% (RS 12–25)	Included only RS 0-11 (37.9%) or RS 12-25/ET response (62.1%)	n.a.
Percentage genomically high-risk group (only for Oncotype DX)	14.3% (RS ≥ 26)	n.a.	24.3% (RS ≥ 26)	n.a.	27.0% (high clinical <u>and</u> high genomic risk)
12-year follow-up	reported	n.r.	n.r.	n.r.	n.r.

Adjuvant Endocrine Therapy

Predictive Factors for DFS

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Therapy	Factor	Oxford		
		LoE	GR	AGO
▪ Endocrine therapy	▪ ER / PR status [%]	1a	A	++
	▪ IHC staining intensity (ER/PR)	1a	A	-
	▪ Ki-67 Re-Evaluation after short preoperative endocrine therapy (2-4 weeks) (ypT and ypN)*	1b	A	+
▪ Extended endocrine therapy (EAT)	▪ Breast Cancer Index [®] MammaPrint	2b	B	+/-
▪ Tamoxifen	▪ CYP2D6-polymorphism	2b	B	-
▪ Ovarian ablation or suppression	▪ Menopausal status	1c	A	++
▪ Aromatase inhibitors vs. tamoxifen	▪ Menopausal status	1c	A	++
	▪ ER / PR / HER2 as single factors	1c	A	-
	▪ Invasiv-lobular breast cancer	2b	B	+
	▪ Ki-67 high	2b	B	+/-
	▪ Obesity (BMI > 30 kg/m ²)	2b	B	+/-

Adjuvant Chemotherapy and Targeted Therapy

Predictive Factors for DFS



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Therapy	Factor	Oxford		
		LoE	GR	AGO
<ul style="list-style-type: none"> Adjuvant Chemotherapy 	70-Gene-signature (Mammaprint®)	1b	A	+
	21-Gene-signature (Oncotype DX RS®)	1b	A	+
	EPclin (Endopredict®)	2b	B	+
	PAM-50 (Prosigna®)	2b	B	+
	Histological type (lobular vs. NST)	2b	B	-
	TIL´s in TNBC	2b	B	+/-
<ul style="list-style-type: none"> Anti-HER2-Therapy 	HER2 (IHC, ISH)	1a	A	++
<ul style="list-style-type: none"> PARP-Inhibitors 	gBRCA1/Mutation (HER2 neg.)	1a	A	+

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*Consider decision according to age/menopausal status, prospective evidence available for Mammaprint and OncotypeDX only (see next slide)

Results for prospectively evaluated biomarkers (LOE1a) in early HR+/HER2- breast cancer

biomarker/ signature	Population (HR+/HER2- patients)	therapy options
Mammaprint (MINDACT n=2140)	Clinically high/genomic low risk (n=1550) N0-1, age >50 yrs N0-1, age ≤50 yrs (patients with OFS in the ET arm: 26%)	ET, no adjuvant CT adjuvant CT→ET*: 2.6% CT-benefit in 5-y DDFS (93.6 vs. 96.2%)
Oncotype DX (TAILORx n=6711)	TailorX (T1b-T2, N0, 74% clinically low risk, 13% OFS in premenopausal women) N0, RS 0-25 age>50 yrs. N0 RS 0-15 age ≤50 yrs N0 RS 16-25 age ≤50 yrs	ET, no adjuvant CHT ET, no adjuvant CHT adjuvant CT→ET*: (3.2-3.4% CT-benefit in 5-y DRFI (93→95-96% 5 y DRFI, in RS 16-20 if clinical high risk only, 16-20: HR=1.4 (n.s.), 21-25: HR=2.19 (sign) for ET vs. CT→ET
RxPonder (n=5018)	RxPonder: N1 RS 0-25: postmenopausal RS 0-25: premenopausal (patients with OFS in the ET arm: 19%)	ET, no adjuvant CT (neo)adjuvant CT→ET* 2.4% CT benefit in 5-y DRFI (5-y DRFI 93.9 vs. 96.3%, HR=0.062, p=0.02) explorative analysis: no effect of CT age 50 and older (p _{interaction} 0.06)
RS + Ki-67post (ADAPT, n=2290 endocrine treated)	clinically intermediate/high risk , RS 0-25 (RS 12, 25+Ki67 _{post} ≤10%) N0-1, age>50 yrs N0, RS 0-11 and age ≤50 yrs N0, RS 12-25 with Ki67 _{post} ≤10% and age ≤50 yrs N1: RS 0-25 (+ Ki-67 _{post} ≤10% in RS 12-25) and age ≤50 yrs N1: RS 0-25 and ki-67 _{post} >10%	ET, no adjuvant CT adjuvant ET, no adjuvant CT adjuvant ET+/- OFS, if RS >16 or clinically high risk +/- CT: 5-yr-DDFS: 97% with ET alone, no significant difference between RS 0-15 and 16-25 adjuvant ET+OFS or CT→ET 5-yrs. DDFS 97% with ET alone (neo)adjuvant CT→ET

* If CT is refused: alternative ET+OFS

DDFS=distant-disease-free-survival, DRFI= distant recurrence free interval, ET= endocrine treatment, CT= chemotherapy, OFS= ovarian function suppression, RS= Recurrence Score

Neoadjuvant Systemic Chemotherapy (NACT)

Predictive Factors for pCR I



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Factor	pCR* Probability	Oxford		
		LoE	GR	AGO
▪ Young age	↑	1a	A	+
▪ Obesity	↓	2a	B	+
▪ cT1 / cT2 tumors o. N0 o. G3	↑↑	1a	A	++
▪ Negative hormone receptor status	↑↑	1a	A	++
▪ Triple negative breast cancer	↑↑	1a	A	++
▪ Positive HER2-status	↑↑	1a	A	++
▪ Early clinical response	↑	1b	A	+
▪ Lobular tumor type	↓	1a	A	+
▪ Metaplastic tumor type	↓↓	4	C	+

* High (↑) or very high (↑↑) probability to reach pCR, low (↓) or very low (↓↓) probability to reach pCR
See also chapter „Prognostic and predictive factors“

Neoadjuvant Systemic Chemotherapy (NACT)

Predictive Factors for pCR II



Oxford

Factor	pCR* Probability	Oxford		
		LoE	GR	AGO
<ul style="list-style-type: none"> Gene expression profiles (gene signatures) (Mammaprint®, Endopredict® Oncotype DX®, Prosigna®, Breast Cancer IndexSM) 	↑	2b	B	+/-
<ul style="list-style-type: none"> Ki-67 	↑	2b	B	+
<ul style="list-style-type: none"> Tumor infiltrating lymphocytes** 	↑	2a	B	+
<ul style="list-style-type: none"> PIK3CA mutation (for HER2-positive BC) 	↑	2a	B	+/-
<ul style="list-style-type: none"> gBRCA-mutation (for the effect of chemotherapy) 	↑	2b	B	+
<ul style="list-style-type: none"> gBRCA-mutation (for the effect of platinum) 	↔	2b	B	+/-

* High (↑) or very high (↑↑) probability of pCR, low (↓) or very low (↓↓) probability of pCR

** Defined as dense lymphocytic infiltration of inner peritumoral stroma outside of the invasion front (lymphocytes make up > 50% of stroma area)

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Metastatic Breast Cancer (mBC)

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Factor	Oxford		
	LoE	GR	AGO
<ul style="list-style-type: none"> ■ Circulating tumor cells (CTC in blood, Cell Search®) <ul style="list-style-type: none"> ■ Prognosis ■ Early response assessment (3w) ■ Therapy decision solely based on dynamics of CTC numbers over time or CTC phenotype ■ Cell-free DNA (cfDNA in blood) 	1a	A	+
	1b	B	+
	1b	A	-*
	2a	A	+/-

Treatment of Metastatic Breast Cancer

Predictive Factors for response

Oxford

Therapy	Factor	LoE	GR	AGO
▪ Endocrine therapy	ER / PR (prim. tumor, better: metastasis)	1a	A	++
	Response to prior therapy	2b	B	++
	Autocrine receptor mutation (<i>ESR1</i>)	2b	B	+
▪ Alpelisib	<i>PIK3CA</i> mutation (prim. tumor, metastases, plasma)	1b	A	++
▪ Trastuzumab Deruxtecan	HER2-low or HER2-positive	1b	A	++
▪ Chemotherapy	Response to prior therapy	1b	A	++
▪ Anti-HER2-therapy	HER2 (prim. tumor, better: metastasis)	1a	A	++
▪ Checkpoint-Inhibitors	PD-L1 positivity [#] (PD-L1ic, CPS) in TNBC (primary tumor or metastasis)	1b	B	++
▪ PARP-Inhibitors	<i>gBRCA1/2</i> -mutation	1a	A	++
▪ Bone modifying drugs	Bone metastasis	1a	A	++

see chapter „pathology“

Mutation Diagnostics* in mBC: „Precision Medicine“ for Targeted Therapies

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Altered genes	Therapeutic relevance	Gene region	Material	Oxford		
				LOE	GR	AGO
BRCA1, BRCA2	Olaparib, Talazoparib Olaparib	All exons	Germline: Blood cells	1b	A	++
			Somatic: Tissue	2b	B	+/-
PALB2	Olaparib		Germline: Blood cells	2b	B	+
PIK3CA	Alpelisib	Exons 7, 9 and 20	Primary tumor, metastases, plasma	1b	A	++
HER2-mutation (independent of HER2-status)	Neratinib, lapatinib	Kinase- and extracellular domains; S310, L755, V777, Y772_A775dup	Primary tumor, metastases, plasma particul. lobular BC	4	C	+/-
ESR1	Resistance against AI Response oral SERDs	Exons 4, 7 and 8	Metastases, plasma	2b	B	+/-
			Metastases, plasma	1b	B	+/-
NTRK gene fusion	Larotrectinib, entrectinib	Fusion- and splice variants	Tumor tissue, particul. secretory breast cancer	2a	B	+
MSI	Pembrolizumab	Microsatellite-instability	Tissue	2a	B	+

* Ideally panel diagnostics

Decision guidance prospectively evaluated biomarkers (LOE1a) and therapy options (mBC)



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Biomarker / Signature-therapy option	Subtyp / Population	Therapy option
PDL-L1 ≥ 1%	TNBC	First line Atezolizumab + nab Paclitaxel
CPS > 10	TNBC	First line Pembro + chemotherapy
PIK3CA mutation	HR+ / HER2-	Fulvestrant + Alplisib after failure of first line ET
BRCA1/2 mutation (OlympiAD, EMBRACA)	HER2 –	Olaparib, Talazoparib

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Therapy-Relevant Mutational Analysis for „Actionable“ Genomic Alterations in BC

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Diagnostic Tool*	Outcome	Oxford		
		LoE	GR	AGO
Evidence from studies with other cancer patients („tumor-agnostic testing“)				
<ul style="list-style-type: none"> Companion Diagnostics for therapies of other tumor entities (e.g. BRAF, FGFR1, ...) 	Efficacy of diverse therapies	4	D	+/-**
<ul style="list-style-type: none"> Large Panel Gene Analysis (e.g. FoundationOne, GPS Cancer, NeoSelect, Molecular Health Guide, local „hand-selected,, panels) 	Efficacy of diverse therapies, prognosis	3a	C	+/-**
<ul style="list-style-type: none"> Next Generation Sequencing (NGS) (recommended only in Tier 1 + 2) 	Efficacy of evaluated drugs	1b	B	+/-**

* Assessment method for somatic mutations (tumor tissue, cf-DNA) is not taken into consideration for LoE

** Participation in clinical trials or structured registries recommended



Joint Consensus Recommendations of AMP, ACMG, ASCO and CAP for Reporting Genetic Variants in Cancer

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Tier	LoE		Explanation
Tier 1	A.1	Biomarkers that predict response or resistance to FDA-approved therapies for a specific type of cancer	Variants of strong clinical significance
	A.2	Biomarkers included in professional guidelines that predict response to therapies for a specific type of tumor	
	B	Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies with consensus from experts in the field	
Tier 2	C.1	Biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different type of tumor	Variants of potential clinical significance
	C.2	Biomarkers that serve as inclusion criteria for clinical trials	
	D	Biomarkers that show plausible therapeutic significance based on preclinical studies	
Tier 3		Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases. No convincing published evidence or cancer association	Variants of unknown clinical significance
Tier 4		Observed at significant allele frequency in the general or specific subpopulation Databases. No existing published evidence of cancer association	Benign or likely benign variants

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