Review

Gene therapy for carcinoma of the breast
Genetic ablation strategies

David T Curiel

Gene Therapy Clinic, The University of Alabama at Birmingham, Birmingham, Alabama, USA

Received: 11 November 1999
Accepted: 11 November 1999
Published: 17 December 1999

Breast Cancer Res 2000, **2**:45–49

Abstract

The gene therapy strategy of mutation compensation is designed to rectify the molecular lesions that are etiologic for neoplastic transformation. For dominant oncogenes, such approaches involve the functional knockout of the dysregulated cellular control pathways provoked by the overexpressed oncoprotein. On this basis, molecular interventions may be targeted to the transcriptional level of expression, via antisense or ribozymes, or post-transcriptionally, via intracellular single chain antibodies (intrabodies). For carcinoma of the breast, these approaches have been applied in the context of the disease linked oncogenes *erb*B-2 and cyclin D_1, as well as the estrogen receptor. Neoplastic revision accomplished in modal systems has rationalized human trials on this basis.

Keywords: antisense, dominant-negative, gene therapy, oncogene, ribozyme

Introduction

The gene therapy approach of mutation compensation involves correction of the genetic lesions that are etiologic for neoplastic transformation [1•,2–4]. For the dominant oncogene type of genetic lesion, such genetic correction approaches are designed to functionally ablate dysregulated patterns of gene expression. In considering this type of gene therapy approach for breast cancer, knowledge of the genetic mechanisms responsible for initiation and progression of malignancy is of relevance. In this regard, breast cancer, like other types of human cancer, develops via a succession of genetic alterations [5–7]. Inherited or somatic genetic changes that occur in oncogenes, tumor suppressor genes, the DNA repair machinery, and cell cycle checkpoints lead to low-risk or high-risk forms of *in situ* carcinoma, and subsequently to invasion and metastasis. For colorectal carcinoma, such changes have been well enumerated by Vogelstein and coworkers [8,9].

A similar analysis of these genetic changes in breast cancer represents a daunting task, because of the greater genetic heterogeneity that characterizes carcinoma of the breast and because few of the relevant genes appear to cause familial syndromes. In this regard, potentially, functional analysis of gene products that are known to be involved in breast cancer (eg p53, Rb, p16, BRCA-1, BRCA-2, ATM), combined with further characterization of other loci implicated by allelic loss or gene amplification, will lead to the identification of pathways that are important to the pathogenesis of many or all breast cancers. Such an understanding of the molecular pathogenesis of breast cancer will allow rational application of novel gene therapeutic and pharmacologic strategies for adjuvant therapy and the early treatment of recurrence.

On this basis, it may be understood that genetic ablation gene therapy strategies may be most successful when such strategies are designed to correct one, or more, of the spe-

EGFR = epidermal growth factor receptor; IGF = insulin-like growth factor; sFv = single-chain variable fragment antibody.

cific genetic changes known *a priori* to be present in tumor cells. Many of these changes, including gene amplification, allelic loss, *p53* mutation, and increased expression of certain oncogenes, are found in preinvasive lesions such as ductal carcinoma *in situ* of the breast [10–15]. Early correction of these defects could prevent subsequent progression of invasion and metastasis. Indeed, such gene therapy approaches for precancer have been attempted in the context of carcinoma of the lung, and are clearly relevant in the context of carcinoma of the breast also [16]. In addition, targeting of the same alterations in more advanced tumors could prove effective in supplementing current therapies: surgery, radiotherapy, and chemotherapy.

Genetic abnormalities in breast cancer

Two major forms of genetic alteration in breast cancer are loss of specific chromosome arms and gene amplification. Loss of heterozygosity analysis of polymorphic DNA markers have implicated chromosomes and subregions of chromosome arms that probably harbor tumor suppressor genes [7]. Nonetheless, in only a few cases have specific genes relevant to allelic loss been identified. Karyotype analysis and chromosome *in situ* hybridization approaches, such as comparative genomic hybridization or fluorescent *in situ* hybridization, point to amplified chromosomal loci likely to harbor oncogenes, and facilitate loss of heterozygosity studies by identifying regions of the genome that are under-represented in tumors [6,17–21]. These studies have shown that breast cancers are unusual among human tumors because of their great degree of genetic heterogeneity, suggesting that breast cancer in reality results from multiple genetic changes. Although characterization of the many unidentified genes that are relevant to allelic loss and gene amplification will undoubtedly suggest additional gene therapy strategies for breast cancer, current knowledge of a few such targets already offers the possibility of effective intervention.

The genetic heterozygosity of breast cancer may thus predicate gene therapy approaches that are targeted to multiple dysregulated alleles. In this regard, the genetic heterogeneity of breast cancer is reflected in the various oncogenes previously implicated. Genetic alteration involving known oncogenes is restricted to six loci that undergo gene amplification. No known genes, including the *ras* family members, have been shown to undergo base mutation or translocation in primary human breast cancer. Gene amplification occurs at the following specific loci at the approximate frequencies indicated: *erb*B-2 (chromosome 17q12, 20% of tumors), c-*myc* (8p24, 20%), *PRAD1/CYD1* (11q13, 15%), the fibroblast growth factor receptors (8p12, 10–15%), *BEK* (10q26, 10–15%), and the insulin-like growth factor receptor (IGF) (15q24-25, 2%). It also involves unidentified genes at chromosomes 13q31, 17q22-24, and 20q12-13.2 [19,20]. In addition to these, other potential oncogenes that are expressed in the absence of genetic alteration include H-*ras*, *erb*B-1/epidermal growth factor receptor (EGFR), *erb*B-3, and others. Thus, a variety of candidate oncogenes have been identified that might be approached via genetic ablation strategies.

With the exceptions of c-*myc* and *PRAD1/CYD1* (encoding the kinase-associated cyclin D_1), gene amplification in breast cancer commonly involves one of several growth factor receptors, as noted above. Although the signal transduction mechanisms of these diverse molecules are currently under study, it is likely that common elements of the signaling machinery are involved. For example, signaling by $p185^{c\text{-}ErbB\text{-}2/1\,(ErbB\text{-}2)}$ utilizes downstream elements such as phospholipase C-γ, phosphatidylinositol 3-kinase, guanosine triphosphatase-activating protein, and the adapter protein SHC [22]. Gene therapeutic modulation of the basal signal transduction apparatus could therefore prove effective in a majority of breast cancer cases. Thus, despite the molecular heterogeneity, common points of dysregulation can provide a limited set of rational targets.

Genetic ablation strategies for breast cancer

In this regard, the dysregulation of oncogenes by mutation, gene amplification, gene rearrangement, or overexpression contributes to oncogenesis by removing controls on normal cell cycle regulation. One approach to gene therapy for breast cancer is to 'knockout' dominant oncogenes and thereby reduce the growth or invasive potential of the tumor. Inhibition or ablation of oncogenic function can occur at three levels. First, the translation of the oncogene can be targeted. This strategy involves the use of antisense molecules to sequester and/or functionally ablate oncogene messenger RNA. Second, the function of the gene product can be targeted. This approach uses polypeptides containing dominant interfering mutations ('dominant-negative') to downregulate signal transduction in tumor cells. Third, the nascent oncogenic protein can be prevented from reaching its proper intracellular location. This approach uses intracellular antibodies ('intrabodies') to pre-empt the cellular localization machinery and sequester growth factor receptors inside the cell.

Antisense molecules

With regard to antisense inhibition of oncogene function, early studies demonstrated inhibition of lymphoma growth by administration of naked antisense DNA to c-*myc* [23]. Somewhat more recently, a *myc* antisense phosphorothioate oligonucleotide that has enhanced stability has been shown to produce cytostatic effects in estrogen-dependent and estrogen-independent breast cancer cell lines [24]. As an alternative approach to delivering antisense molecules, inhibition of K-*ras* expression and its normally potent tumorigenicity was achieved in nonsmall cell lung cancer cells *in vitro* through the use of a mammalian plasmid vector that produces antisense RNA oligo-

nucleotides [25]. The same investigators showed a dramatic reduction in tumor growth in nude mice [26]. Additional genes relevant to breast cancer have been successfully targeted by antisense oligonucleotides. Antisense molecules to cyclin Di inhibited growth and reversed the transformed phenotype in esophageal cancer cells [27], antisense RNA to type 1 IGF receptor suppressed rat prostate tumor growth and invasion [28], and transforming growth factor-α antisense messenger RNA inhibited estrogen-induced proliferation in estrogen responsive breast cancer cells [29]. In addition, antisense approaches have been developed targeting the ErbB-2 oncoprotein. In those studies, antisense oligonucleotides delivered *in vitro* to breast cancer cell lines could accomplish downregulation of ErbB-2 with modest inhibition of cellular proliferation [30]. In addition, the adenoviral protein E1A has also been shown to allow selective ErbB-2 downmodulation with reversion of the transformed phenotype [31–33].

In the context of clinical trials, a messenger RNA antisense approach for breast cancer is being entered into human trials in a protocol by Arteaga and Holt [34•], which uses disabled mouse mammary tumor virus to drive the tissue-specific expression of antisense oligonucleotides to c-*fos* and c-*myc* in late-stage breast cancer patients with lung, meningeal or peritoneal metastases. These investigators have shown that delivery of this vector encoding antisense to c-*fos* results in inhibition of breast tumor growth and increased survival in a mouse model. Similarly, the E1A approach has been translated into the clinical context [35].

The use of antisense oligonucleotides must overcome several obstacles in order to be clinically useful. These include attaining stable intracellular levels by frequent administration or by constant production internally, and the need to inhibit oncogenes that are amplified or expressed at high levels. As for other therapeutic approaches, the molecule must be delivered to nearly every tumor cell in order to be effective in inhibiting growth or invasive potential. Thus, the enthusiasm for antisense approaches to human gene therapy trials has been limited.

Dominant negative mutations

A second approach to ablation of oncogene function is expression of dominant interfering or 'dominant negative' mutant proteins. Especially attractive targets for such an approach are the receptor tyrosine kinases (eg ErbB-1/EGFR, ErbB-2, and ErbB-3), which appear to be amplified and/or overexpressed in breast cancer. Two different mechanisms have proved effective in blocking receptor function. One approach disrupts dimerization, which is required for intracellular signaling. This was accomplished by transfection of cytoplasmic domain mutants of EGFR into cells expressing wild-type receptor. The resulting heterodimers failed to show high affinity EGF binding, receptor endocytosis, or biological signaling [36]. Alternatively, growth factor binding to the receptor can be prevented by expression of a mutant growth factor, or by sequestering the growth factor extracellularly. NIH3T3 cells that produced a mutant platelet-derived growth factor molecule showed a reverted phenotype with a reduced growth rate in culture, and reduced invasive potential [37]. Extracellular sequestering was demonstrated for IGF-1. Cells were transfected with IGF binding protein-3, which bound IGF-1 in the extracellular space. This inhibited growth of Balb/c cells even in the presence of high concentrations of insulin and IGF-1 [38]. In addition, this type of approach has been applied in the context of the estrogen receptor, whereby neoplastic reversion has been achieved [39].

Intracellular single-chain antibodies

In addition to antisense strategies and dominant-negative mutations, another strategy aims at disrupting normal subcellular localization of growth factor receptors. We have recently developed an approach that prevents growth factor receptors from reaching the cell surface. By transfecting human ovarian cancer cells with a gene encoding an anti-ErbB-2 single-chain variable fragment antibody (sFv), we were able to demonstrate downregulation of cell surface ErbB-2 and a corresponding specific growth inhibition of cells overexpressing the receptor [40,41••]. This sFv-mediated oncogene downmodulation triggered apoptotic cell death in cells that overexpress the receptor [42]. Furthermore, it could be shown that breast cancer cells that overexpressed ErbB-2 were also eradicated in this manner [43]. Interestingly, the level of ErbB-2 that characterized breast cancer tumor targets was predictive of their response to this genetic intervention. The ability to accomplish selective abrogation of oncogenes by the use of intracellular sFvs opens a wide variety of possibilities in breast cancer investigation and therapy. These results were corroborated by Hynes and coworkers, who demonstrated ErbB-2 downregulation with impairment of receptor activation in breast cancer cells [44] and reversion of the transformed phenotype in ErbB-2 overexpressing cells [45].

Strategies for gene delivery

As for all mutation compensation approaches, the delivery context is an important predicate of the viability of the genetic intervention. In this regard, contexts whereby target cells can be manipulated *ex vivo* may allow the achievement of a level of specificity and efficiency of gene delivery that is commensurate with a meaningful therapeutic outcome. Thus, in the context of carcinoma of the breast it may be rational to employ such an *ex vivo* approach to achieve genetic purging of bone marrow, as has been attempted in a variety of neoplastic contexts [46,47].

Alternatively, locoregional and disseminated disease require direct *in vivo* delivery of the knockout gene. This

delivery context imposes a greater stringency, and current generation vectors are limited in their ability to achieve this goal [48,49•,50,51]. In this regard, such *in vivo* gene delivery approaches have been limited to disease contexts whereby tumor is sequestered in a compartment context. On this basis, the relatively limited presentation of breast cancer in this manner, combined with vector limitations in this regard, have restricted direct clinical application of genetic ablation strategies for carcinoma of the breast. This recognition has led to the exploration of vector approaches that possess the capacities for efficient, and cell-specific gene delivery *in vivo*. Such systems, based on both viral and nonviral schemes, have been attempted [52–54]. Thus, despite the fact that genetic ablation strategies offer the possibility of a tumor cell-specific effect, this utility can not be realized until vector approaches can achieve a requisite level of efficiency in the stringent *in vivo* gene delivery context.

Acknowledgement

We wish to thank Patty Parker for her administrative assistance.

References

Articles of particular interest have been highlighted as:
- • of special interest
- •• of outstanding interest

1. Grushcow J, Curiel DT: **Gene therapy for carcinoma of the breast.** • *Cancer Gene Ther* 1995, **4**:195–202.
An excellent subject review is presented.

2. Curiel DT: **Gene therapy approaches for treatment of cancer.** In: *Cecil Textbook of Medicine, 20th ed.* Edited by Claude Bennett J, Plumb F. WB Saunders Company, 1996:98–117.

3. Rosenfeld ME, Curiel DT: **Gene therapy strategies for novel cancer therapeutics.** *Curr Opin Oncol* 1996, **8**:72–77.

4. Bilbao G, Curiel DT: **Gene therapy for cancer therapeutics.** *Oncol Endocr Metab* 1997, **3**:1267–1284.

5. Mars WM, Saunders GF: **Chromosomal abnormalities in human breast cancer.** *Cancer Metast Rev* 1990, **9**:35–43.

6. Cox LA, Chen G, Lee EY: **Tumor suppressor genes and their roles in breast cancer.** *Breast Cancer Res Treat* 1994, **32**:19–38.

7. Devilee P, Cornelisse CJ: **Somatic genetic changes in human breast cancer.** *Biochem Biophys Acta* 1994, **1198**:113–130.

8. Parsons R, Li GM, Longley MJ, *et al*: **Hypermutability and mismatch repair deficiency in RER+ tumor cells.** *Cell* 1993, **75**:1227–1236.

9. Cho KR, Vogelstein B: **Suppressor gene alterations in the colorectal adenomia-carcinoma sequence.** *J Cell Biochem* 1992, **16G** (suppl):137–141.

10. Liu E, Thor A, He M, *et al*: **The HER2 (c-erbB-2) oncogene is frequently amplified in *in situ* carcinoma.** *Oncogene* 1992, **7**:1027–1032.

11. Elledge RM, Allred DC: **The p53 tumor suppressor gene in breast cancer.** *Breast Cancer Res Treat* 1994, **32**:39–47.

12. Zhuang ZP, Merino MJ, Chuaqui R, Liotta LA, Emmertbuck MR: **Identical allelic loss on chromosome 11q13 in microdissected *in situ* and invasive human breast cancer.** *Cancer Res* 1995, **55**:467–471.

13. Radford DM, Fair KL, Phillips NJ, *et al*: **Allelotyping of ductal carcinoma *in situ* of the breast: deletion of loci on 8p, 13q, 16q, 17p, 17q.** *Cancer Res* 1995, **55**:3399–3405.

14. Munn KE, Walker EA, Varley JM: **Frequent alterations of chromosome 1 in ductal carcinoma *in situ* of the breast.** *Oncogene* 1995, **10**:1653–1657.

15. Weinstat-Saslow D, Merino MJ, Manrow RE, *et al*: **Overexpression of cyclin D mRNA distinguishes invasive and in situ breast carcinomas from non-malignant lesions.** *Nature Med* 1995, **1**:1257–1260.

16. Zou Y, Zong G, Ling YH, *et al*: **Effective treatment of early endobronchial cancer with regional administration of liposome-p53 complexes.** *J Natl Cancer Inst* 1998, **90**:1130–1137.

17. Trent J, Yang J-M, Emerson J, *et al:* **Clonal chromosome abnormalities in human breast carcinomas. Thirty four cases with metastatic disease.** *Genes Chrom Cancer* 1993, **7**:194–203.

18. Thompson F, Emerson J, Dalton W, *et al*: **Clonal chromosome abnormalities in human breast carcinomas. Twenty eight cases with primary disease.** *Genes Chrom Cancer* 1993, **7**:185–193.

19. Kallioniemi A, Kallioniemi OP, Piper J, *et al*: **Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization.** *Proc Natl Acad Sci USA* 1994, **91**:2156–2160.

20. Guan X-Y, Meltzer PS, Dalton WS, Trent JM: **Identification of cryptic sites of DNA sequence amplification in human breast cancer by chromosome microdissection.** *Nature Genet* 1994, **8**:155–161.

21. Pandis N, Jin Y, Gorunova L, *et al*: **Chromosome analysis of 97 primary breast carcinomas: identification of eight karyotypic subgroups.** *Gene Chrom Cancer* 1995, **12**:173–185.

22. Dougall WC, Qian X, Peterson NC, *et al*: **The neu-oncogene: signal transduction pathways, transformation mechanisms and evolving therapies.** *Oncogene* 1994, **9**:2109–2123.

23. McManaway ME, Neckers LM, Loke SL, *et al*: **Tumor-specific inhibition of lymphoma growth by an antisense oligonucleotide.** *Lancet* 1990, **335**:808–811.

24. Watson PH, Pon RT, Shiu RP: **Inhibition of c-*myc* expression by phosphorothioate antisense oligonucleotide identifies a critical role for c-*myc* in the growth of human breast cancer.** *Cancer Res* 1991, **51**:3996–4000.

25. Mukhopadhyay T, Tainsky M, Cavender AC, Roth JA: **Specific inhibition of K-ras expression and tumorigenicity of lung cancer cells by antisense RNA.** *Cancer Res* 1991, **51**:1744–1748.

26. Georges RN, Mukhopadhyay T, Zhang Y, Yen N, Roth JA: **Prevention of orthotopic human lung cancer growth by intratracheal instillation of a retroviral antisense K-ras construct.** *Cancer Res* 1993, **53**:1743–1746.

27. Zhou P, Jaing W, Zhang Y, *et al:* **Antisense to cyclin D_1 inhibits growth and reverses the transformed phenotype of human esophageal cancer cells.** *Oncogene* 1995, **11**:571–580.

28. Burfeind P, Chernicky CL, Rininsland F, Ilan J, Ilan J: **Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells *in vivo*.** *Proc Natl Acad Sci USA* 1996, **93**:7263–7268.

29. Kenney NJ, Saeki T, Gottardis M, *et al:* **Expression of transforming growth factor α antisense mRNA inhibits the estrogen-induced production of TGFα and estrogen-induced proliferation of estrogen-responsive human breast cancer cells.** *J Cell Physiol* 1993, **156**:497–514.

30. Bertram J, Killian M, Brysch W, Schlingensiepen K-H, Kneba M: **Reduction of erbB-2 gene product in mammary carcinoma cell lines by erbB-2 mRNA-specific and tyrosine kinase consensus phosphorothioate antisense oligonucleotides.** *Biochem Biophys Res Commun* 1994, **200**:661–667.

31. Yan DH, Chang LS, Hung MC: **Repressed expression of the HER-2/c-ErbB-2 proto-oncogene by the adenovirus E1A gene products.** *Oncogene* 1991, **6**:343–345.

32. Yu D, Hamada J, Zhang H, Nicolson GL, Hung MC: **Mechanisms of c-erbB-2/*neu* oncogene-induced metastasis and repression of metastatic properties by adenovirus 5 E1A gene products.** *Oncogene* 1992, **7**:2263–2270.

33. Yu D, Wolf JK, Scanlon M, Price JE, Hung MC: **Enhanced c-erbB-2/*neu* expression in human ovarian cancer cells correlates with more severe malignancy that can be suppressed by E1A.** *Cancer Res* 1993, **53**:891–898.

34. • Arteaga CL, Holt J: **Tissue-targeted antisense c-fos retroviral vector inhibits established breast cancer xenografts in nude mice.** *Cancer Res* 1996, **56**:1098–1103.
An interesting treatment study is presented.

35. Hortobagyi GN, Carey MM, Hung M-C, Berestein-Lopez G: **A phase I multicenter study of E1A gene therapy for patients with metastatic breast cancer and epithelial ovarian cancer that overexpresses HER-2/*neu* or epithelial ovarian cancer.** *Hum Gene Ther* 1998, **9**: 1775–1798.

36. Kashles O, Yarden Y, Fischer R, Ullrich A, Schlessinger J: **A dominant negative mutation suppresses the function of normal epidermal growth factor receptors by heterodimerization.** *Mol Cell Biol* 1991, **11**:1454–1463.

37. Vassbotn FS, Andersson M, Westermark B, Heldin C-H, Ostman A: **Reversion of autocrine transformation by a dominant negative platelet-derived growth factor mutant.** *Mol Cell Biol* 1993, **13**: 4066–4076.

38. Cohen P, Lamson G, Okajima T, Rosenfeld RG: **Transfection of the human insulin-like growth factor binding protein-3 gene into Balb/c fibroblasts inhibits cellular growth.** *Mol Endocrinol* 1993, **7**: 380–386.

39. Lazennec G, Alcorn JL, Katzenellenbogen BS: **Adenovirus-mediated delivery of a dominant negative estrogen receptor gene abrogates estrogen-stimulated gene expression and breast cancer cell proliferation.** *Mol Endocrinol* 1999, **13**:969–980.

40. Deshane J, Loechel F, Conry RM, *et al*: **Intracellular single-chain antibody directed against erbB2 down regulates cell surface erbB2 and exhibits a selective anti-proliferative effect in erbB-2 overexpressing cancer cell lines.** *Gene Ther* 1994, **1**:332–337.

41. •• Deshane J, Siegal GP, Alvarez RD, *et al*: **Targeted tumor cell killing via an intracellular antibody directed against the erbB-2.** *J Clin Invest* 1995, **96**:2980–2989.
A highly novel approach to achieve mutation compensation is presented.

42. Deshane J, Grim J, Loechel F, *et al*: **Intracellular antibody against erbB-2 mediates targeted tumor cell eradication by apoptosis.** *Cancer Gene Ther* 1996, **3**:89–98.

43. Wright M, Grim J, Deshane J, *et al*: **An intracellular anti-erbB-2 single-chain antibody is specifically cytotoxic to human breast carcinoma cells overexpressing erbB-2.** *Gene Ther* 1997, **4**: 317–322.

44. Graus-Porta D, Beerli RR, Hynes NE: **Single-chain antibody-mediated Intracellular retention of erbB-2 impairs neu differentiation factor and epidermal growth factor signalling.** *Mol Cell Biol* 1995, **15**:1182–1191.

45. Beerli RR, Wels W, Hynes NE: **Intracellular expression of single chain antibodies reverts erbB-2 transformation.** *J Biol Chem* 1994, **269**:23931–23936.

46. Kim M, Wright M, Deshane J, *et al*: **A novel gene therapy strategy for elimination of prostate carcinoma cells from human bone marrow.** *Hum Gene Ther* 1997, **8**:157–170.

47. Marini III, FC, Snell V, Yu Q, *et al*: **Purging of contaminating breast cancer cells from hematopoietic stem cell grafts by adenoviral GAL-TEK gene therapy and magnetic antibody cell separation.** *Clin Cancer Res* 1999, **5**:1557–1568.

48. Bilbao G, Contreras JL, Gomez-Navarro J, Curiel DT: **Improving adeno-viral vectors for cancer gene therapy.** *Tumor Targeting* 1998, **3**:59–79.

49. • Reynolds PN, Curiel DT: **Strategies to adapt adenoviral vectors for gene therapy applications: targeting and integration.** In: *The Development of Human Gene Therapy*, vol. 6. Cold Spring Harbor Laboratory Press, 1999:111–130.
Excellent summaries of the state of vector art are presented.

50. Douglas JT, Curiel DT: **Targeted adenoviral vectors for cancer gene therapy.** *Int J Oncol* 1997, **11**:341–348.

51. Curiel DT: **The promise of cancer gene therapy [letter].** *Curr Res in Mol Ther* 1998, **1**:323.

52. Boxhorn HKE, Eck SL: **Gene therapy for breast cancer.** *Gene Ther* 1998, **12**:665–675.

53. Lebkowski JS, Philip R, Okarma TB: **Breast cancer: cell and gene therapy.** *Cancer Invest* 1997, **15**:568–576.

54. Ruppert JM, Wright M, Rosenfeld M, *et al*: **Gene therapy strategies for carcinoma of the breast.** *Breast Cancer Res Treat* 1997, **44**:93–114.

Author affiliation: Gene Therapy Center, The University of Alabama at Birmingham, Birmingham, Alabama, USA

Sponsorship: The research of the authors reported in the present review was supported by the following grants: National Institutes of Health (R01 CA74242); National Institutes of Health (R01 CA72532); National Cancer Institute (N01 C097110); and the United States Department of Defense.

Correspondence: David T Curiel, Gene Therapy Center, The University of Alabama at Birmingham, 1824 6th Ave. South, WTI 620, Birmingham, AL 35294-3300, USA. Tel: +1 205 934 8627; fax: +1 205 975 7476; e-mail: david.curiel@ccc.uab.edu