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ORIGINAL ARTICLE

Pathologic complete remission rate after cisplatin-based primary chemotherapy in breast cancer: correlation with p63 expression

Andrea Rocca · Giuseppe Viale · Richard D. Gelber · Luca Bottiglieri · Shari Gelber · Giancarlo Pruneri · Raffaella Ghisini · Alessandra Balduzzi · Elisabetta Pietri · Claudia D'Alessandro · Aron Goldhirsch · Marco Colleoni

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Abstract
Purpose p63, a gene that shares structural and functional homologies with p53, codes for different isoforms, with (TA) and without (ΔN) transactivating properties. The anti-apoptotic ΔN isoform is often expressed in breast cancer (BC). DNA damaging drugs such as cisplatin (C) induce its degradation and stabilization of the TA, proapoptotic isoform. This supports the role of these drugs in the treatment of tumors expressing p63. The aim of the present study was to ascertain the predictive value of p63 immunoreactivity in patients treated preoperatively with regimens including cisplatin and/or anthracyclines.

Methods We reviewed the pretreatment biopsies of 189 patients with large or locally advanced BC (cT1–4d, N0-2, M0) treated with preoperative chemotherapy, performing p63 immunohistochemistry. The rate of pathological complete remission (pCR) at final surgery was assessed with respect to cisplatin administration and p63 immunoreaction.

Results pCR was identified in 20 patients (11%); 147 patients (78%) had an objective response, 39 (21%) stable disease, and 3 (1%) disease progression. One hundred forty seven patients (78%) received a cisplatin-containing regimen. Only regimens including cisplatin without anthracy-

A. Rocca (✉) · R. Ghisini · A. Balduzzi · E. Pietri · C. D'Alessandro · M. Colleoni
Research Unit in Medical Senology,
European Institute of Oncology, Milan, Italy
e-mail: andrea.rocca@ieo.it

R. Ghisini
e-mail: raffaella.ghisini@ieo.it

A. Balduzzi
e-mail: alessandra.balduzzi@ieo.it

E. Pietri
e-mail: elisabetta.pietri@ieo.it

C. D'Alessandro
e-mail: claudia.dalessandro@ieo.it

M. Colleoni
e-mail: marco.colleoni@ieo.it

G. Viale · G. Pruneri
Department of Pathology,
European Institute of Oncology, Milan, Italy
e-mail: giuseppe.viale@ieo.it

G. Pruneri
e-mail: giancarlo.pruneri@ieo.it

G. Viale · L. Bottiglieri
University of Milan School of Medicine, Milan, Italy
e-mail: luca.bottiglieri@ieo.it

R. D. Gelber · S. Gelber
Department of Biostatistics and Computational Biology
and Harvard Medical School, Dana-Farber Cancer Institute,
Boston, USA
e-mail: gelber@jimmy.harvard.edu

S. Gelber
e-mail: shari@jimmy.harvard.edu

A. Goldhirsch
Department of Medicine,
European Institute of Oncology, Milan, Italy
e-mail: aron.goldhirsch@ibcsg.org

clines yielded a higher rate of pCR in p63-positive compared with p63-negative tumors (23 vs. 0%, $P = 0.048$). No significant difference in the pCR rate was observed for regimens containing anthracycline without cisplatin.

Conclusions Administration of cisplatin without anthracyclines correlates with a high rate of pCR after primary chemotherapy in patients with p63-positive BC. The role of cisplatin-based chemotherapy should be further studied in these patients.

Keywords Breast carcinoma · Cisplatin · p63 · Neoadjuvant chemotherapy · Pathological complete remission · Predictive factor

Introduction

Preoperative treatment is indicated for patients with operable breast cancer for whom a reduction of primary tumor size may permit breast conservation [15]. The introduction of new drugs in preoperative chemotherapy regimens might provide some survival benefit as recently indicated in randomized trials [6, 28]. The identification of reliable predictive markers and the definition of tumor subtypes, through proper studies of gene expression profiling [29] and/or immunohistochemistry [25], may lead to more effective targeted therapies.

Aberrations in the p53 family of transcription factors have been reported to be predictive of response to chemotherapy. p63 shares structural and functional homologies with p53, and is capable of binding DNA, transactivating p53-responsive genes and inducing apoptosis [4, 18]. At variance with p53, p63 codes for multiple mRNA transcripts under two different promoters and via three alternative splicing modalities of the C-terminal ends. In particular, TAp63-α, β and γ have transactivating properties, while ΔNp63-α, β and γ lack the NH2-terminal transactivation domain and function as dominant negative proteins, capable of blocking the activities of the full-length homologues [31]. DNA-damaging agents, such as cisplatin, promote TAp63 stabilization and ΔNp63 degradation, eventually leading to cancer cell apoptosis [22]. DNA-damaging drugs such as cisplatin may therefore be very effective against tumors expressing p63. Both the full-lengths and truncated isoforms of p63 have been reported to be expressed in breast carcinoma, particularly, but not exclusively, in the basal like subtype [26].

On the basis of these considerations, using immunohistochemistry we retrospectively analyzed 189 core biopsies of breast carcinoma from patients treated with primary chemotherapy. The aims of the study were: (1) to assess the association among the expression of p63 and that of hormone receptors, Ki-67 and HER2/neu; (2) to assess the predictive value of p63 for pathological complete remission (pCR); (3) to assess if the expression of p63 is associated with different likelihood of response to different chemotherapeutic regimens.

Materials and methods

Patients

The study population includes 189 patients with clinical stage [1] T1–4d, N0-2, M0 breast carcinoma, treated with preoperative chemotherapy mainly within single arm clinical trials conducted from February 1995 through October 2004 in a single institution, for whom prospectively-collected data on treatment and follow up were available. Eligibility criteria for preoperative chemotherapy were: histological diagnosis of invasive breast carcinoma, no evidence of distant metastases, no previous chemotherapy or endocrine therapy, performance status 0–2 (Eastern Cooperative Oncology Group scale), measurable lesions, age between 18 and 70 years, no relevant concomitant illnesses, and adequate bone marrow reserve, renal function and hepatic function. The only patient with cT1 tumor had positive axillary lymph nodes. Further criteria were specific for some clinical trials: estrogen receptor (ER) and/or progesterone receptor (PgR) > 20% or Ki-67 < 20% for the FLN (fluorouracil, lederfolin, vinorelbine) regimen, Ki-67 $\geq$ 20% for the VFUP (vinorelbine, fluorouracil, cisplatin) regimen, ER and PgR < 20% or absence of PgR for 58 patients treated with the ECF (epirubicin, fluorouracil, cisplatin) regimen. Baseline workup included bilateral mammography and breast ultrasound, breast core-needle biopsy, chest X-ray, abdominal ultrasound, bone scan, and laboratory tests. Each patient provided written informed consent.

Treatment

Chemotherapeutic regimens used during the study period are classified as follows: (1) regimens without either cisplatin or anthracyclines: FLN, fluorouracil 350 mg/m^2/day i.v. days 1–3, lederfolin 100 mg/m^2/day i.v. days 1–3, vinorelbine 20–25 mg/m^2 i.v. days 1 and 3 (13 patients); (2) regimens with anthracyclines without cisplatin: AC, doxorubicin 60 mg/m^2 i.v. day 1, cyclophosphamide 600 mg/m^2 i.v. day 1 (14 patients); FAC, as AC plus fluorouracil 600 mg/m^2 i.v. day 1 (3 patients); AT, doxorubicin 60 mg/m^2 i.v. day 1, docetaxel 75 mg/m^2 i.v. day 1 (3 patients); TAX, docetaxel 70 mg/m^2 i.v. days 1 and 8, doxorubicin 50 mg/m^2 i.v. days 1 and 8, capecitabine 2,000 mg/m^2/day orally days 1–14 (8 patients); VEFU, vinorelbine 20 mg/m^2 i.v. days 1 and 3, epirubicin 20 mg/m^2/day i.v. days 1–3,

fluorouracil 200 mg/m^2/day i.v. as continuous infusion, days 1–21 (1 patient); (3) regimens containing cisplatin without anthracyclines: VFUP, vinorelbine 20 mg (fixed dose) i.v. days 1 and 3, cisplatin 60 mg/m^2 i.v. day 1, fluorouracil 200 mg/m^2/day i.v. as continuous infusion, days 1–21 (34 patients); (4) regimens containing both anthracyclines and cisplatin: ECF, epirubicin 25 mg/m^2/day i.v. days 1 and 2, cisplatin 60 mg/m^2 i.v. day 1, fluorouracil 200 mg/m^2/day i.v. as continuous infusion, days 1–21 (113 patients).

Courses were repeated every 3 weeks, with standard dose modifications due to toxicity as previously reported [9]. Patients received a median of six cycles of chemotherapy (range 1–6). Antitumor activity was evaluated with mammography and breast ultrasound after three and six cycles, and by clinical examination after each cycle. After three cycles, patients with stable disease received surgery, and those with objective response proceeded up to a maximum of six cycles before surgery. Fourteen patients also received preoperative radiotherapy after primary chemotherapy with AC. Surgery was offered at any time in case of progressive disease. After surgery, patients with hormone receptor-positive tumors received adjuvant endocrine therapy; those with endocrine non-responsive disease completed chemotherapy for a total duration of at least 6 months.

Response criteria

Clinical response was evaluated by both imaging (breast ultrasound and mammography) and physical examination, and graded according to WHO response criteria [21]. Pathological complete remission was defined as absence of infiltrating carcinoma either in the breast or in the axillary lymph nodes (residual in situ carcinoma, without any infiltrating area, was considered a pathological complete remission).

Pathology and immunohistochemistry

Immunoreactivity for estrogen receptor (ER), progesterone receptor (PgR) and HER2/neu, as well as the Ki-67 labeling index, had been previously ascertained for clinical purposes, as described [9], while p63 was evaluated specifically for this study. The cases were considered negative for ER, PgR, and p63 if no staining (0% of neoplastic cells) was observed, and positive otherwise. The cases showing a strong and complete membranous Her2/neu immunoreactivity in more than 10% of the neoplastic cells (3+) were considered positive. For assessing p63 immunoreactivity, 3–5 μm-thick sections were immunostained with the 4A4 monoclonal antibody (Dako, Glostrup, Denmark), at a working dilution of 2 μl/ml, using an automated immunostainer (Autostainer, DakoCytomation, Glostrup, Denmark) and a commercially available detection kit (Dako EnVision Plus-HRP), according to the manufacturer's instructions. In each case, at least 1,000 neoplastic cells were evaluated at ×400 magnification, and the percentage of cells showing any definite nuclear immunoreactivity was recorded. Built-in positive controls were myoepithelial cells in normal breast tissue. All the slides immunostained for p63 were reviewed by a blinded second investigator, and the interobserver agreement was 98%.

Statistical methods

The primary endpoint was rate of pCR. The associations among categorical variables and between categorical variables and pCR were assessed using Fisher's exact tests. *P* values less than 0.05 were considered statistically significant; no adjustments were made for multiple testing. Statistical analyses were performed with R [24].

Results

All 189 patients had assessment of p63, ER and PgR, while Ki-67 and HER2/neu were unavailable in 2 and 7 cases, respectively.

Overall, 20 patients (11%) achieved a pCR; 147 (78%) had an objective response, either complete or partial, 39 (21%) had stable disease, and 3 (1%) had disease progression.

Immunoreactivity for p63 was found in 66 cases (35%). There was a nonstatistically significant negative association between the expression of p63 and that of ER: 51 of 130 (39%) ER-absent tumors showed p63 expression, compared with 15 of 59 (25%) ER-positive tumors ($P = 0.07$). No association was found between the expression of p63 and that of either PgR or HER2/neu: 56 of 149 (38%) PgR-absent tumors showed p63 expression, compared with 10 of 40 (25%) PgR-positive tumors ($P = 0.19$); 47 of 135 (35%) HER2/neu-absent tumors showed p63 expression, compared with 17 of 47 (36%) HER2/neu-overexpressing tumors ($P = 0.86$). By contrast, a significant association was found between p63 and Ki-67: 5 out of 30 (17%) tumors with low Ki-67 (<20%) had p63 expression, compared with 61 out of 157 (39%) tumors with high Ki-67 (≥20%) ($P = 0.02$).

The rates of pCR according to pathological and clinical features are reported in Table 1. Overall, there were no differences in the rates of pCR between p63-positive and p63-negative tumors (11 vs. 11%). The lack of expression of hormone receptors was significantly associated with a higher pathological response rate: 16% of the patients with ER and PgR absent tumors achieved a pCR, compared with 0% for those with tumors positive for either ER and/or PgR

Table 1 Pathological complete response rates according to baseline features and type of treatment

Features	Patients, *N*	pCR, *N* (%)	P^b
Total	189	20 (11)	
p63 status			
p63 positive	66	7 (11)	1
p63 negative	123	13 (11)	
ER status			
ER positive	59	0 (0)	0.0005
ER negative	130	20 (15)	
PgR status			
PgR positive	40	0 (0)	0.009
PgR negative	149	20 (13)	
Hormone receptor status			
ER & PgR positive	39	0 (0)	0.003
Either ER or PgR positive	21	0 (0)	
ER and PgR negative	129	20 (16)	
HER2 status			
HER2 overexpressed	47	7 (15)	0.42
HER2 not overexpressed	135	13 (10)	
HER2 unknown	7	0 (0)	
Ki-67 status			
Ki-67 < 20%	30	1 (3)	0.21
Ki-67 ≥ 20%	157	19 (12)	
Ki-67 unknown	2	0 (0)	
Menopausal status			
Premenopausal	114	17 (15)	0.02
Postmenopausal	75	3 (4)	
Clinical tumor stage			
T1–2	87	13 (15)	0.21
T3	53	3 (6)	
T4	49	4 (8)	
Clinical nodal stage			
N0	36	6 (17)	0.23
N1	138	14 (10)	
N2	15	0 (0)	
Chemotherapy regimen			
FLN	13	0 (0)	0.81
AC[a]	14	1 (7)	
FAC	3	0 (0)	
AT	3	0 (0)	
TAX	8	0 (0)	
VEFU	1	0 (0)	
VFUP	34	3 (9)	
ECF	113	16 (14)	
Regimen types			
With cisplatin	147	19 (13)	0.051
Without cisplatin	42	1 (2)	
Without cisplatin and anthracyclines	13	0 (0)	0.25
With anthracyclines, without cisplatin	29	1 (3)	
With cisplatin, without anthracyclines	34	3 (9)	
With cisplatin and anthracyclines	113	16 (14)	

pCR pathological complete remission; *ER* estrogen receptors; *PgR* progesterone receptors; *VFUP* vinorelbine, fluorouracil, cisplatin; *ECF* epirubicin, cisplatin, fluorouracil; *TAX* docetaxel, doxorubicin, capecitabine; *AC* doxorubicin, cyclophosphamide; *FLN* fluorouracil, lederfolin, vinorelbine; *VEFU* vinorelbine, epirubicin, fluorouracil; *FAC* fluorouracil, doxorubicin, cyclophosphamide; *AT* doxorubicin, docetaxel

[a] Patients treated with AC also received radiation therapy before surgery

[b] Fisher's exact test. All tests do not include the unknown category

($P = 0.003$). Tumors with high Ki-67 index (≥20%) had a higher rate of pCR (12 vs. 3%, $P = 0.21$) than those with low Ki-67 index (<20%), but the difference was not statistically significant. The overexpression of HER2/neu was not associated with the pathological response rate in this cohort.

Among clinical features, premenopausal status was associated with an increased probability of pCR compared with postmenopausal status (15 vs. 4%, $P = 0.02$). Although a higher pathological response rate was seen in cohorts with smaller tumors (T1–2) and in those without nodal involvement, the clinical tumor stage and nodal stage were not significantly related to response to therapy. The rate of pCR was higher after treatment with cisplatin-containing regimens (13%) than after regimens without cisplatin (2%), with a difference approaching statistical significance ($P = 0.051$). This might in part be due to the higher percentage of hormone receptor negative tumors in patients treated with regimens containing cisplatin (105/147, 71%) compared with patients treated without cisplatin (24/42, 57%), although this difference was not significant ($P = 0.09$).

Since only patients with endocrine non-responsive tumors experienced a pCR, and because the expression of p63 has been more frequently reported in basal-like tumors, we analyzed the effect of p63 immunoreactivity within the hormone receptor-absent cohort, as well as within the "triple negative" cohort (hormone receptors negative and HER2/neu negative), showing no association with response in either group (data not shown). Indeed, the lack of expression of hormone receptors drives the overall response to primary chemotherapy in this series of patients.

Table 2 reports the response rates to different chemotherapeutic regimens according to the immunoreactivity for p63. Chemotherapy regimens are grouped according to: (1) the presence or absence of cisplatin (two categories); (2) the presence or absence of both cisplatin and anthracyclines

Table 2 Rates of pCR for different types of chemotherapeutic regimens according to the expression of p63

Chemotherapy regimen	p63−		p63+		*P*
	Total *N*	pCR *N* (%)	Total N	pCR *N* (%)	
Without cisplatin	32	1 (3)	10	0 (0)	1
With cisplatin	91	12 (13)	56	7 (13)	1
P		0.18		0.58	
Without cisplatin and anthracyclines	10	0 (0)	3	0 (0)	1
With anthracyclines, without cisplatin	22	1 (5)	7	0 (0)	1
With cisplatin, without anthracyclines	21	0 (0)	13	3 (23)	0.048
With cisplatin and anthracyclines	70	12 (17)	43	4 (9)	0.28
P		0.07		0.50	

pCR pathological complete remission

(four categories). Regimens including cisplatin yielded more pCR than those without cisplatin, both within the p63 positive cohorts (13 vs. 0%, $P = 0.58$) and within the p63-negative cohorts (13 vs. 3%, $P = 0.18$) but differences were not significant possible due to the small number of cases. Patients who received cisplatin-based regimens without anthracycline achieved significantly higher pCR rate if their tumor was p63 positive compared with p63 negative (23 vs. 0%, $P = 0.048$). The three patients who achieved pCR in this group were young (36, 39 and 40 years old) and had "triple negative" tumors with high Ki-67 (30, 50 and 60%). The percentage of tumor cells expressing p63 in these patients did not differ significantly from the percentage of expression found in patients with p63 positive tumors who did not achieve a pCR after treatment with cisplatin-based regimens without anthracycline (data not shown). Regimens including both cisplatin and anthracyclines yielded pCR rates of 9 and 17% in the p63-positive and p63-negative cohorts, respectively ($P = 0.28$).

Because only patients with hormone receptor-negative tumors had pathologic complete remissions, we analyzed that cohort of 129 women separately, as well as the cohort of 84 women with "triple negative" tumors. The trends in pCR rates according to treatment regimen and p63 status were the same as for the total cohort, but results were not statistically significant (data not shown) possible due to the small number of cases.

Discussion

Primary systemic treatment is an ideal setting to assess the activity of different regimens of chemotherapy. A variety of potential predictors of response have been studied. The absence of hormone receptors has been reported to be associated with a higher rate of response to chemotherapy [9], although it does not portend responsiveness to specific agents or drug combinations. The overexpression of topoisomerase IIα, resulting from gene amplification that is encountered in a fraction of tumors also bearing HER2/neu amplification, has been significantly associated with response to anthracycline-based regimens in retrospective studies [19, 20], and its role is currently being evaluated in a prospective randomized trial [5]. Data on HER2/neu overexpression indicate a possible higher pCR rate with the administration of trastuzumab [7], whereas data on p53 mutations as predictors of response to primary chemotherapy are conflicting, and cannot direct treatment choices [5]. Although the study of gene expression profiling may provide response prediction [2, 8, 12, 27], it has not been proven superior to conventional pathologic predictors, and prospective validation is ongoing.

We report the results of a retrospective analysis assessing the association between p63 immunoreactivity and pathological complete response to different regimens of primary chemotherapy for breast cancer. Overall the expression of p63 was not associated with responsiveness to preoperative chemotherapy. However, cisplatin-based regimens (those including cisplatin but not anthracyclines) yielded a significantly higher rate of pCR in p63-positive compared with p63-negative tumors (23 vs. 0%, $P = 0.048$). By contrast, anthracycline-based regimens (including anthracyclines but without cisplatin) achieved one pCR in p63-negative tumors but none in p63-positive ones. Cisplatin-based regimens appear to be more active in p63-positive than in p63-negative tumors, and potentially more active than anthracycline-based regimens in p63-positive tumors.

Although our study has several limitations, including the retrospective nature of the analysis, the limited sample size, and the different kinds of chemotherapy regimens employed, these results suggest that p63-positive breast tumors might be treated more effectively with regimens containing cisplatin. The monoclonal antibody used in this study does not distinguish between the transactivating and truncated isoforms of p63, but previous data indicate that

breast carcinomas preferentially express the truncated isoform [3]. This is the main isoform found in the basal layers of stratified epithelia as well [3, 23].

Different preclinical models have related the expression of ΔNp63 with response to cisplatin. In an immortalized mammary epithelial cell line showing a cytokeratin profile consistent with a basal phenotype, and expressing exclusively the ΔNp63-α isoform, a negative autoregulation of this protein in response to genotoxic stress was demonstrated [13]. The ΔN-specific p63 promoter was shown to endow a p53 binding element, through which p53 induces the expression of ΔNp63-α. In response to genotoxic stress such as ultraviolet irradiation or cisplatin, ΔNp63-α is recruited to this element, preventing p53 binding and down-regulating the expression of ΔNp63-α itself. By contrast, doxorubicin failed to down-regulate ΔNp63-α in these cells. Another possible mechanism is that cisplatin enhances the stratifin-mediated nuclear export of ΔNp63. Within the cytoplasm, ΔNp63 interacts with the receptor for activated C kinase 1 (RACK1), which acts as an E3 ubiquitin ligase, causing proteasomal degradation of ΔNp63 [10]. A further mechanism recently reported is based on the coexpression of ΔNp63 and TAp73 in a subset of "triple negative" breast cancers, usually in presence of a mutated p53, where ΔNp63 binds TAp73 inhibiting its proapoptotic activity. Contrary to other chemotherapeutic agents studied, cisplatin has been shown to induce the dissociation of the ΔNp63/TAp73 complex thereby promoting apoptosis [17].

There is, however, very limited evidence directly correlating ΔNp63 expression with response to cisplatin in clinical reports. In patients treated with cisplatin-based chemotherapy for head and neck squamous cell carcinoma, a robust expression of ΔNp63 was found in responders, and no expression in nonresponders [32]. Neoadjuvant single agent cisplatin showed considerable activity in patients with "triple negative" early breast cancer, with 64% rate of objective remissions and 23% pathological complete remissions [11]. The expression of p63 has been shown to be correlated with a reduced expression of BRCA1 in sporadic basal-like tumors [26]. BRCA1 is involved in DNA repair, and its reduced expression seems to confer increased sensitivity to DNA-damaging drugs such as cisplatin, and has been suspected to confer resistance to spindle poisons [16].

In our series of patients, no pCR was obtained in p63 expressing tumors with regimens not including cisplatin, e.g. with anthracycline-based regimens. Anthracyclines could be unable to induce TAp63 stabilization and ΔNp63 degradation in these tumors. As previously reported, doxorubicin was unable to diminish ΔNp63-α levels in immortalized mammary cells [13]. In p53 deficient breast cancer cell lines, TAp73, but not TAp63, has been shown to functionally replace p53 in triggering apoptosis [30]. Wild type p53 down-regulates topoisomerase IIα expression [14], and it could be hypothesized that the pro-apoptotic isoform TAp63 might do the same, reducing responsiveness to anthracyclines.

Treatment with regimens including both cisplatin and anthracyclines in a cohort of 113 patients did not result in a better pCR rate in p63 expressing tumors in our study. This raises the possibility that anthracyclines could reduce the beneficial effect of cisplatin in p63 expressing tumors, although the lack of a difference is also explained by the higher rate of pCR in p63-negative tumors in this cohort (17%) compared with the cohort treated with regimens including cisplatin without anthracyclines (0%).

In conclusion, our data indicate that regimens including cisplatin without anthracyclines may yield a significantly higher rate of pCR in p63-positive compared with p63-negative tumors, thus suggesting that cisplatin-based chemotherapy should be further studied in patients whose tumors express p63.

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