

ARTICLES

Multidrug Resistance in Breast Cancer: a Meta-analysis of MDR1/gp170 Expression and Its Possible Functional Significance

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Background: P-glycoprotein (gp170; encoded by the MDR1 gene [also known as P-glycoprotein]) is a membrane protein capable of exporting a variety of anticancer drugs from cells. MDR1/gp170 expression has been studied in breast cancer, but the prevalence of this expression and its role in breast tumor drug resistance are unclear. **Purpose:** We conducted a critical review and meta-analysis of studies examining MDR1/gp170 expression in breast cancer to estimate the likely prevalence and clinical relevance of this expression. We also explored reasons for differences in the findings from individual studies. **Methods:** Published papers on MDR1/gp170 expression in breast cancer were identified by searching several literature databases and reviewing the bibliographies of identified papers. Variability across the studies in the proportion of tumors expressing MDR1/gp170 was assessed by use of chi-squared tests of homogeneity, weighted means, and weighted linear regression. Pooled relative risks (RRs) for the association between the induction of MDR1/gp170 expression and prior chemotherapy and associations between MDR1/gp170 expression and several clinical outcomes were estimated by use of Mantel-Haenszel methods. Heterogeneity among the pooled RRs was explored by use of chi-squared tests. Reported *P* values are two-sided. **Results:** Thirty-one studies were identified and evaluated. The proportion of breast tumors expressing MDR1/gp170 in all of the studies was 41.2%, but there was substantial heterogeneity in the values across individual studies ($P < .0001$). Regression analyses demonstrated that a considerable portion of the observed heterogeneity was a consequence of the change, over time, from RNA hybridization-based assays to immunohistochemistry-based assays of MDR1/gp170 expression. Measuring MDR1/gp170 expression before versus after chemotherapy and use of cytotoxic drugs that are not substrates for gp170 also contributed to the heterogeneity. Treatment with chemotherapeutic drugs or hormonal agents was associated with an increase in the proportion of tumors expressing MDR1/gp170 (RR = 1.77; 95% confidence interval [CI] = 1.46-2.15). Patients with tumors expressing MDR1/gp170 were three times more likely to fail to respond to chemotherapy than patients whose tumors were MDR1/gp170 negative (RR = 3.21; 95% CI = 2.28-4.51); this RR increased to 4.19 (95% CI = 2.71-6.47) when considering

only patients whose tumor expression of MDR1/gp170 was measured after chemotherapy. MDR1/gp170 expression was not associated with lymph node metastases, estrogen receptor status, tumor size, tumor grade, or tumor histology. **Conclusions and Implications:** MDR1/gp170 expression in breast tumors is associated with treatment and with a poor response to chemotherapy. The data are consistent with a contributory role for MDR1/gp170 in the multidrug resistance in some breast tumors. [J Natl Cancer Inst 1997;89:917-31]

Breast cancer is often considered to be one of the more chemoresponsive solid tumors. Many structurally diverse cytotoxic drugs, when administered either as single agents or in combination, can induce remissions in previously untreated breast cancer patients (1). While the overall response rate can be high, the duration of response is relatively short (2), and most of the initially responsive breast tumors acquire a multidrug resistance phenotype. This phenotype is frequently characterized by a cross-resistance to drugs to which the tumors have not been exposed. The development of a multidrug resistant phenotype in metastatic breast cancer is primarily responsible for the failure of current treatment regimens. The precise nature of this phenotype remains unclear. However, several mechanisms may be involved and include kinetic resistance (3,4), gp170 (PGP, P-glycoprotein) expression, glutathione transferases (5,6), superoxide dismutases (7), topoisomerases (8), and the multidrug resistance-associated protein (MRP) (9). These may occur independently or in combination, thereby conferring resistance in heavily treated patients exposed to structurally and functionally diverse agents.

In experimental models, the multidrug-resistant phenotype is often accompanied by the expression of the MDR1 gene (also

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known as PGY1) and/or its gp170 glycoprotein product (MDR1/gp170, where MDR1 refers to the gene and its messenger RNA and gp170 denotes the glycoprotein gene product) (10). A 170-kd cell membrane glycoprotein, gp170 appears to work by actively effluxing substrates from cells (11). The glycoprotein possesses two adenosine triphosphate (ATP)-binding sites and exhibits significant ATPase activity (12). The drug-binding sites are close to gp170's cytosolic transmembrane domains (13), the drugs apparently being removed from within the membrane bilipid layer (14). Activation of gp170 may be regulated through phosphorylation by protein kinases, e.g., protein kinase C (15-17), and its efflux function may be affected by membrane lipid composition (18) and fluidity (19-21).

The multidrug-resistant phenotype conferred by gp170 is characterized by resistance to various structurally unrelated anticancer agents, including the anthracyclines, epipodophyllotoxins, vinca alkaloids, and taxanes (22). The majority of the most widely used combination chemotherapy regimens for breast cancer include gp170 substrates as either first-line or second-line treatments, most frequently in combination with non-gp170 substrates. For example, doxorubicin (Adriamycin) and vinblastine are gp170 substrates and are among the most effective antineoplastic drugs in breast cancer (22). Paclitaxel (Taxol) when administered as a single agent (23) or in combination with doxorubicin also produces significant responses in breast cancer (24) and is likely to become more widely used as an agent for breast cancer treatment.

The role of MDR1/gp170 in human breast cancer remains unclear, partly because of an apparent lack of a consensus on whether MDR1/gp170 is expressed in breast tumors. While various small studies (25-27) have readily detected MDR1/gp170 expression, one of the largest single studies published to date (28) and several smaller studies (29,30) have failed to detect any expression of MDR1/gp170 in breast tumors. Even among those studies detecting MDR1/gp170 expression, there has been no clear consensus regarding its likely functional significance.

Our primary goals were to clarify the prevalence and clinical relevance of MDR1/gp170 expression in breast cancer and to explore the causes of heterogeneity across the studies evaluated. We have undertaken a critical review and meta-analysis of the published literature describing the expression and potential function of the MDR1 gene and its gp170 product in breast cancer. Meta-analysis has rarely been applied to basic science research. However, it may be useful for translational studies of molecular mechanisms in cancer, since the rapid pace of basic research is associated with considerable variation in methods, reagents, and quality-control measures, and such variation may obscure the clinical relevance of a given molecule.

The specific questions that we addressed are as follows: 1) Is MDR1/gp170 expressed in breast tumors and, if so, what is the frequency of expression? 2) Is MDR1/gp170 expression associated with cytotoxic and/or hormonal treatment and, if so, what is the magnitude of this association? 3) What is the potential functional relevance of MDR1/gp170 expression in breast cancer with respect to response to chemotherapy and predicting prognosis? 4) For each of the above questions, if there is a significant lack of agreement among studies, what factors contribute to this heterogeneity?

Both the validity and the most appropriate methodology for

performing reviews of this type are controversial (31-34). We have emphasized objective quantitative criteria in our evaluation and have followed explicit guidelines for the conduct of meta-analyses and critical research reviews (35-37). Moreover, we considered an assessment of the sources of heterogeneity to be a goal of the analysis rather than a preliminary step.

The treatment and exposures of interest are the systemic therapies administered to breast cancer patients. Such therapies include cytotoxic chemotherapy, which we considered in two subsets, i.e., regimens including at least one known gp170 substrate and regimens not including any known substrates. For example, the CAF regimen (cyclophosphamide, doxorubicin, and fluorouracil) is considered to be an MDR1/gp170-related drug regimen because of the presence of doxorubicin. In contrast, the CMF regimen (cyclophosphamide, methotrexate, and fluorouracil) is considered to be a non-MDR1/gp170-related drug regimen. We also have included studies that utilized the antiestrogen tamoxifen (38,39), which is known to reverse gp170-mediated resistance *in vitro*.

Materials and Methods

Criteria for Conducting the Review and Meta-analysis

Formal research reviews and meta-analyses have become increasingly common for evaluating large, often diverse bodies of research to resolve apparent lack of agreement among the individual studies. These often take the form of purely synthetic exercises, where the individual study results are combined in a summary measure, without regard to the appropriateness of combining the studies. Such analyses often also lack a careful exploration of the reasons for disagreement among studies. The latter can often be as important as or more important than the summary measure, since the factors underlying disagreement may point to informative patient subgroups or methodologic issues.

This review and meta-analysis focused not only on quantifying the prevalence of MDR1/gp170 expression and its functional relevance in breast cancer, but also on exploring sources of variation or heterogeneity in results across studies. The appropriate criteria for research reviews and meta-analyses have been widely discussed (37,40-42), most recently in this Journal (43). The general guidelines from these sources were adapted and applied for the conduct of this review as follows:

- 1) We include a clear statement of the specific purpose(s) of the review, with reference to the population to be generalized, the treatment or exposure of interest, and the major outcome(s) of interest (*see above*).
- 2) We describe the sources and define the methods of citation searches.
- 3) We formulated specific guidelines, in advance, to determine which studies were to be excluded or included and detail the reasons for exclusion or inclusion.
- 4) We established, and subsequently applied, a consensual assessment of the validity of the methods used in the studies reviewed and provide a determination of what conclusions are justified by these methods. More than one author was involved in determining the assessment.
- 5) We integrate results of individual studies in a quantitative, weighted fashion, with consideration of data limitations and/or inconsistencies. Where significant heterogeneity is present, we attempt to determine its sources. This may be more important than any summary measure.
- 6) We summarize all major or relevant findings.
- 7) We identify specific directions or considerations for new research by identifying gaps in our present knowledge.
- 8) We provide suggestions for the design of future studies, particularly where this relates to eliminating confounding factors and identified sources of heterogeneity.

Sources of Data and Methods of Citation Search

To identify studies that evaluated MDR1/gp170 expression in human breast cancers, we performed an extensive literature search of the following databases: Medline (National Library of Medicine, National Institutes of Health, Bethesda, MD), CANCERLIT (National Library of Medicine), *Current Contents* (Institute for Scientific Information, Philadelphia, PA), *Current Advances in Cancer Research* (Current Awareness in Biological Sciences, Elsevier Science Inc., Tarrytown, NY), *Knowledge Finder* (Aries Systems Corp., North Andover, MA), and the *Science Citation Index* (Institute for Scientific Information). For keyword-based Boolean searches, we used 44 different keywords that were either words or variations of words, e.g., spelling, punctuation, and abbreviation, which represent the multidrug resistance subject. We also used the bibliography listings of relevant papers found in the above databases as leads for identifying additional studies.

Criteria for Inclusion or Exclusion

For studies to be included, they had to describe original research involving measurement of MDR1/gp170 expression in human breast cancers. Reviews were excluded. If more than one report described the same data, we used only the latest report. To be included, a published report also must have enabled determination of the number of MDR1/gp170-positive and -negative tumors. Since amplification is often an unreliable indicator for gene expression and the contribution of other relevant coamplified genes cannot be assessed readily (44,45), studies based only on amplification of the MDR1 gene were not included. All other studies, which primarily used immunologic detection of protein (western blot or immunohistochemistry) and/or RNA hybridization methods (northern blot or dot blot), were included. Study eligibility was determined independently by all three investigators. When determining eligibility, the investigators were not formally blinded to study results because it was often necessary to read the results to determine whether the number of MDR1/gp170-positive tumors was given. However, the inclusion criteria are quite unambiguous, and it is unlikely that lack of blinding induced bias (only four papers were excluded—see below).

Three studies (28,46,47) used more than one assay method to evaluate the same group of tumor samples. These results were included in all analyses of subgroups of studies that used a specific method. For example, the report by Merkel et al. (28) analyzed many of the same tumors by use of both western blot and RNA hybridization methods and was represented in both the subgroups of western blot and RNA hybridization studies. (Data from the study by Merkel et al. that were based on gene amplification were not included.) In analyses that pooled all studies, the results obtained by Merkel et al. were represented by the assay method based on the largest number of tumors (western blot). The results obtained by Kim (46), who included the same number of tumors for each assay method, were represented by the method yielding the highest expression of MDR1/gp170, i.e., immunohistochemistry. The study by Chevillard et al. (47) is represented here among the studies of immunohistochemistry. There were insufficient studies using the polymerase chain reaction (PCR) to enable analysis of data obtained by this methodology.

Assessment of Studies and Methods

Studies of MDR1/gp170 expression in breast cancer are essentially translational research. Thus, methodologic considerations must be coupled with the ability to derive clinical inferences from the results. With this in mind, we chose to assess the methodologic rigor and the clinical relevance of the studies by applying a series of objective technical criteria. These criteria were used to describe the studies, but a "quality score" was not obtained for use as a stratification factor for the meta-analysis. Such quality scores are often confounded and highly subjective. Stratification or exploration of heterogeneity can be better accomplished on the basis of the individual components of the quality score (33).

The criteria that we used were based on factors that we deemed a priori to be potential contributors to heterogeneity and that also comprised a set of important criteria for the conduct of this type of translational research. The criteria included the following:

- 1) Appropriate consideration of statistical precision, i.e., adequate sample size, use of confidence intervals (CIs), or consideration of sample size in interpretation of results. Twenty-five patients were considered to be the minimum adequate sample size because this size would produce an upper confidence bound of less than 10% if the study found no tumors expressing MDR1/gp170.

- 2) Adequate description of tumors and treatment, such that MDR1/gp170-positive and -negative tumors could be classified according to whether the assay was performed on primary or metastatic tumors (before or after chemotherapy) and according to whether treatment included multidrug-resistant substrates (e.g., doxorubicin) or non-multidrug-resistant substrates (e.g., cyclophosphamide).
- 3) A clear description of the criteria for MDR1/gp170 positivity, sufficient to allow comparison among studies.
- 4) A clear description of the characteristics of the patient population and criteria for accrual.
- 5) The presence of positive controls in the assay for MDR1/gp170 expression.
- 6) The presence of negative controls in the assay for MDR1/gp170 expression.
- 7) The use of more than one antibody for assessing gp170 expression (immunohistochemistry studies only) and the use of non-cross-reacting antibodies or confirmatory methods such as PCR.

These criteria were established in advance by all three authors, who also scored each study independently. Each criterion was scored simply as (+) or (–) by each assessor.

Statistical Methods, Integration of Individual Studies, and Assessment of Heterogeneity

The proportion of tumors expressing MDR1/gp170 was calculated in each study and in subgroups where appropriate. A 95% CI for the proportion was calculated by use of standard methods for the binomial distribution. The proportions were pooled across studies as the weighted average of the proportions with the use of individual study sample sizes as weights (48). A chi-squared test for homogeneity (χ^2_{homog}) of the proportions was calculated by use of standard methods for $2 \times C$ contingency tables (49). It should be noted, however, that the test for heterogeneity may be too sensitive when the number of studies (number of columns in the contingency table) is large.

To explore sources of significant heterogeneity among the studies, we examined whether the proportion of MDR1/gp170-expressing tumors exhibited a linear trend with any characteristics of the individual studies. We applied a weighted regression model, with the study-specific proportion as the dependent variable and weighted by the sample size. This analysis was performed by use of the weighted regression option in the procedure PROC REG in SAS (Statistical Analysis Systems Institute Inc., Cary, NC). Since each "observation" was an average over all patients in a study, the standard errors of the regression coefficients were corrected by dividing by the square root of the residual mean square (50).

We also examined heterogeneity by classifying studies according to potential sources of heterogeneity and recalculating the weighted mean proportions and χ^2_{homog} within these subgroups. To compare subgroups of studies, e.g., studies in which MDR1/gp170 was measured before versus after chemotherapy, we calculated the weighted mean of the proportion expressing MDR1/gp170 for each subgroup of studies. The difference between means was divided by the standard deviation of the difference to give a z-score as described by Greenland (50).

We examined the potential association between MDR1/gp170 expression and 1) prognostic factors, 2) prior treatment, 3) clinical response to chemotherapy, and 4) in vitro doxorubicin resistance. We determined the relative risk (RR) of the particular clinical outcome in MDR1/gp170-positive versus -negative tumors for each study and pooled the RRs across studies by using a Mantel-Haenszel approach (51). Cochran-Mantel-Haenszel statistics (52), as implemented in the SAS subroutine PROC FREQ, were used to test the significance of the pooled RR. A similar approach was used to investigate the effect of chemotherapy on MDR1/gp170 induction, but the RR compared the probability of MDR1/gp170 induction between patients with and without prior chemotherapy at the time of MDR1/gp170 measurement. CIs for the pooled RRs and a χ^2_{homog} of RRs were calculated by use of the method of Breslow and Day (51). These analyses were conducted by the procedure PROC FREQ in SAS. Since many studies had small cell sizes or cells with zero patients, we included the continuity correction of 0.5 added to each cell for the calculation of RRs. We used pooled RRs rather than odds ratios because the latter can overestimate the RR when the outcome being considered occurs in more than 10% of the study sample (53). Since the samples in these studies were sampled in a cohort fashion, there is no need to use the odds ratio because the RR provides a clearer indication of the clinical impact of MDR1/gp170 expression. All *P* values are two-sided.

Results

Results of Citation Search

Thirty-one studies (25-30,46,47,54-76) were identified that met the criteria for inclusion in the meta-analysis. These included 21 studies (26,46,47,55,57-59,61,62,64-75) with MDR1/gp170 expression based on immunohistochemistry, eight studies (27-29,46,54,56,60,63) based on RNA hybridization methods, three studies (25,28,30) based on immunoblot (western blot) methods, and two studies (47,76) based on the reverse transcription-PCR. [Three studies (28,46,47) used more than one method.] One additional study (77) was excluded from the analysis because it used only gene amplification as an indicator of MDR1/gp170 activity. Twelve studies (30,46,54,56,59,61,64-66,70,72,75) measured MDR1/gp170 expression on tumors prior to any chemotherapy or hormonal therapy, 13 studies (25-28,47,55,57,62,63,67,73,74,76) included tumor measurements both before and after treatment, two studies (68,69) had only post-treatment tumor measurements, and in four studies (29,58,60,71) the authors did not indicate the timing of the assay with respect to treatment. In addition to the studies that met the

inclusion criteria, four published reports identified by the search were excluded for the following reasons: Only gene amplification was used as an indicator of MDR1 activity (77), results for breast tumors could not be separated from those for all tumors (78), no samples from breast cancers were tested (79), and the report described earlier results that are included in a later report (80).

Assessment of Methodologic Rigor of Studies

Table 1 lists the degree to which the individual studies complied with the criteria for methodologic rigor. Only two of the criteria, i.e., a definition of MDR1/gp170 positivity and the use of positive and negative controls, were met by nearly all studies. For each of the other four criteria, there were many studies that did not adhere to at least one criterion. Thirty-five percent of the studies had a sample size of fewer than 25 patients. None of these 11 studies (26,29,30,46,54,55,58,59,61,74,76) used CIs to indicate the degree of uncertainty in their data, and they did not discuss the possible influence of sample size on their results. Because small samples produce relatively imprecise estimates of the prevalence of MDR1/gp170 expression and its association with clinical parameters, sample size may contribute to hetero-

Table 1. Methodologic and clinical criteria for studies of MDR1/gp170 expression in human breast cancer*

Authors, year of publication (reference No.)	Criteria									
	Sample size	Description of tumor, treatment†	Definition of MDR1/gp170 positivity‡	Description of patient base§	Controls§		Adequacy of antibody methods			
					Positive	Negative				
Goldstein et al., 1989 (27)	+	-	+	-	-	+	+	NA	NA	
Kacinski et al., 1989 (54)	-	+	+	-	+	+	+	NA	+	
Merkel et al., 1989 (28)	+	+	+	-	-	+	-	-	(+)	
Moscow et al., 1989 (29)	-	-	+	-	-	+	+	NA	NA	
Ronchi et al., 1989 (30)	-	+	+	-	-	+	+	-	-	
Schneider et al., 1989 (55)	-	+	+	-	-	+	+	-	-	
Salmon et al., 1989 (26)	-	-	+	-	-	+	+	+	+	
Keith et al., 1990 (56)	+	+	+	-	-	+	+	NA	NA	
Ro et al., 1990 (57)	+	+	+	+	+	+	+	-	-	
Wishart et al., 1990 (66)	+	+	+	-	-	+	+	+	+	
Sugawara, 1990 (59)	+	-	-	-	-	+	+	-	-	
Kim, 1990 (46)	-	+	+	-	-	+	+	-	+	
Cordon-Cardo et al., 1990 (58)	-	-	-	-	-	+	+	+	+	
Wallner et al., 1991 (60)	+	-	+	-	+	+	+	NA	NA	
Verelle et al., 1991 (61)	-	+	+	-	+	+	+	-	-	
Sanfilippo et al., 1991 (25)	+	-	+	-	-	+	+	-	-	
Dixon et al., 1992 (68)	+	-	+	-	-	+	+	-	-	
Koh et al., 1992 (62)	+	+	+	-	-	+	+	-	-	
Hennequin et al., 1993 (63)	+	+	+	-	+	+	+	NA	NA	
Botti et al., 1993 (69)	+	+	+	-	+	+	+	-	-	
Schneider et al., 1994 (9)	+	+	-	-	+	-	+	+	+	
Veneroni et al., 1994 (64)	+	+	+	-	-	+	+	-	-	
Charpin et al., 1994 (65)	+	+	+	-	+	+	+	-	(+)	
Keen et al., 1994 (67)	+	-	+	-	-	-	-	-	-	
Schneider and Romero, 1995 (75)	+	+	+	+	+	+	+	-	-	
Seymour et al., 1995 (73)	+	+	+	-	+	+	+	-	-	
Linn et al., 1995 (71)	+	-	+	-	+	+	+	+	+	
Decker et al., 1995 (74)	-	+	+	+	+	+	+	+	+	
Luowen et al., 1995 (72)	+	+	+	+	+	+	+	-	-	
Chevillard et al., 1996 (47)	+	+	+	-	+	+	+	+	+	
O'Driscoll et al., 1996 (76)	-	+	-	-	-	+	+	NA	NA	

*+ = criterion present; - = criterion absent; (+) = criterion partially achieved; NA = not applicable.

†This criterion addresses whether MDR1/gp170 expression could be classified as to primary versus metastatic tumor, treated versus untreated patients, and treatments that are or are not gp170 substrates.

‡This criterion addresses whether the description is sufficient to allow comparison of MDR1/gp170 positivity among studies.

§The first rating concerns the description of how samples were accrued; i.e., is it sufficient to assess the likely sources of bias? The second rating concerns the description of characteristics of the study population; i.e., can individual characteristics be related to multidrug resistance?

||Refers to whether studies based on antibody methods were likely to have guarded against artifacts by (first column) use of more than one antibody (indicated by +) or (second column) by use of non-cross-reacting antibodies or other confirmatory methods (indicated by +).

genuity. Thirty-two percent of the studies did not provide a sufficient description to determine whether tumor samples came from primary or metastatic tumors or from treated or untreated patients and the type of treatment(s) used, or they did not use treatments known to be gp170 substrates. As a result, it is difficult to interpret the observed prevalence of MDR1/gp170 expression. Most studies did not adequately describe the patient base or accrual procedure. This information is important for determining the representativeness of patients in an individual study and the ability to generalize study results. It also allows the reader to determine a study's susceptibility to various forms of bias (e.g., size bias, defined as bias toward larger tumors that allow multiple sampling).

Fourteen studies (46,55,57,59,61,62,64,65,67-69,72,73,75) of the 21 (67%) studies based on immunohistochemical detection of gp170 and all three studies (25,28,30) using the western blot technique used only one antibody (Table 1), despite the commercial availability of several antibodies with nonoverlapping epitopes (81-84). Unfortunately, several of the antibodies used in isolation have well-defined cross-reactivity with proteins other than gp170. For example, both JSB-1 (85) and C494 (86) cross-react with pyruvate carboxylase. The C219 monoclonal

antibody recognizes MDR3, a member of the same gene family that is not involved in drug resistance (87). This antibody also cross-reacts with the heavy chain of muscle myosin (84), and myofibroblasts are a major component of the desmoplastic response to breast cancer (88). Thus, there may be a tendency for an increased frequency of false-positive results in some single-antibody studies, where the adequacy of the negative controls becomes critical for assessing the likely validity of gp170 estimates.

Proportion of MDR1/gp170-Positive Tumors

Panels A and B of Fig. 1 show the proportion of MDR1/gp170-positive tumors and the associated 95% CIs for studies with MDR1/gp170 measured before or after treatment, respectively. Expression varies considerably across studies; this variation is apparent regardless of whether the tumor material was assayed before or after chemotherapy. Some potential sources of heterogeneity are indicated by stratifying the studies according to the type of assay method (Fig. 1, A) or the type of treatment received (Fig. 1, B). Fig. 1, A, shows that most of the studies that found no expression of MDR1/gp170 were based on western blot or RNA hybridization methods. Only one immunohisto-

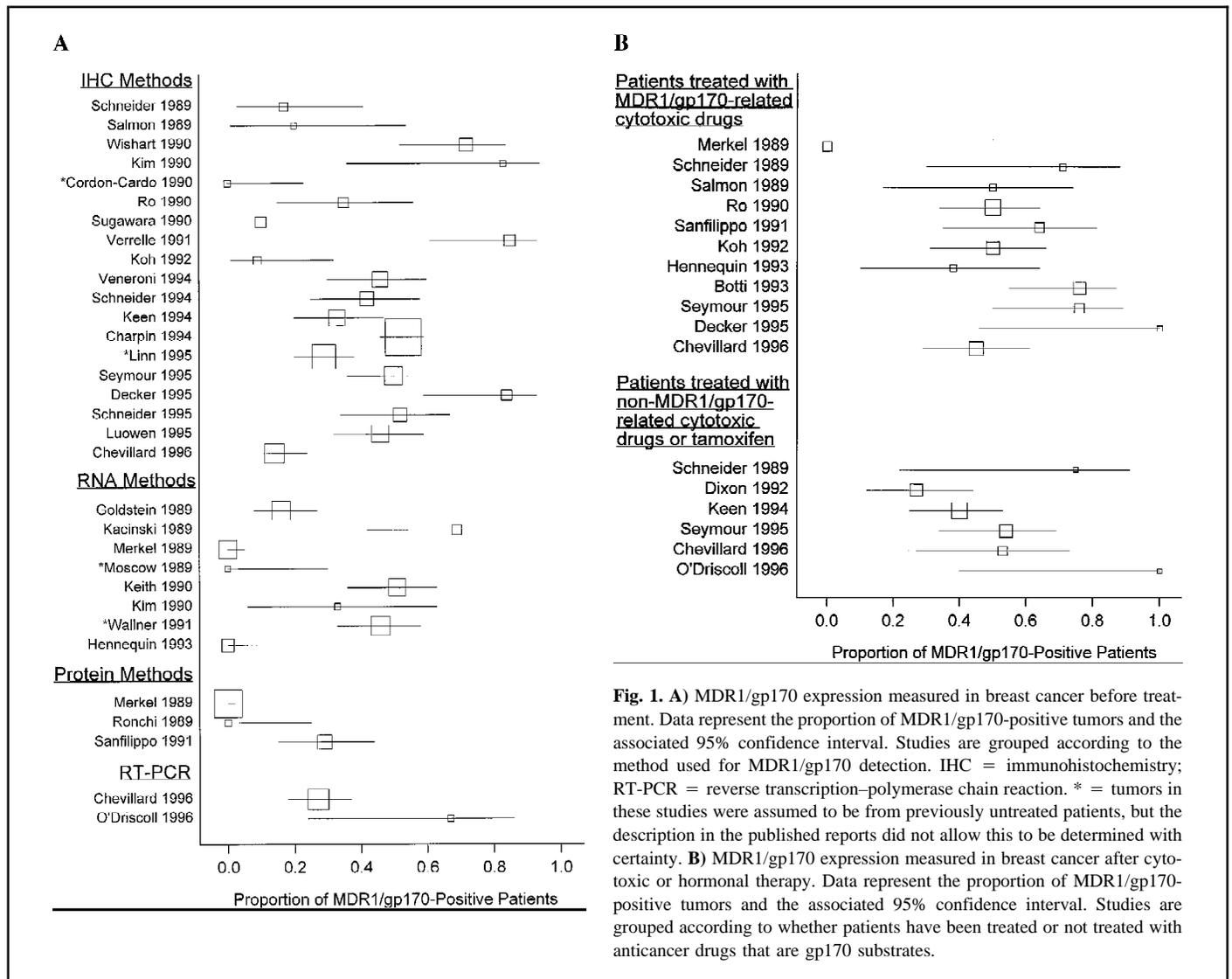


Fig. 1. A) MDR1/gp170 expression measured in breast cancer before treatment. Data represent the proportion of MDR1/gp170-positive tumors and the associated 95% confidence interval. Studies are grouped according to the method used for MDR1/gp170 detection. IHC = immunohistochemistry; RT-PCR = reverse transcription-polymerase chain reaction. * = tumors in these studies were assumed to be from previously untreated patients, but the description in the published reports did not allow this to be determined with certainty. B) MDR1/gp170 expression measured in breast cancer after cytotoxic or hormonal therapy. Data represent the proportion of MDR1/gp170-positive tumors and the associated 95% confidence interval. Studies are grouped according to whether patients have been treated or not treated with anticancer drugs that are gp170 substrates.

chemistry study (58) found no immunoreactivity (based on only eight breast tumors), but this study reported some staining of "apparently normal parenchymal" cells in some specimens.

Based on the weighted average over all 31 studies listed in Table 1, the percentage of tumors expressing MDR1/gp170 was 41.2% (95% CI = 36.0%-46.5%). However, the proportions were highly variable across individual studies ($P < .0001$). These studies were performed over an 8-year period (1989-1996). To evaluate whether potential sources of heterogeneity may have changed over time, we performed a weighted linear regression of the proportion of MDR1/gp170-positive tumors in each study against the calendar year when the study was published. Table 2 shows a highly significant trend with time, there being a slope coefficient of 0.040, with $z = 3.52$ ($P = .0005$). This trend indicates that, on average, there was an increase of about 4.0% per year in the reported detection of MDR1/gp170 expression in breast tumors.

There was a shift away from RNA hybridization methods toward immunohistochemical methods over this time, so we stratified the studies according to whether they used RNA hybridization or immunohistochemical methods and conducted separate regressions within each group of studies. (No regression was conducted for the western blot studies because there were only three such studies.) Within groups of studies using either RNA hybridization or immunohistochemical methods, no trend of MDR1/gp170 expression with time was evident (Table 2). Thus, a significant degree of variability among the studies appears to be due to the shift over time from RNA hybridization methods (primarily northern blot hybridizations) to the more sensitive immunohistochemical methodologies.

We next examined heterogeneity separately within both the RNA hybridization and immunohistochemistry studies. The average percentage of tumors expressing MDR1/gp170 was 27.1%

(95% CI = 15.4%-38.6%) for studies using RNA hybridization methods and 48.5% (95% CI = 42.0%-55.0%) for immunohistochemistry studies. However, even within these subgroups, there was still significant heterogeneity ($P < .0001$ for both RNA hybridization and immunohistochemistry studies).

We further stratified these subgroups according to whether MDR1/gp170 had been measured before or after chemotherapy or hormonal therapy. The difference between values before and after chemotherapy or hormonal therapy was relatively small for both immunohistochemistry and RNA hybridization studies, and significant heterogeneity still existed within these subgroups (Table 3). Among studies of values after chemotherapy or hormonal therapy, we compared MDR1/gp170 expression among tumors from patients treated with and without drugs associated with the multidrug-resistant phenotype (data not shown). As expected, MDR1/gp170 expression was higher for patients treated with MDR1/gp170-related drugs, with an average of 57.2% of tumors being positive. However, a sizable percentage (44.6%) of patients treated with non-MDR1/gp170-related drugs also expressed MDR1/gp170. The studies of MDR1/gp170-related and non-MDR1/gp170-related drugs exhibited marginally significant heterogeneity ($P = .052$ and $P = .054$, respectively).

Associations With Clinical Parameters

It has been suggested that the expression of MDR1/gp170 is more likely to be a surrogate marker for a worse prognosis than an indicator of potential response to cytotoxic chemotherapy (89). To address the clinical relevance of MDR1/gp170 expression, we considered the evidence linking it to various clinical parameters, including induction by cytotoxic or hormonal treatment and association with 1) established prognostic attributes, 2) response to chemotherapy, 3) recurrence and survival, and 4) in vitro doxorubicin resistance.

Induction of MDR1/gp170 expression. The effect of chemotherapy on induction of MDR1/gp170 expression is shown in Table 4. This analysis was performed by comparing the proportion of patients whose tumors expressed MDR1/gp170 before chemotherapy with that of patients whose tumors expressed MDR1/gp170 after chemotherapy. We used only studies that provided both pretreatment and post-treatment data, although most were not consecutive measurements on the same patients. In the 13 studies meeting this criterion (25-28,47,55,57,62,63,67,73,74,76), treatment with cytotoxic or hormonal agents was associated with a significant increase in the proportion of tumors expressing MDR1/gp170 (RR = 1.77 [95% CI = 1.46-2.15]; $P < .0001$). Unlike the analysis performed on all stud-

Table 2. Regression of proportion of tumors expressing MDR1/gp170 on year in which the study was conducted (expressed as year - 1900)

Studies	Regression coefficient	Corrected standard error	z-statistic	P*
All studies† (n = 31)	0.040	0.011	3.52	.0005
Immunohistochemistry studies (n = 21)	0.004	0.017	0.23	.82
RNA hybridization studies (n = 8)	0.019	0.044	0.43	.67

*Two-sided.

†Includes two studies based only on western blot methods (25,30) and one study that used both western blot and RNA hybridization methods (28).

Table 3. Pooled proportion of tumors expressing MDR1/gp170, according to timing of assay with respect to treatment (pretreatment versus post-treatment), and assay method (immunohistochemistry based versus RNA hybridization based)

Timing (No. of studies)	Assay	No. of patients	Pooled proportion	Test of homogeneity	
				χ^2 (df*)	P†
Pretreatment (17)	Immunohistochemistry	661	0.458	91.6 (16)	<.001
Pretreatment (8)	RNA hybridization	270	0.274	72.8 (7)	<.0001
Post-treatment (10)	Immunohistochemistry	279	0.520	23.8 (9)	.005
Post-treatment (3)	RNA hybridization	22	0.227	10.6 (2)	.005

*df = degrees of freedom for the test of homogeneity.

†Two-sided.

Table 4. MDR1/gp170 expression in association with cytotoxic therapy*

Type of treatment	No. of studies (No. of patients)	Summary: RR (95% CI)	RR <i>P</i> value†	Test for homogeneity	
				χ^2 (df)	<i>P</i> †
All cytotoxic drugs	13 (726)	1.77 (1.46-2.15)	<.0001	20.22 (12)	.056
MDR1/gp170-related drugs only	10 (499)	1.99 (1.56-2.54)	<.0001	12.81 (9)	.19

*RR = relative risk, i.e., the probability of MDR1/gp170-positive tumor in patients treated with cytotoxic therapy versus untreated patients; CI = confidence interval; df = degrees of freedom for the test of homogeneity.

†Two-sided.

ies, there was only marginally significant heterogeneity in this subgroup ($P = .056$). When we excluded the three studies that used drugs not commonly related to MDR1/gp170 (27,67,76), the effect was somewhat stronger (RR = 1.99 [95% CI = 1.56-2.54]; $P < .0001$), and there was no significant heterogeneity ($P = .19$). The results were unchanged when we repeated the analyses by reclassifying as negative those tumors exhibiting weakly positive expression (25,55,57).

Association with prognostic attributes. Only 11 studies (54,57,60,61,63,65,69,70,73-75) included data associating MDR1/gp170 expression with one or more known prognostic attributes, including lymph node status at diagnosis, tumor size, tumor histology, tumor grade, and estrogen receptor status. We considered only attributes examined in three or more studies. Table 5 shows that none of these attributes were significantly associated with MDR1/gp170 expression. This result suggests that MDR1/gp170 expression is not acting as a surrogate for another prognostic factor in its ability to predict outcome or response to chemotherapy.

Association with response to chemotherapy. Since studies provided different levels of detail about clinical response, we considered all studies in which it was possible to determine the number of patients who exhibited at least a clinical partial response, i.e., either partial response (PR) or complete response (CR). These responses were defined according to widely accepted usage, where CR indicates complete disappearance of the tumor, while PR indicates a greater than 50% reduction in the largest diameter of the tumor. There were nine studies in which these data could be determined (47,57,61-64,68,69,74). An additional three studies either did not permit classification of the responses of patients into CR/PR versus less than PR (46,67) or did not permit the association between MDR1/gp170 positivity versus negativity (73).

Table 6 shows that patients whose tumors expressed MDR1/gp170 were 3.21 times more likely to exhibit a worse than PR

than patients whose tumors did not express MDR1/gp170 ($P < .0001$). There was no evidence of significant heterogeneity among these studies ($P = .27$). Excluding the single study using treatment with non-MDR1/gp170-related drugs did not significantly change the association. Furthermore, the association between MDR1/gp170 expression and clinical response became stronger when the studies were restricted to those in which expression was measured after any treatment (RR = 4.19 [95% CI = 2.71-6.47]; $P < .0001$) or after treatment with MDR1/gp170-related drugs (RR = 3.87 [95% CI = 2.44-6.14]; $P < .0001$) (Table 6). This is consistent with the ability of MDR1/gp170-related drugs to induce MDR1/gp170 expression and the ability of this induced expression to confer cross-resistance among classical substrates for gp170 but not for other drugs (10,90-92). When we repeated the analyses by reclassifying as negative those tumors exhibiting weak positive expression (61,69,74), the results were unchanged.

An important clinical concern is whether the expression of MDR1/gp170 before initial chemotherapy will predict response. Only five studies (47,57,61,64,74), with a total of 115 patients, measured expression before treatment in patients evaluated for response to subsequent chemotherapy. When combined ($\chi^2_{\text{homog}} = 1.98$; $P = .74$), these studies indicate a suggestive, but not statistically significant, association between MDR1/gp170 expression prior to treatment and a worse than partial clinical response to cytotoxic regimens containing gp170 substrates (RR = 1.47 [95% CI = 0.94-2.29]; $\chi^2_{\text{homog}} = 2.91$; $P = .088$).

Association with recurrence and survival. Eight studies (61,63,69,71-75) associated MDR1/gp170 expression with either recurrence-free survival or overall survival. Unfortunately, conducting any formal statistical evaluation of the combined studies was not possible because the necessary data were included in only two studies that evaluated recurrence-free survival (63,69) and in three studies that evaluated overall survival

Table 5. Associations between MDR1/gp170 expression and breast cancer prognostic factors*

Prognostic factor	No. of studies (No. of patients)	Summary: RR (95% CI)	RR <i>P</i> value†	Test for homogeneity	
				χ^2 (df)	<i>P</i> †
Positive lymph nodes: yes versus no	9 (255)	1.08 (0.89-1.32)	.43	0.52 (8)	.99
Estrogen receptor status: negative versus positive	5 (426)	1.08 (0.84-1.39)	.57	4.56 (4)	.35
Tumor size: T3/T4 versus T1/T2	7 (184)	0.92 (0.75-1.13)	.44	1.63 (6)	.98
Histology: ductal versus lobular	5 (176)	0.96 (0.86-1.06)	.42	0.04 (4)	.99
Tumor grade: grade 3 versus grade <3	3 (58)	1.74 (0.65-4.64)	.27	1.56 (2)	.47

*RR = relative risk, i.e., the risk of adverse prognostic factor category among those patients with MDR1/gp170-positive versus MDR1/gp170-negative tumors; CI = confidence interval; df = degrees of freedom for the test of homogeneity. See (120,121) for staging and grading systems used for the tumors.

†Two-sided.

Table 6. Association between MDR1/gp170 expression and failure to respond (i.e., less than a clinical partial response) to cytotoxic drugs*

Type of treatment	No. of studies (No. of patients)	Summary: RR (95% CI)	RR <i>P</i> value†	Test for homogeneity	
				χ^2 (df)	<i>P</i> †
All cytotoxic agents, pretreatment or post-treatment MDR1/gp170 expression measurements	9 (260)	3.21 (2.28-4.51)	<.0001	10.0 (8)	.27
MDR1/gp170-related drugs, pretreatment or post-treatment MDR1/gp170 expression measurements	8 (215)	2.97 (2.08-4.25)	<.0001	9.18 (7)	.24
All cytotoxic agents, post-treatment MDR1/gp170 expression measurement	7 (193)	4.19 (2.71-6.47)	<.0001	7.64 (6)	.27
MDR1/gp170-related drugs, post-treatment MDR1/gp170 expression measurement	6 (135)	3.87 (2.44-6.14)	<.0001	7.41 (5)	.21

*RR = relative risk, i.e., the risk of less than a clinical partial response to chemotherapy among those patients with MDR1/gp170-positive versus MDR1/gp170-negative tumors; CI = confidence interval; df = degrees of freedom for the test of homogeneity.

†Two-sided.

(63,74,75). However, of the four studies that associated MDR1/gp170 expression with recurrence-free survival, two (61,69) found reduced survival associated with expression, whereas the other two (63,71) did not. Similarly, three studies (71,72,75) found that expression was associated with a significant reduction in overall survival, whereas three other studies (63,73,74) did not. Only two studies (71,72) used multivariable analysis to control for other major prognostic factors, although both studies observed significant survival decrements even in univariate analyses.

Association with in vitro doxorubicin resistance. Three studies (25,26,64), with a total of 93 patients, examined the association between MDR1/gp170 expression and resistance to doxorubicin using an in vitro clonogenic assay on cells from patients' tumors. Patients whose tumors expressed MDR1/gp170 were 2.5 times more likely to exhibit in vitro resistance to doxorubicin (RR = 2.50; 95% CI = 1.77-3.52) ($P < .0001$). There was no evidence of significant heterogeneity in this group of studies ($\chi^2_{\text{homog}} = 4.15$ [2 degrees of freedom]; $P = .14$). These studies indicate that some breast tumor cell subpopulations express sufficient levels of MDR1/gp170 to confer a functional level of in vitro drug resistance.

Discussion

The considerable variability across studies of MDR1/gp170 expression explains the controversy regarding the presence of this expression and its relevance in breast cancer. A cursory evaluation of this literature could lead to apparently equally justifiable support for quite divergent opinions. For example, it could be argued and supported by several citations either that MDR1/gp170 expression is rarely detected in breast cancer and has no clinical relevance or that expression is widely detected and may have considerable clinical relevance. With such variability across many independent studies, a careful review and meta-analysis can provide a critical and objective approach to the body of research.

Purpose and Interpretation of Meta-analyses

In its simplest form, meta-analysis is a systematic approach to combining, in a quantitative fashion, the results of related studies. The approach and its justification are conceptually similar to those used in either multicenter studies or studies with a large number of potentially relevant subgroups or strata. Combining the results of individual studies is performed in a manner similar

to that of combining the results across strata in a single large study. However, the validity of such data pooling and the generation of a summary statistic require that the studies be sufficiently alike in the association being measured. Thus, both the study population and methods should be sufficiently similar that it is reasonable to consider all the studies as if they had been generated as subgroups of a single large study. This is rarely the case, or there would be little controversy with regard to the overall inference from the studies and there would be little need to conduct a meta-analysis (33).

As occurred with the MDR1/gp170 analyses, groups of studies typically exhibit meaningful variability, and the search for the source of the variability is important. This search may also identify subgroups of studies that are sufficiently alike to make data pooling appropriate. For example, this meta-analysis showed that significant heterogeneity in the proportion of tumors expressing MDR1/gp170 could be attributed to differences in types of assays (immunohistochemistry based versus RNA hybridization based), complicating meaningful interpretation of a summary measure of this proportion.

Where subgroups of studies are chosen on a rational and predefined basis and the data from the integrated studies do not exhibit significant heterogeneity, meaningful associations among variables can be identified. For example, all nine of the studies that contain information on MDR1/gp170 expression and response to chemotherapy (47,57,61-64,68,69,73) can be validly combined and explored because there is no significant heterogeneity (Table 6; $\chi^2_{\text{homog}} = 10.0$; $P = .27$).

One concern in the conduct of any meta-analysis is publication bias, i.e., the tendency for studies reporting "negative" results to be underrepresented in the literature. Fig. 2 shows a "funnel plot," where the proportion of MDR1/gp170-positive tumors in each study is plotted against the sample size (as an indicator of standard error). In the absence of publication bias, such a plot should resemble a funnel lying on its side with the narrow end pointing to the right, with study results scattered around an expected "true" value and the degree of scatter decreasing with sample size. Publication bias would be suggested by a lack of small "negative" studies, i.e., studies with zero or low percentages of tumors expressing MDR1/gp170 (93). Fig. 2 shows no apparent bias against negative studies, since six studies (28-30,58,59,63) demonstrated expression in 10% or less of tumors, and four of these studies (29,30,58,59) had 20 patients or fewer.

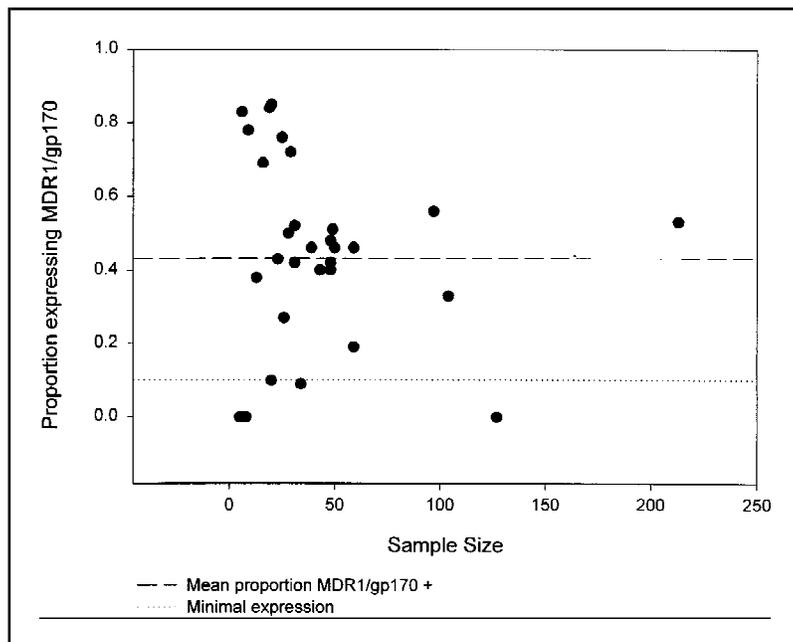


Fig. 2. Funnel plot evaluation of publication bias in studies of MDR1/gp170 expression and breast cancer. Data represent the proportion of MDR1/gp170-positive tumors (from untreated plus treated patients) in each study, plotted against the study sample size.

Frequent but Heterogeneous Expression of MDR1/gp170 in Breast Cancer

Northern and western blot hybridization techniques suffer from the possible loss of the signal by dilution from surrounding MDR1/gp170-negative tissues. This result may account for the generally lower estimates of positivity in the studies using these techniques when compared with immunohistochemistry. For many years, the single largest study in breast cancer provided the most compelling evidence for a lack of expression and, therefore, of any functional role of MDR1/gp170 in breast cancer (28). Merkel et al. (28) acknowledge that their analysis, which utilized northern, Southern, and western blotting analyses of homogenates of breast tumor tissues, may be too insensitive to adequately quantitate MDR1/gp170 expression. This may be the most likely explanation for the lack of detectable MDR1/gp170 expression in breast tumors in their study, since expression has been readily detected in more recent studies of comparable size (65,71,73).

As the results of our analysis indicate, the increase over time in the use of more sensitive immunohistochemistry-based assays has been accompanied by an increase in the reported prevalence of MDR1/gp170 expression. An inability to detect gene expression by use of earlier RNA hybridization or immunoblot assay techniques is not surprising, given the generally heterogeneous and low levels of expression now apparent in many breast tumors (62,66,73). Immunoblotting has recently been recommended as one means to detect MDR1/gp170 expression in tumors (94). However, it is apparent from our study that this approach is inappropriate for analysis of the relatively low levels of MDR1/gp170 expression in breast tumors.

While one source of heterogeneity arises from the use of different assays for MDR1/gp170 expression, significant heterogeneity remains when studies using similar assay techniques are combined. This applies to both RNA hybridization and immu-

nohistochemical analyses. There are clearly many other sources of heterogeneity (e.g., differences in patient populations resulting from patient selection criteria; whether there has been prior treatment, the nature of the treatment, and whether it includes gp170 substrates; the timing of sampling relative to diagnosis and treatment; the use of primary versus metastatic tumor tissue; and sampling artifacts reflecting heterogeneous expression within the tissue). Additional sources of heterogeneity specific to different types of assays include tissue handling and preparation, the choice of antibody(ies) or probes, general laboratory procedures for the particular assay, and the choice of an immunodetection method. Differences in the criteria for scoring tumors as positive or negative also contribute significantly to heterogeneity. Several workshops and individual laboratories (94-98) have described their protocols for optimal detection of MDR1/gp170. With such diversity of opinion and laboratory practice, obtaining an internationally recognized standardized approach may be difficult in the short term.

The significant heterogeneity across studies prevents the generation of a statistically valid summary estimate to describe the overall prevalence of MDR1/gp170 expression in breast cancer. However, the weighted average over all 31 studies provides a very general approximation and implies that approximately 40% of all breast tumors express detectable levels of MDR1/gp170. This may be an underestimate, since it combines the typically lower positivity rates found in the less sensitive RNA hybridization studies. While less than the frequency of positivity for estrogen receptor expression, which is almost 70% in all breast tumors when assayed by immunohistochemistry (99-101), the frequency of gp170 positivity exceeds that of other prognostic indicators. For example, overexpression of erbB2 when detected by immunohistochemistry approaches 20% in all breast cancers and 25% in invasive ductal cell carcinomas (102). This overexpression also may be associated with reduced sensitivity to some chemotherapeutic regimens (103). p53 mutations are considered the most frequent genetic changes in breast cancer; they are found in approximately 15%-50% of breast cancers (104,105), a frequency that compares with our estimate of 40% MDR1/gp170 positivity. With such a high relative frequency for detection of MDR1/gp170, a definitive assessment of its functional role in breast cancer is an important goal.

MDR1/gp170 Expression in Untreated and Treated Breast Cancers

Many previously untreated breast cancers, 27% in the RNA hybridization studies and 46% in the immunohistochemistry studies, express MDR1/gp170. These observations strongly suggest that many breast tumors already acquire MDR1/gp170 expression before clinical detection. Among previously untreated breast cancer patients, 20%-55% obtain a worse than PR when they are treated with cytotoxic chemotherapy. Furthermore, among those who respond to first-line chemotherapy, the median duration of response is relatively short (only 5-13 months) (2).

These observations are consistent with the likely acquired expression of low levels of MDR1/gp170 and/or other multiple drug resistance mechanisms early in the biology of breast cancer progression. This acquisition may not require the selective pressure imposed by chemotherapy. It also is not clear whether the expression detected after chemotherapy represents either an induced expression or a selection that increases the proportion of MDR1/gp170-expressing tumor subpopulations.

A few studies (58,66,106) have looked for MDR1/gp170 expression in the normal breast. The results of these studies suggest that expression is low, absent, or predominantly stromal. Pavelic et al. (106), using four independent antibodies, detected MDR1/gp170 expression in normal ductal epithelia. Greater than 80% of normal breast ductal epithelium stained positively with the MRK-16 anti-gp170 antibody, and staining was confined to the luminal surface (79). These observations suggest that some MDR1/gp170-positive breast tumors may arise in those previously untreated patients with tumors of intraductal origin.

Selection against MDR1/gp170-related drugs in vitro frequently induces MDR1/gp170 expression (10). Furthermore, the meta-analysis results demonstrate that MDR1/gp170 expression is twice as likely to be detected in tumors from treated versus untreated patients. This increased incidence of detectable MDR1/gp170 expression may be a direct consequence of the cytotoxic drug therapy.

Association of MDR1/gp170 Expression With a Worse Than PR

Because of its ability to function as a drug efflux pump, gp170 confers multidrug resistance in vitro. Thus, it would be predicted that the detection of its expression in breast cancer would be associated with clinical resistance. In our analysis, patients with detectable levels of MDR1/gp170 were three times more likely to exhibit a less than partial clinical response to cytotoxic chemotherapy. Since the incidence of MDR1/gp170 expression increases with treatment, and the response rate to second-line chemotherapy is worse than that to first-line treatment, we would expect MDR1/gp170 expression measured after treatment to show a stronger association with a poor clinical response. The post-treatment analysis for all drugs indicated that MDR1/gp170 expression in treated tumors was associated with a fourfold increase in risk for a patient having a worse than PR. These data infer that the expression of MDR1/gp170 may already have been present in the tumors prior to and/or during chemotherapy. Furthermore, they demonstrate a strong association between detectable MDR1/gp170 expression and a poor clinical response to cytotoxic chemotherapy.

There were only limited data concerning the predictive utility of MDR1/gp170 expression measured before treatment. However, in the five evaluated studies (47,57,61,64,74), there is a marginally significant 50% increase in the probability of an associated worse than partial clinical response. While this finding suggests that de novo gp170 expression also may play a role in drug resistance, it is inconclusive. The relatively small number of patients and the differences in immunohistochemistry-based assay techniques across studies may contribute to the inability of these studies to demonstrate clearly, either way, the role of gp170 expression in previously untreated breast cancer

patients. Additional prospective, randomized, clinical trials are clearly needed to resolve this issue.

Expression of MDR1/gp170 and Overall Survival

The data relating MDR1/gp170 expression to recurrence-free and overall survival could not be analyzed because the required data were not available in most published reports. The individual studies were essentially equally grouped in favor of and against an association between expression and survival. This is not entirely unexpected, since response to therapy generally is not associated with survival (107,108). Until sufficient studies are completed and the data are reported in a way that can be readily accessed, any association of MDR1/gp170 expression with survival, whether positive or negative, will remain unknown.

Expression of MDR1/gp170 and Other Prognostic Attributes

Expression of MDR1/gp170, rather than being associated with treatment, may merely reflect a more aggressive phenotype (89). Individual studies have found an association between the detectable expression of MDR1/gp170 in breast tumors and a poor prognosis (61), poor survival (61,69), lymph node metastasis (109), and low progesterone receptor expression (54). Among all the studies that have examined associations with other prognostic attributes, however, there is little evidence to support the contention that MDR1/gp170 expression is merely a surrogate for other indicators of an aggressive phenotype. We found no association of MDR1/gp170 with either positive lymph nodes, tumor size, grade, histology, or estrogen receptor expression. However, relatively few studies examined such associations. The most commonly evaluated markers, lymph node status and tumor size, were examined in nine studies (54,57,60,61,63,69,70,74,75) and seven studies (54,60,61,63,70,74,75), respectively.

Establishing the Functional Relevance of MDR1/gp170 Expression—Considerations and Future Directions

The meta-analysis data clearly indicate that MDR1/gp170 is expressed in a significant proportion of breast tumors. A direct examination of the clinical relevance of MDR1/gp170 expression is clearly the next most important step. The precise delineation of this role is likely to be difficult and complex. Clinical studies have almost exclusively assessed MDR1/gp170 expression either before or after therapy, but not during treatment. In experimental models, MDR1/gp170 expression is frequently induced by exposure to a substrate (110), and expression becomes constitutive only after repeated or prolonged in vitro exposure. Thus, in some tumors, expression may be detectable only during either therapy or the later cycles of therapy. The level of expression before or after treatment may underestimate the level induced during treatment and may produce a false-negative impression with regard to the role of MDR1/gp170.

Studies that have begun to address gp170 function in patients have used response to a combination chemotherapy regimen as their end point. This may be a suboptimal approach. Determining the individual contribution of each drug to the response in a specific tumor is currently impossible. For example, when a regimen is used in which each drug is effective as a single agent,

e.g., CAF, what proportion of the response, or lack thereof, will be due to the gp170 substrate? Furthermore, doxorubicin is the major or sole gp170 substrate in chemotherapy regimens used in most studies. However, doxorubicin is subject to several drug resistance mechanisms unrelated to gp170, including an altered expression of manganese superoxide dismutase (7) and increased activities of glutathione transferase and topoisomerase II (111,112). Thus, when using doxorubicin as the gp170 substrate, lack of a clinical response can be attributed only cautiously to gp170. This is also a concern for the present meta-analysis and suggests that patients previously treated with a non-MDR1/gp170-related regimen should be excluded from future studies, since this treatment may induce cross-resistance mechanisms to some MDR1/gp170-related drugs.

Suggestions for Design of Future Clinical Trials

We wish to raise several issues for consideration, since these issues are likely to have contributed to the significant heterogeneity apparent in previously published studies. We hope that these suggestions will provide some degree of consistency within, if not among, future studies. Several groups (94,95) have recently attempted to derive specific guidelines for the assessment of MDR1/gp170 positivity. Our analysis of the sources of heterogeneity among published studies supports some suggestions raised in these reports and raises the following additional issues for consideration:

1) In general, well-designed prospective studies with adequate statistical power are preferable to retrospective studies. Thus, MDR1/gp170 expression should be measured before the commencement of cytotoxic chemotherapy. If a neoadjuvant design is adopted, investigators should consider the feasibility of also measuring MDR1/gp170 expression in biopsy specimens obtained during chemotherapy. Any tissue removed, or accessible either after treatment or upon relapse, also should be examined for MDR1/gp170 expression.

2) Where possible, the study population should comprise patients not previously treated with any systemic therapies. These patients may have fewer other endogenous cross-resistance mechanisms present, e.g., altered topoisomerase or glutathione transferase expression, or at least express other mechanisms at relatively low levels.

3) Measuring gp170 expression may be preferable to measuring MDR1 expression, since immunohistochemistry is more sensitive and can discriminate between stromal and tumor cell expression.

4) Where immunohistochemistry is adopted, there should be a clear definition of positivity and negativity of expression compared with well-defined positive and negative controls. Since some anti-gp170 antibodies cross-react with epitopes on proteins other than gp170 (58,84,87), more than one antibody with nonoverlapping cross-reactivities should be considered. Samples should be screened and scored in a blinded fashion by more than one pathologist using criteria established before the analysis. Experimental criteria discussed in detail by others (94-98) also should be considered.

5) When the data are presented, the methods of accruing patients (samples), characteristics of the study population, and the treatments (exposures) should be described in detail. Data should be presented so that MDR1/gp170 positivity can be de-

scribed among the following patient subgroups: those whose tumors are assayed before or after treatment, those whose tumors are treated with MDR1/gp170-related or non-MDR1/gp170-related substrates, and those whose tumors are primary or metastatic.

MDR1/gp170 expression might be associated with the induction of other drug resistance mechanisms. It is possible that exposure to cytotoxic drugs induces a cellular stress response that co-induces several stress and detoxification mechanisms. This response could include increased expression of the heat shock proteins (113), glutathione transferases (5), and superoxide dismutases (7). The activity of some DNA repair processes, including the activity of the topoisomerases, also may be altered (8). Since each subpopulation of cells within a tumor may express a different pattern of these various resistance mechanisms, future studies should determine whether MDR1/gp170 expression either is a general marker for drug resistance or arises independently of other resistance mechanisms. A recent study (114) suggests that combining assessments of MDR1/gp170 with those of MRP provides a more accurate predictor of clinical drug resistance in patients with acute myeloid leukemia.

Studies with agents that can reverse gp170 function provide another approach to determining the functional relevance of expression. However, this approach is likely to be complicated by several additional factors. While there is no clear consensus on how best to detect MDR1/gp170, several studies with reversing agents (115-118) have been performed on breast cancer patient populations in whom the MDR1/gp170 status is either unknown or not reported. Antibodies and complementary DNA probes have been available for several years, and such study designs are discouraged.

One "inpatient" study design has been suggested that might alleviate some of these concerns and is worthy of consideration (119). Previously untreated breast cancer patients with comparable disease status could receive a single MDR1/gp170-related substrate regimen until relapse. These patients are then screened for MDR1/gp170 expression in accessible lesions, and patients with MDR1/gp170-positive tumors are assigned to subsequent groups where the same drug is administered with the reversing agent. The pharmacokinetics of the cytotoxic agent would be established in each patient, both when the drug is administered alone and subsequently when the drug is given with the reversing agent. This will enable each patient essentially to act as his or her own control and reduce the effects of interpatient variation (119). To control for unrelated effects on clinical response and pharmacokinetics, selecting patients with broadly comparable performance status may be necessary. Patients should be evaluated for MDR1/gp170 expression before any systemic therapy is initiated.

Implications of This Analysis for Future Translational Research Studies

The studies reviewed in this analysis varied substantially with respect to the level of information presented in the published reports. Because of the nature of translational research, it is vital that researchers provide the information necessary to readily understand the clinical implications of a particular study. Results should be presented in a way that allows differences in the critical subgroups to be discerned, particularly when the activity of the molecule is thought to vary with clinical attributes, e.g.,

expression levels in patients before and after treatment. A clear description of the patient population and method of accrual also is important. Tumor samples assayed in a particular study will rarely come from randomized clinical trials, so it is important to consider the selection factors, implicit or explicit, that influence the study sample's composition. Tumor specimens accrued to tumor banks or to individual studies generally represent a number of clinical and institutional factors, including tumor size, referral patterns, tissue-handling procedures, procurement policies, consent procedures, other protocols competing for tissue, and recruitment into clinical trials. All these factors can influence the biologic characteristics of patients or specimens comprising a particular study.

Conclusions

The data from our meta-analysis indicate that many breast tumors express detectable levels of MDR1/gp170. There is considerable heterogeneity across studies of MDR1/gp170 expression, resulting partly from the different techniques and end points utilized. The incidence of expression is higher in patients who have received cytotoxic chemotherapy and in those who either will have or have had a worse than partial clinical response to chemotherapy. While the functional relevance of this expression remains to be established, these data are strongly supportive of a likely role for MDR1/gp170 in conferring clinical resistance to gp170 substrates in a significant proportion of breast tumors. We found no evidence to support the assumption that MDR1/gp170 expression has no role in breast cancer.

While the precise role of MDR1/gp170 in breast cancer remains to be established definitively, it seems likely that, in tumors where expression is detectable, this expression contributes to the multidrug-resistant phenotype. However, it seems equally likely that multidrug resistance in breast cancer is a multifactorial phenomenon and may include MRP and the altered expression of superoxide dismutases, glutathione transferases, heat shock proteins, and other resistance genes. The precise contribution of each potential multidrug resistance mechanism is unclear, and it is likely that more than one mechanism can operate within the same tumor cell subpopulation and/or within different subpopulations of the same tumor. If correct, then the establishment of a role for any of these other resistance mechanisms will become as complex and controversial as is the study of MDR1/gp170.

The potential complexity that applies to the study of drug resistance in breast cancer likely also applies to other solid tumors. Clearly, the design of future clinical trials to establish the functional relevance of drug resistance mechanisms will require careful and detailed consideration of the patient population, the drugs used to induce response, and the potential contribution of other drug resistance mechanisms.

One purpose of meta-analyses is to identify potential directions for future studies and, if possible, to provide suggestions for incorporation into improved study designs. In this respect, the issues we have raised regarding study design are provided in the hope that they will both raise awareness and generate some discussion of the complexities and difficulties associated with establishing the role of MDR1/gp170 and other drug resistance mechanisms.

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