Prevalence of CA 27.29 in Primary Breast Cancer Patients before the Start of Systemic Treatment

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Abstract. *Background: Several trials show that tumor markers at primary diagnosis of cancer have prognostic relevance and can predict dissemination of the disease. While MUC-1 markers are frequently used to monitor treatment efficacy in metastatic breast cancer, their role at primary diagnosis or during follow-up remains unclear. This translational research project within the SUCCESS trial evaluates the role of the tumor marker CA 27.29 before and after adjuvant chemotherapy, as well as after two and then five years in patients with early breast cancer. Patients and Methods: The SUCCESS trial compared FEC (500/100/500)-docetaxel (100) vs. FEC (500/100/500)-docetaxel/gemcitabine (75/2000) and two vs. five years of zoledronate treatment in node-positive and high-risk node-negative patients with primary breast cancer. CA 27.29 was measured before chemotherapy in 2669 patients with the reagent ST AIA-PACK CA 27.29 for AIA-600II-Analyzer (Tosoh Bioscience, Belgium). Results of CA 27.29 above 31 U/ml were regarded as positive. Results: 7.6% of patients had elevated marker levels after the completion of primary surgical treatment but before initiation of chemotherapy (n=202, mean 19, range 3-410 U/ml). No correlation between nodal status (p=0.55), grading (p=0.85), hormonal status (p=0.21), HER2/neu status on the primary tumor (p=0.58) and CA 27.29 was shown. However, larger tumor size (p=0.02), lobular histology (p<0.0001), older age (p<0.001) and postmenopausal hormone status before the start of treatment (p=0.006) were significantly associated with higher CA 27.29 levels. Conclusion: These data indicate a close relationship between CA 27.29 and tumor mass persisting even several weeks after surgery, but also identify potential confounding factors that should be considered in interpreting tumor marker results. Further follow-up of the SUCCESS trial will clarify whether CA 27.29 measured after surgery but before the start of systemic treatment is prognostically relevant and whether it is a useful marker for treatment monitoring in the adjuvant setting.*

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Mucin MUC-1 is physiologically present at the luminal surface of glandular epithelia. Since this glycoprotein is up-regulated in many adenocarcinomas and released into the blood stream in higher amounts than in healthy individuals, it has been investigated extensively in breast cancer patients. Several studies identified MUC-1 markers such as Ca15-3 and CA 27.29 at primary diagnosis as being independent predictors for disease outcome, in addition to the traditional prognostic markers such as tumor size and nodal status (1-3). After primary therapy, the level of these markers can predict disease recurrence about six months earlier than available methods (4, 5). However, according to the current tumor marker guidelines of the American Society of Clinical Oncology, CA 15-3 and CA 27.29 are not recommended as prognostic markers for routine clinical use because there are

no trials available demonstrating a clear benefit regarding improved survival or diminished toxicity resulting from a timely detection of recurrence and early treatment initiation (6). Furthermore, clinical use of these markers, especially in early breast cancer, is hampered by a low sensitivity and specificity of the available assays, which show an elevation of tumor markers in healthy individuals and in benign diseases (7, 8). Therefore, it will be crucial to identify a comprehensive spectrum of factors influencing tumor marker results. These factors have to be considered in a valid interpretation of tumor markers and might lead to adapted normal values in prespecified subpopulations.

Despite these limitations, testing for the existence of tumor markers is widely used in disease surveillance and treatment monitoring in daily practice. As non-invasive, reproducible and easily accessible tests are available at any point in time during disease progression for MUC-1 markers they are a highly suitable measure by which to select patients at risk of recurrence, both at primary diagnosis and during follow-up, and to monitor treatment efficacy. In this trial, we prospectively evaluated the role of the tumor marker CA 27.29 after surgery but before the start of taxane-based adjuvant chemotherapy in 2669 primary breast cancer patients.

Patients and Methods

Study design. The SUCCESS study is a German, multicenter prospectively randomized, open label, phase III trial, evaluating the role of gemcitabine in the treatment of primary breast cancer patients, as well as the optimal duration of adjuvant zoledronate treatment. In this 2×2 factorial design trial, 3754 node-positive or high risk node-negative patients were randomized to receive FEC-Doc (3 cycles of 5-fluorouracil (5-FU) 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 *q3w*, followed by 3 cycles of docetaxel 100 mg/m^2 *q3w*) or FEC-Doc/gemcitabine (3 cycles of 5-FU 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 *q3w*, followed by 3 cycles of docetaxel 75 mg/m^2, gemcitabine 1.000 mg d1,8 *q3w*). In a second randomization, all patients were assigned to two *vs*. five years of zoledronate (4 mg ×*q3m*×24m *vs*. *q3m*×24m followed by q6m×36m). Node-negative patients were included in case of pT≥2, histopathological grade 3, age ≤35 years or negative hormone receptor status.

As part of the study, blood samples were collected before the start of systemic treatment, after chemotherapy and after two and five years. Analyses of the tumor marker CA 27.29 before chemotherapy are presented.

Patients. Serum samples from 2669 primary breast cancer patients recruited from 251 German centers were used. The study was approved by relevant ethical boards involved in Germany. The tumor stage at primary diagnosis was classified according to the revised AJCC tumor-node-metastasis (TNM) classification (9). Histopathological grading of the primary tumors was performed according to the Bloom-Richardson system (10). The primary surgical treatment consisted of either breast conservation or modified radical mastectomy, leading to R0 resection in all reported cases. Sentinel lymph node excision alone was performed in all cases with no histopathological evidence of tumor in the sentinel nodes. Routine axillary dissection in patients with metastases in the sentinel nodes included lymph nodes of levels I and II, while those of level III were excised only in cases with macroscopic metastatic involvement of the lower levels. For the diagnosis of lymph node metastasis, single embedded lymph nodes were screened up to three levels. In all patients treated with breast conservation, external beam radiation therapy was administered. Chest wall irradiation following mastectomy was performed in patients with more than 3 involved lymph nodes or T3 and T4 tumors.

All patients received either FEC-Doc or FEC-Doc/gemcitabine chemotherapy according to their randomization. Following chemotherapy, premenopausal patients with hormone receptor positive disease were treated with tamoxifen for five years. Additionally patients below 40 years of age or that had premenopausal blood status after chemotherapy, were treated with two years of gosereline. Postmenopausal patients were switched from tamoxifen to anastrozole after two years.

Methods. Laboratory analysis was performed centrally at the Ludwig Maximilians-University of Munich Women's Hospital. CA 27.29 concentrations were measured by the ST AIA-Pack 27.29 reagent using the AIA-600II Analyzer (Tosoh Bioscience, Tessenderlo, Belgium) according to the manufacturer instructions. The ST AIA-Pack 27.29 assay is an automated monoclonal fluorometric assay directed against the MUC-1 antigen. Samples were shipped at room temperature and either analyzed immediately on their arrival in the laboratory or stored at –20˚C until analysis at a later date. Serum (150 μl) was diluted by 1:20 and the monoclonal antibody bound to magnetic pearls was then added. The pearls were washed and incubated at 37˚C with the fluorogenic substrate 4MUP. Assay results above 31 U/ml were regarded as positive. In all positive samples, two repeat assays were performed and the mean was used for analysis. Sixty-six controls without malignant disease were analyzed, from whom one sample was found to be above 31 U/ml (1%). Clinical information was obtained directly from the electronic study documentation of the SUCCESS trial.

Statistical analysis. The χ^2-test was used to compare categorical variables. The two-tailed *t*-test was used to calculate the differences of the mean of the independent samples that had continuous variables. *P*-values of less than 0.05 were considered significant in two-sided tests. No adjustment for the error probability for multiple testing was performed. The Statistical Package for the Social Sciences 16.0 (SPSS Inc., Chicago, IL, USA) was used throughout.

Results

Prevalence of CA 27.29 positivity at primary diagnosis. The serum samples were acquired after primary surgical treatment leading to R0 resection and before the start of chemotherapy. At that time point, 7.6% of patients presented with elevated marker levels (n=202). The mean CA 27.29 value was 19 U/ml, with a range from 3 U/ml to 410 U/ml). CA 27.29 levels categorized by percentiles are depicted in Figure 1.

The median age of all patients was 53 years (range 21 to 76 years). The majority of patients had small tumors (41.6% T1, 51.5% T2, 5.7% T3, 1.2% T4) and 34.3% were node-

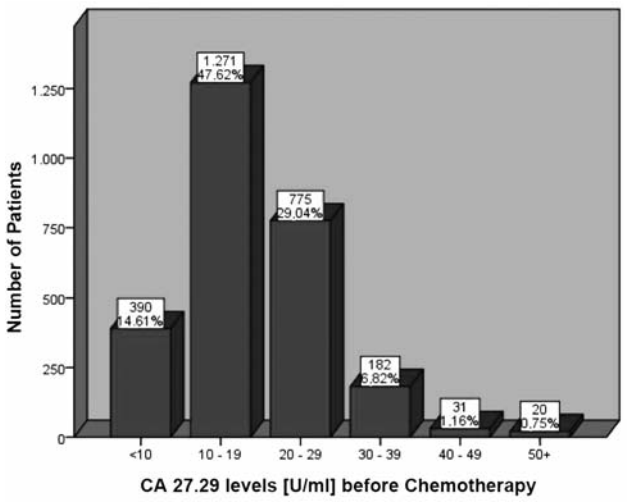

Figure 1. *CA 27.29 values after surgical treatment but before the start of systemic chemotherapy.*

negative (45.5% N1, 14.2% N2, 6.1% N3). Overall, 4.7% presented with G1 tumors, 71.4% of patients were hormone-receptor positive and 25.5% Her2/neu positive on the primary tumor.

Correlation of CA 27.29 at primary diagnosis with classical prognostic markers. Table I summarizes the patient characteristics at the time of primary diagnosis in relation to CA 27.29 positivity. Elevated CA 27.29 levels did not correlate with the majority of tumor characteristics at the time of primary diagnosis, such as nodal status (p=0.55), histopathological grading (p=0.85), hormone receptor status (p=0.21) or Her2/neu status on the primary tumor (p=0.58), nor with the surgical treatment (p=0.08 for breast surgery and p=0.31 for axillary treatment) or systemic chemotherapy randomization (p=0.5). However, larger tumor size (p=0.02) and lobular histological tumor type (<0.0001), as well as older age (p<0.001) and postmenopausal status before the start of treatment (p=0.006) were significantly associated with higher CA 27.29 levels.

Table I. *Patient characteristics at the time of primary diagnosis.*

	CA 27.29 positive pts (%)	CA 27.29 negative pts (%)	*P*-value
Number of patients	202	2467	
Age (years)	57 (28-74)	53 (21-76)	<0.001
Tumor size[a]			0.01
pT1	64 (33.3)	1012 (42.3)	
pT2	104 (54.2)	1228 (51.3)	
pT3	19 (9.9)	127 (5.3)	
pT4	5 (2.6)	25 (1.0)	
LNM			0.55
Absent (pN0)	65 (32.2)	839 (34.0)	
1-3 axillary LNM (pN1)	85 (42.1)	1146 (46.5)	
4-9 axillary LNM (pN2)	24 (11.9)	336 (13.6)	
≥10 axillary LNM (pN3)	28 (13.9)	140 (5.7)	
NX	0 (0.0)	6 (0.2)	
Grading			0.85
G1	10 (5.0)	115 (4.7)	
G2-3	192 (95.0)	2352 (95.3)	
Hormone receptor status			0.21
Negative	50 (24.8)	714 (28.9)	
Positive	152 (75.2)	1753 (71.1)	
Her2/neu status[b]			0.58
Negative	144 (76.2)	1757 (74.4)	
Positive	45 (23.8)	605 (25.6)	
Histological type[c]			<0.0001
Ductal	137 (71.4)	1975 (82.5)	
Lobular	39 (20.3)	262 (10.9)	
Other	16 (8.3)	157 (6.6)	
Menopausal status			0.006
Premenopausal	68 (33.7)	1077 (43.7)	
Postmenopausal	134 (66.3)	1390 (56.3)	
Primary operation[a]			
Breast-conserving	126 (65.6)	1714 (71.6)	0.08
Mastectomy	66 (32.7)	679 (28.4)	
SLNE	37 (19.3)	520 (21.7)	0.31
Axillary dissection	155 (80.7)	1856 (77.6)	
No axillary staging	0 (0.0)	16 (0.7)	
Systemic therapy			0.50
FEC-Doc	99 (49.0)	1270 (51.5)	
FEC-Doc/gemcitabine	103 (51.0)	1197 (48.5)	

[a]Tumor size and information on primary operation missing in 85 cases; [b]Her2/neu status missing in 118 cases; [c]Histological type missing in 83 cases. LNM: Lymph node metastasis classification.

Discussion

In this trial, we prospectively analyzed the role of the MUC-1 marker CA 27.29 in a large group of 2669 primary breast cancer patients before adjuvant taxane-based chemotherapy. CA 27.29 is an established, standardized assay for the detection of the MUC-1 antigen. Mucins are complex membrane-associated glycoproteins that interact with the cytoskeleton and are frequently up-regulated and are shed into the blood stream in patients with adenocarcinomas (11). MUC-1 is reported to be expressed in about 90% of breast tumors (12-15). Several trials have compared different MUC-1 assays and found comparable sensitivity and specificity for CA 27.29 and Ca 15-3 (11,16-18). Some authors even assume a higher sensitivity for CA 27.29 in cases with low antigen concentrations or limited variations in tumor extension (18, 19). Albeit the difference between the two antibodies was small, it may support a possible advantage when the test is used during follow-up for early detection of disease recurrence.

In our trial, we found 8% of patients with CA 27.29 levels above the cut-off level when blood was drawn after primary surgery with R0 resection of the tumor but before the start of

adjuvant systemic treatment. As multiple assays are used for tumor marker detection, and cut-offs are not clearly defined, it is difficult to compare positivity rates across different trials. Overall, our positivity rate of 8% at primary diagnosis is below the 9% to 75% reported for stage I to IV disease (20-23). However, in contrast to most other trials, blood sampling in our study was performed after excision of the primary tumor, which should result in a drop of shedded MUC-1 antigen in the circulation compared to preoperative samples.

The interpretation of tumor marker assays is complicated by several factors influencing MUC-1 antigen expression. In addition to assay- or treatment-related factors such as G-CSF application, patient characteristics can also have an influence on MUC-1 antigen expression. In our trial, increased CA 27.29 levels were observed in older patients ($p<0.001$) and patients with postmenopausal hormonal status before the start of treatment ($p=0.006$). Similar findings were reported by others (16, 24) and were confirmed in healthy women (25). This observation can be explained by a diminished sialylation caused by aging and unmasking of MUC-1 antigenic sites recognized by the assay.

We also evaluated the association of CA 27.29 at primary diagnosis with other established prognostic factors and primary surgical treatment, and found no significant correlation with nodal status ($p=0.55$), grading ($p=0.85$), hormonal status ($p=0.21$) or HER2/neu status on the primary tumor ($p=0.58$). As shown in Table I and reported by others (18, 24, 26, 27), elevated CA 27.29 was associated with increased tumor size ($p=0.02$). Reflecting the higher tumor burden in the CA 27.29-positive group, these patients were treated by mastectomy more frequently than were CA 27.29 -negative patients (33% *vs*. 28%, $p=0.08$), but this difference did not reach statistical significance. However, we were unable to prove an association of CA 27.29 with lymph node positivity in our patient group.

Another finding in our data set is the association of CA 27.29 positivity with lobular histological tumor type. While we found 11% of lobular carcinomas in the CA 27.29-negative group, this rate increased to 20% in the CA 27.29-positive group ($p<0.0001$). A high MUC-1 expression in up to 90% of tumors has been reported both for ductal and lobular breast carcinoma tissue, whereas mucinous carcinomas were less MUC-1 reactive (12-14). However, high MUC-1 tissue expression does not always coincide with elevated MUC-1 serum levels (15, 28). As different glycoforms of MUC-1 are expressed on cancer cells, serum tests and immunohistochemistry might detect different glycoforms. Consequently, the screening for different glycoforms could be a reasonable approach. To our knowledge, a correlation between elevated CA 27.29 and lobular histological type has not been reported before.

These data indicate a close relationship between CA 27.29 and the tumor mass persisting even several weeks after primary surgery. Furthermore, we identified tumor and patient characteristics that should be considered in tumor marker interpretation. Longer follow-up within the SUCCESS trial will provide information on the prognostic relevance of CA 27.29 before the start of systemic treatment and will demonstrate the role of this tumor marker in adjuvant chemotherapy and endocrine, as well as bisphosphonate, treatment monitoring.

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References

1 Ebeling FG, Stieber P, Untch M, Nagel D, Konecny GE, Schmitt UM, Fateh-Moghadam A and Seidel D: Serum CEA and CA 15-3 as prognostic factors in primary breast cancer. Br J Cancer *86*: 1217-1222, 2002.

2 Gion M, Boracchi P, Dittadi R, Biganzoli E, Peloso L, Mione R, Gatti C, Paccagnella A and Marubini E: Prognostic role of serum CA15.3 in 362 node-negative breast cancers. An old player for a new game. Eur J Cancer *38*: 1181-1188, 2002.

3 Kumpulainen EJ, Keskikuru RJ and Johansson RT: Serum tumor marker CA 15.3 and stage are the two most powerful predictors of survival in primary breast cancer. Breast Cancer Res Treat *76*: 95-102, 2002.

4 Kokko R, Holli K and Hakama M: CA 15-3 in the follow-up of localised breast cancer: a prospective study. Eur J Cancer *38*: 1189-1193, 2002.

5 Nicolini A, Tartarelli G, Carpi A, Metelli MR, Ferrari P, Anselmi L, Conte M, Berti P and Miccoli P: Intensive postoperative follow-up of breast cancer patients with tumour markers: CEA, TPA or CA15.3 *vs*. MCA and MCA-CA15.3 *vs*. CEA-TPA-CA15.3 panel in the early detection of distant metastases. BMC Cancer *6*: 269, 2006.

6 Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF and Bast RC Jr: American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol *25*: 5287-5312, 2007.

7 Laessig D, Nagel D, Heinemann V, Untch M, Kahlert S, Bauerfeind I and Stieber P: Importance of CEA and CA 15-3 during disease progression in metastatic breast cancer patients. Anticancer Res *27*: 1963-1968, 2007.

8 Stieber P, Molina R, Chan DW, Fritsche HA, Beyrau R, Bonfrer JM, Filella X, Gornet TG, Hoff T, Jager W, van Kamp GJ, Nagel D, Peisker K, Sokoll LJ, Troalen F, Untch M and Domke I: Clinical evaluation of the Elecsys CA 15-3 test in breast cancer patients. Clin Lab *49*: 15-24, 2003.

9 Singletary ES, Allred C, Ashley P, Bassett LW, Berry D, Bland KI, Borgen PI, Clark G, Edge SB, Hayes DF, Hughes LL, Hutter RV, Morrow M, Page DL, Recht A, Theriault RL, Thor A, Weaver DL, Wieand HS and Greene FL: Revision of the American Joint Committee on Cancer Staging System for Breast Cancer. J Clin Oncol *20*: 3628-3636, 2002.

10 Le D, V, Tubiana HM, Friedman S, Hacene K, Spyratos F and Brunet M: Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson (SBR). An improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. Cancer *64*: 1914-1921, 1989.

11 Klee GG and Schreiber WE: MUC1 gene-derived glycoprotein assays for monitoring breast cancer (CA 15-3, CA 27.29, BR): are they measuring the same antigen? Arch Pathol Lab Med *128*: 1131-1135, 2004.
12 Baldus SE, Wienand JR, Werner JP, Landsberg S, Drebber U, Hanisch FG and Dienes HP: Expression of MUC1, MUC2 and oligosaccharide epitopes in breast cancer: prognostic significance of a sialylated MUC1 epitope. Int J Oncol *27*: 1289-1297, 2005.
13 Rakha EA, Boyce RW, Abd El-Rehim D, Kurien T, Green AR, Paish EC, Robertson JF and Ellis IO: Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. Mod Pathol *18*: 1295-1304, 2005.
14 Rahn JJ, Dabbagh L, Pasdar M and Hugh JC: The importance of MUC1 cellular localization in patients with breast carcinoma: an immunohistologic study of 71 patients and review of the literature. Cancer *91*: 1973-1982, 2001.
15 Croce MV, Isla-Larrain MT, Demichelis SO, Gori JR, Price MR and Segal-Eiras A: Tissue and serum MUC1 mucin detection in breast cancer patients. Breast Cancer Res Treat *81*: 195-207, 2003.
16 Bon GG, van Kamp GJ, Verstraeten RA, Mensdorff-Pouilly S, Hilgers J and Kenemans P: Quantification of MUC1 in breast cancer patients. A method comparison study. Eur J Obstet Gynecol Reprod Biol *83*: 67-75, 1999.
17 Frenette PS, Thirlwell MP, Trudeau M, Thomson DM, Joseph L and Shuster JS: The diagnostic value of CA 27-29, CA 15-3, mucin-like carcinoma antigen, carcinoembryonic antigen and CA 19-9 in breast and gastrointestinal malignancies. Tumour Biol *15*: 247-254, 1994.
18 Gion M, Mione R, Leon AE, Luftner D, Molina R, Possinger K and Robertson JF: CA27.29: A valuable marker for breast cancer management. A confirmatory multicentric study on 603 cases. Eur J Cancer *37*: 355-363, 2001.
19 Chan DW, Beveridge RA, Muss H, Fritsche HA, Hortobagyi G, Theriault R, Kiang D, Kennedy BJ and Evelegh M: Use of Truquant BR radioimmunoassay for early detection of breast cancer recurrence in patients with stage II and stage III disease. J Clin Oncol *15*: 2322-2328, 1997.
20 Eskelinen M, Tikanoja S and Collan Y: Efficient test for cancer antigens: decreased levels of cancer antigen in serum after excision of breast tumor. Anticancer Res *9*: 437-439, 1989.
21 Fehm T, Gebauer G and Jager W: Clinical utility of serial serum c-erbB-2 determinations in the follow-up of breast cancer patients. Breast Cancer Res Treat *75*: 97-106, 2002.
22 Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology. J Clin Oncol *14*: 2843-2877, 1996.
23 Park BW, Oh JW, Kim JH, Park SH, Kim KS, Kim JH and Lee KS: Preoperative CA 15-3 and CEA serum levels as predictor for breast cancer outcomes. Ann Oncol *19*: 675-681, 2008.
24 Duffy MJ, Duggan C, Keane R, Hill AD, McDermott E, Crown J and O'Higgins N: High preoperative CA 15-3 concentrations predict adverse outcome in node-negative and node-positive breast cancer: study of 600 patients with histologically confirmed breast cancer. Clin Chem *50*: 559-563, 2004.
25 Lynch DM, Rogers PE, Love JC, Salkas MJ, Skarphol KA, Gross ME, Lu MG, Petrides VH, Bruzek DJ, Cox JL, Jones KA, Kelley CA and Chan DW: Clinical evaluation comparing AxSYM CA 15-3, IMx CA 15-3 and Truquant BRTM RIA. Tumour Biol *19*: 421-438, 1998.
26 Canizares F, Sola J, Perez M, Tovar I, De Las HM, Salinas J, Penafiel R and Martinez P: Preoperative values of CA 15-3 and CEA as prognostic factors in breast cancer: a multivariate analysis. Tumour Biol *22*: 273-281, 2001.
27 Shering SG, Sherry F, McDermott EW, O'Higgins NJ, and Duffy MJ: Preoperative CA 15-3 concentrations predict outcome of patients with breast carcinoma. Cancer *83*: 2521-2527, 1998.
28 Cannon PM, Ellis IO, Blamey RW, Bell J, Elston CW and Robertson JF: Expression of tumour-associated antigens in breast cancer primary tissue compared with serum levels. Eur J Surg Oncol *19*: 523-527, 1993.

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