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(54) Title: DENDRITIC CELLS TRANSDUCED WITH A WILD-TYPE SELF GENE ELICIT POTENT ANTITUMOR IMMUNE RESPONSES

(57) Abrégé/Abstract:
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DESCRIPTION

DENDRITIC CELLS TRANSDUCED WITH A WILD-TYPE SELF GENE ELICIT POTENT ANTITUMOR IMMUNE RESPONSES

BACKGROUND OF THE INVENTION

The present application claims the benefit of U.S. Provisional Application Serial Number 60/124,482 and U.S. Provisional Application Serial Number 60/124,388, both of which were filed on March 15, 1999. The government owns rights in the present invention pursuant to grant number CA61242 from the National Cancer Institute.

A. FIELD OF THE INVENTION

The present invention relates generally to the fields of immunology and cancer therapy. More particularly, it concerns a method of eliciting a cytotoxic T lymphocyte response directed against self gene antigens presented by hyperproliferative cells.

B. DESCRIPTION OF RELATED ART

Normal tissue homeostasis is a highly regulated process of cell proliferation and cell death. An imbalance of either cell proliferation or cell death can develop into a cancerous state (Solyanik *et al.*, 1995; Stokke *et al.*, 1997; Mumby and Walter, 1991; Natoli *et al.*, 1998; Magi-Galluzzi *et al.*, 1998). For example, cervical, kidney, lung, pancreatic, colorectal and brain cancer are just a few examples of the many cancers that can result (Erlandsson, 1998; Kolmel, 1998; Mangray and King, 1998; Gertig and Hunter, 1997; Mougin *et al.*, 1998). In fact, the occurrence of cancer is so high, that over 500,000 deaths per year are attributed to cancer in the United States alone.

The maintenance of cell proliferation and cell death is at least partially regulated by proto-oncogenes. A proto-oncogene can encode proteins that induce cellular proliferation (*e.g., sis, erbB, src, ras* and *myc*), proteins that inhibit cellular

proliferation (*e.g., Rb, p53, NF1* and *WT1*) or proteins that regulate programmed cell death *(e.g., bcl-2*) (Ochi *et al.*, 1998; Johnson and Hamdy, 1998; Liebermann *et al.*, 1998). However, genetic rearrangements or mutations to these proto-oncogenes, results in the conversion of a proto-oncogene into a potent cancer causing oncogene. Often, a single point mutation is enough to transform a proto-oncogene into an oncogene. For example, a point mutation in the p53 tumor suppressor protein results in the complete loss of wild-type p53 function (Vogelstein and Kinzler, 1992; Fulchi *et al.*, 1998) and acquisition of "dominant" tumor promoting function.

Currently, there are few effective options for the treatment of many common cancer types. The course of treatment for a given individual depends on the diagnosis, the stage to which the disease has developed and factors such as age, sex and the general health of the patient. The most conventional options of cancer treatment are surgery, radiation therapy and chemotherapy. Surgery plays a central role in the diagnosis and treatment of cancer. Typically, a surgical approach is required for biopsy and to remove cancerous growth. However, if the cancer has metastasized and is widespread, surgery is unlikely to result in a cure and an alternate approach must be taken. Radiation therapy, chemotherapy and immunotherapy are alternatives to surgical treatment of cancer (Mayer, 1998; Ohara, 1998; Ho *et al.*, 1998). Radiation therapy involves a precise aiming of high energy radiation to destroy cancer cells and much like surgery, is mainly effective in the treatment of non-metastasized, localized cancer cells. Side effects of radiation therapy include skin irritation, difficulty swallowing, dry mouth, nausea, diarrhea, hair loss and loss of energy (Curran, 1998; Brizel, 1998).

Chemotherapy, the treatment of cancer with anti-cancer drugs, is another mode of cancer therapy. The effectiveness of a given anti-cancer drug therapy is often limited by the difficulty of achieving drug delivery throughout solid tumors (el-Kareh and Secomb, 1997). Chemotherapeutic strategies are based on tumor tissue growth, wherein the anti-cancer drug is targeted to the rapidly dividing cancer cells. Most chemotherapy approaches include the combination of more than one anti-cancer drug,

which has proven to increase the response rate of a wide variety of cancers (U.S. Patent 5,824,348; U.S. Patent 5,633,016 and U.S. Patent 5,798,339). A major side effect of chemotherapy drugs is that they also affect normal tissue cells, with the cells most likely to be affected being those that divide rapidly (*e.g.*, bone marrow, gastrointestinal tract, reproductive system and hair follicles). Other toxic side effects of chemotherapy drugs are sores in the mouth, difficulty swallowing, dry mouth, nausea, diarrhea, vomiting, fatigue, bleeding, hair loss and infection.

Immunotherapy, a rapidly evolving area in cancer research, is yet another option for the treatment of certain types of cancers. For example, the immune system identifies tumor cells as being foreign and thus are targeted for destruction by the immune system. Unfortunately, the response typically is not sufficient to prevent most tumor growths. However, recently there has been a focus in the area of immunotherapy to develop methods that augment or supplement the natural defense mechanism of the immune system. Examples of immunotherapies currently under investigation or in use are immune adjuvants (*e.g., Mycobacterium bovis*, *Plasmodium falciparum*, dinitrochlorobenzene and aromatic compounds) (U.S. Patent 5,801,005; U.S. Patent 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998), cytokine therapy (*e.g.,* interferons α, β and γ; IL-1, GM-CSF and TNF) (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.,* 1998) gene therapy (*e.g.*, TNF, IL-1, IL-2, p53) (Qin *et al.*, 1998; Austin-Edward and Villaseca, 1998; U.S. Patent 5,830,880 and U.S. Patent 5,846,945) and monoclonal antibodies (*e.g.*, anti-ganglioside GM2, anti-HER-2, anti-p185) (Pietras *et al.*, 1998; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311).

As mentioned above, proto-oncogenes play an important role in cancer biology. For example, Rb, p53, NF1 and WT1 tumor suppressors, are essential for the maintenance of the non-tumorogenic phenotype of cells (reviewed by Soddu and Sacchi, 1998). Approximately 50% of all cancers have been found to be associated with mutations of the p53 gene, which result in the loss of p53 tumor suppressor properties (Levine *et al.*, 1991; Vogelstein and Kinzler, 1992; Hartmann *et al.*, 1996a;

Hartmann *et al.*, 1996b). Mutations in the p53 gene also result in the prolongation of the p53 half-life in cells and the overexpression of p53 protein. In normal cells, p53 is undetectable due to its high turnover rate. Thus, p53 overexpression in cancerous cells results in multiple immunogenic p53 epitopes which can be used in immunotherapy. The high incidence of cancer related to mutations of the p53 gene has prompted many research groups to investigate p53 as a route of cancer treatment via gene replacement. The proto-oncogenes sis, erbB, src, ras and myc, encoding proteins that induce cellular proliferation, and the proto-oncogenes of the Bcl-2 family that regulate programmed cell death also play important roles in the non-tumorogenic phenotype of cells.

A few also have explored the use of p53 in immunotherapy. For example, in an *in vitro* assay, p53 mutant peptides capable of binding to HLA-A2.1 and inducing primary cytotoxic T lymphocyte (CTL) responses were identified (Houbiers *et al.*, 1993). In a study in which synthetic p53 mutant and wild-type peptides were screened for immunogenicity in mice, it was observed that only mutant p53 epitopes were capable of eliciting a CTL response (Bertholet *et al.*, 1997). In contrast, the immunization of BALB/c mice with bone marrow-derived dendritic cells (DC) in the presence of GM-CSF/IL-4 and prepulsed with the H-2Kd binding wild-type p53 peptide (232-240) was observed to induce p53 anti-peptide CTL response (Ciernik *et al.*, 1996; Gabrilovich *et al.*, 1996; Yanuck *et al*, 1993; DeLeo, 1998; Mayordomo *et al.*, 1996). Further, the intradermal and intramuscular injection of naked plasmid DNA encoding human wild-type p53 and the intravenous injection of human wild-type p53 presented by a recombinant canarypox vector have been successful in the destruction of tumors (Hurpin *et al.,* 1998).

Despite the foregoing, there currently exist no methods of self gene-based immunotherapy capable of utilizing wild-type self genes to generate an antitumor immune response specific for a variety of cells overexpressing different mutant self proteins. This would permit the treatment of any cancerous or pre-cancerous cell associated with increased or altered expression of the self gene. Further, it would

eliminate the need to identify the site of self gene mutation in each patient and generate customized self gene mutant peptides for immunotherapy. Thus, the need exists for an immunotherapy that is capable of attenuating or enhancing the natural immune systems CTL response against hyperproliferative cells with increased or altered expression of mutant self gene antigens.

SUMMARY OF THE INVENTION

Therefore, there exists a need for an immunotherapy that is capable of augmenting the natural immune systems CTL response against hyperproliferative cells or pathogen infected cells expressing an altered self gene antigen or pathogenic antigen, respectively. The present invention also provides a method of eliciting a cytotoxic T lymphocyte response directed against p53 antigens presented by hyperproliferative cells. In one embodiment of the invention, there is provided a method for treating a subject with a hyperproliferative disease.

The treatment of a hyperproliferative disease in the present invention comprises the steps of identifying a subject with a hyperproliferative disease, characterized by alteration or increased expression of a self gene product in at least some of the hyperproliferative cells in the patient. Following identification of a subject with a hyperproliferative disease, an expression construct comprising a self gene under the control of a promoter operable in eukaryotic dendritic cells is intradermally administered to the subject. The self gene product is expressed by dendritic cells and presented to immune effector cells, thereby stimulating an anti-self gene product response.

In one embodiment, the self-gene product is an oncogene, wherein the oncogene may be selected from the group consisting of tumor suppressors, tumor associated genes, growth factors, growth-factor receptors, signal transducers, hormones, cell cycle regulators, nuclear factors, transcription factors and apoptic factors. In preferred embodiments, the tumor suppressor is selected from the group

consisting of Rb, p53, p16, p19, p21, p73, DCC, APC, NF-1, NF-2, PTEN, FHIT, C-CAM, E-cadherin, MEN-I, MEN-II, ZAC1, VHL, FCC, MCC , PMS1, PMS2, MLH-1, MSH-2, DPC4, BRCA1, BRCA2 and WT-1. In preferred embodiments, the tumor suppressor is p53. In preferred embodiments, the growth-factor receptor is selected from the group consisting of FMS, ERBB/HER, ERBB-2/NEU/HER-2, ERBA, TGF-β receptor, PDGF receptor, MET, KIT and TRK. In preferred embodiments, the signal transducer is selected from the group consisting of SRC, ABl, RAS, AKT/PKB, RSK-1, RSK-2, RSK-3, RSK-B, PRAD, LCK and ATM. In preferred embodiments, the transcription factor or nuclear factor is selected from the group consisting of JUN, FOS, MYC, BRCA1, BRCA2, ERBA, ETS, EVII, MYB, HMGI-C, HMGI/LIM, SKI, VHL, WT1, CEBP-α, NFKB, IKB, GL1 and REL. In preferred embodiments, the growth factor is selected from the group consisting of SIS, HST, INT-1/WT1 and INT-2. In preferred embodiments, the apoptic factor is selected from the group consisting of Bax, Bak, Bim, Bik, Bid, Bad, Bcl-2, Harakiri and ICE proteases. In preferred embodiments, the tumor-associated gene is selected from the group consisting of CEA, mucin, MAGE and GAGE.

The expression construct may be a viral vector, wherein the viral vector is an adenoviral vector, a retroviral vector, a vaccinia viral vector, an adeno-associated viral vector, a polyoma viral vector, an alphavirus vector, or a herpesviral vector. In preferred embodiments, the viral vector is an adenoviral vector.

In certain embodiments, the adenoviral vector is replication-defective. In another embodiment, the replication defect is a deletion in the E1 region of the virus. In certain embodiments, the deletion maps to the E1B region of the virus. In other embodiments, the deletion encompasses the entire E1B region of the virus. In another embodiment, the deletion encompasses the entire E1 region of the virus.

In one embodiment of the present invention, the promoter operable in eukaryotic cells may be selected from the group consisting of CMV IE, dectin-1, dectin-2, human CD11c, F4/80 and MHC class II. In preferred embodiments, the

promoter is CMV IE. In another embodiment the expression vector further comprises a polyadenylation signal.

It is contemplated, in one embodiment of the present invention, that the hyperproliferative disease is cancer, wherein the cancer may be selected from the group consisting of lung, head, neck, breast, pancreatic, prostate, renal, bone, testicular, cervical, gastrointestinal, lymphoma, brain, colon, skin and bladder. In other embodiments, the hyperproliferative disease is non-cancerous and may be selected from the group consisting of rheumatoid arthritis (RA), inflammatory bowel disease (IBD), osteoarthritis (OA), pre-neoplastic lesions in the lung and psoriasis.

In other embodiments, the subject treated for a hyperproliferative disease is a human. It is contemplated in certain embodiments administering to the subject at least a first cytokine selected from the group consisting GM-CSF, IL-4, C-KIT, Steel factor, TGF-β, TNF-α and FLT3 ligand. In yet another embodiment, a second cytokine, different from the first cytokine, is administered to the subject. In another embodiment, the cytokine is administered as a gene encoded by the expression construct. In other embodiments, the immune effector cells are CTLs.

Also contemplated in the present invention is intradermal administration of the expression construct by a single injection or multiple injections. In one embodiment, the injections are performed local to a hyperproliferative or tumor site. In another embodiment, the injections are performed regional to a hyperproliferative or tumor site. In still another embodiment, the injections are performed distal to a hyperproliferative or tumor site. It is further contemplated, that the injections are performed at the same time, at different times or via continuous infusion.

The present invention comprises a method for inducing a p53-directed immune response in a subject comprising the steps of obtaining dendritic cells from a subject, infecting the dendritic cells with an adenoviral vector comprising a p53 gene under the control a promoter operable in eukaryotic cells and administering the adenovirus-

infected dendritic cells to the subject, whereby p53 expressed in the dendritic cells is presented to immune effector cells, thereby stimulating an anti-p53 response.

In another aspect of the present invention, there is provided a method for treating a pathogen-induced disease in a subject comprising the steps of identifying a subject with a pathogen-induced disease characterized by alteration or increased expression of a pathogen gene product in at least some of the pathogen-induced cells in the patient and intradermally administering to the subject an expression construct comprising a pathogen gene under the control of a promoter operable in eukaryoticdendritic cells, whereby the pathogen gene product is expressed by dendritic cells and presented to immune effector cells, thereby stimulating an anti-pathogen gene product response. In one embodiment, the dendritic cells are obtained from peripheral blood progenitor cells. In another embodiment, multiple injections of adenovirus-infected dendritic cells is contemplated.

In one embodiment of the present invention, the pathogen may be selected from the group consisting of bacterium, virus, fungus, parasitic worm, amoebae and mycoplasma. In certain embodiments, the bacterium may be selected from the group consisting of richettsia, listeria and histolytica. In other embodiments the virus may be selected from the group consisting of HIV, HBV, HCV, HSV, HPV, EBV and CMV. In yet another embodiment, the fungus may be selected from the group consisting of hitoplasma, coccidis, immitis, aspargillus, actinomyces, blastomyces, candidia and streptomyces.

In certain embodiments for the treatment of a pathogen-induced disease, the expression construct is a viral vector and may be selected from the group consisting of an adenoviral vector, a retroviral vector, a vaccinia viral vector, an adeno-associated viral vector, a polyoma viral vector, an alphavirus vector, or a herpesviral vector. In a preferred embodiment, the viral vector is an adenoviralvector, wherein said adenoviral vector is replication-defective. In one embodiment, the replication defect is a deletion in the E1 region of the virus. In other embodiments, the deletion maps to the E1B

region of the virus. In yet other embodiments, the deletion encompasses the entire E1B region of the virus. In still other embodiments, the deletion encompasses the entire E1 region of the virus.

The promoter operable in eukaryotic cells may be selected from the group consisting of CMV IE, dectin-1, dectin-2, human CD11c, F4/80 and MHC class II. In preferred embodiments, the promoter is CMV IE. In certain embodiments, the expression vector further comprises a polyadenylation signal.

It is contemplated in embodiments where the expression construct is delivered intradermally, that administration may be by injection. In other embodiments, intradermal administration comprises multiple injections. It is contemplated in the present invention, that the injections are performed local, regional or distal to the pathogen-induced disease site.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1A, FIG. 1B, and FIG. 1C. Expression of p53 protein in DC infected with Ad-p53. DCs generated from bone marrow were infected with 100 MOI Ad-c or Ad-p53 for 48 h, washed, fixed, permeablized and stained with anti-p53 antibody and analyzed. Non-specific staining - Ad-p53 infected DCs stained only with secondary antibody. Ad-c and Ad-p53, DC infected with corresponding virus stained with anti-p53 antibody. Typical results of one of three studies performed are shown.

FIG. 2A, FIG. 2B and FIG. 2C. Ad-p53 transduced DCs induce anti-p53 immune responses. FIG 2A. CTL response. Mice were immunized twice with DC

infected with either Ad-c (Ad-c DC) or with Ad-p53 (Ad-p53 DC) (iv injections). Ten days after the last immunization, T cells from these mice were restimulated with Ad-p53 DC and a CTL assay was performed. P815-Ad and P815-Ad-p53 targets were prepared by overnight incubation of P815 cells with adenovirus at MOI 100 pfu/ml. Mean±SE of cytotoxicity from four studies is shown. FIG. 2B. CTL responses against MethA mouse tumor sarcoma cells (expressing mutant mouse p53). Mice were immunized, T cells were restimulated and CTL assay was performed exactly as described in FIG. 2A. Target MethA sarcoma cells were pre-incubated with 50 U/ml IFNγ for three days prior the assay. Two studies with the same results were performed. FIG. 2C. T cell proliferation. Mice were immunized as described in FIG. 2A. T cells were isolated and cultured in triplicates with either control untreated DC, Ad-c DC or Ad-p53 DC. ^{3}H-thymidine uptake was measured on day 3. Mean ± SE of thymidine incorporation from two studies is shown.

FIG. 3A and FIG. 3B. Immunization with Ad-p53 protects from tumor challenge. Mice were immunized as described in FIG. 2A. Ten days after the second immunization, mice were challenged with 2×10^5 D459 (mouse cell expressing human p53) cells or with 6×10^5 MethA sarcoma cells. In studies with D459 cells, each group included 20 mice, in studies with MethA sarcoma they included 11 mice. Differences between groups were statistically significant ($p<0.05$).

FIG. 4. Treatment with Ad-p53 DC slowed the growth of established tumors. 2×10^5 D459 cells were inoculated sc into the shaved backs of mice. Treatment with 2×10^5 Ad-c or Ad-p53 DC was initiated when tumor became palpable (day 5). DC were injected on day 5, 9 and 13. Mice in the control group were sacrificed on day 31 due to bulky tumors, mice that received treatment with Ad-p53 DC were sacrificed on day 49. Ten mice per group were treated. Mean ± SE is shown.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention contemplates the treatment of hyperproliferative disease by identifying patients with a hyperproliferative disease in which self gene expression is increased or altered in these hyperproliferative cells. The treatment of such a hyperproliferative disease in one embodiment involves the intradermal administration of a p53 expression construct to dendritic cells, which subsequently present the processed p53 wild-type antigens to immune effector cells. The immune effector cells then mount an anti-p53 response, resulting in the destruction or lysis of hyperproliferative cells presenting mutant p53 antigen. In another embodiment, dendritic cells are obtained from a patient in which p53 expression is upregulated in hyperproliferative cells. The dendritic cells obtained are infected with an adenoviral vector comprising a p53 gene and the p53 adenovirus-infected dendritic cells are administered to the patient. It is contemplated that infected dendritic cells will present self gene antigens to immune effector cells, stimulate an anti- self gene response in the patient and result in the destruction or lysis of hyperproliferative cells presenting mutant self gene antigen.

A. HYPERPROLIFERATIVE DISEASE

Cancer has become one of the leading causes of death in the Western world, second only behind heart disease. Current estimates project that one person in three in the U.S. will develop cancer, and that one person in five will die from cancer. Cancers can be viewed from an immunologic perspective as altered self cells, that have lost the normal growth-regulating mechanisms.

There are currently three major categories of oncogenes, reflecting their different activities. One category of oncogenes encode proteins that induce cellular proliferation. A second category of oncogenes, called tumor-suppressors genes or anti-oncogenes, function to inhibit excessive cellular proliferation. The third category of oncogenes, either block or induce apoptosis by encoding proteins that regulate programmed cell death.

In one embodiment of the present invention, the treatment of hyperproliferative disease involves the intradermal administration of a self gene expression construct to dendritic cells. It is contemplated that the dendritic cells present the processed self gene wild-type antigens to immune effector cells, which mount an anti-self gene response, resulting in the destruction or lysis of hyperproliferative cells presenting mutant self antigen. The three major categories of oncogenes are discussed below and listed in Table 1.

1. INDUCERS OF CELLULAR PROLIFERATION

The proteins that induce cellular proliferation further fall into various categories dependent on function. The commonality of all of these proteins is their ability to regulate cellular proliferation. For example, a form of PDGF, the sis oncogene is a secreted growth factor. Oncogenes rarely arise from genes encoding growth factors, and at the present, sis is the only known naturally occurring oncogenic growth factor.

The proteins fms, erbA, erbB and neu are growth factor receptors. Mutations to these receptors result in loss of regulatable function. For example, a point mutation affecting the transmembrane domain of the nue receptor protein results in the nue oncogene. The erbA oncogene is derived from the intracellular receptor for thyroid hormone. The modified oncogenic erbA receptor is believed to compete with the endogenous thyroid hormone receptor, causing uncontrolled growth.

The largest class of oncogenes are the signal transducing proteins (*e.g.*, src, abl and ras) are signal transducers. The protein src, is a cytoplasmic protein-tyrosine kinase, and its transformation from proto-oncogene to oncogene in some cases, results via mutations at tyrosine residue 527. In contrast, transformation of GTPase protein ras from proto-oncogene to oncogene, in one example, results from a valine to glycine mutation at amino acid 12 in the sequence, reducing ras GTPase activity.

The proteins jun, fos and myc are proteins that directly exert their effects on nuclear functions as transcription factors. Table 1 lists a variety of the oncogenes described in this section and many of those not described.

2. INHIBITORS OF CELLULAR PROLIFERATION

The tumor suppressor oncogenes function to inhibit excessive cellular proliferation. The inactivation of these genes results destroys their inhibitory activity, resulting in unregulated proliferation. The tumor suppressors p53, p16 and C-CAM are described below.

High levels of mutant p53 have been found in many cells transformed by chemical carcinogenesis, ultraviolet radiation, and several viruses. The p53 gene is a frequent target of mutational inactivation in a wide variety of human tumors and is already documented to be the most frequently-mutated gene in common human cancers. It is mutated in over 50% of human NSCLC (Hollstein *et al.*, 1991) and in a wide spectrum of other tumors. A variety of cancers have been associated with mutations of the p53 gene, which result in the loss of p53 tumor suppressor properties. Mutations in the p53 gene further account for approximately 50% of all cancers that develop (Vogelstein and Kinzler, 1992; Levine *et al.*, 1991), with the majority of these mutations being single-base missense mutations (Kovach *et al.*, 1996). It has been observed that mutations resulting in a loss of p53 function also result in high nuclear and cytoplasmic concentrations (*i.e.* overexpression) of mutant p53 protein (Oldstone *et al.*, 1992; Finlay *et al.*, 1988). In contrast, functional wild-type p53 protein is expressed at very low levels in cells.

The high cellular concentrations of p53 mutant protein has recently received much attention as an avenue for cancer immunotherapy. The general concept is to elicit an immune response against tumor cells presenting mutant p53 peptides bound to MHC molecules on the cell surface. The generation of an anti-tumor response using mutant p53 peptides as antigens has been demonstrated in several studies (McCarty *et al.*, 1998; Gabrilovich *et al.*, 1996; Mayordomo *et al.*, 1996; Zitvogel *et*

al., 1996) However, this approach to cancer immunotherapy has several limitations. For example, p53 mutations can occur at many different sites in the protein, making it necessary to identify the site of the mutation in each patient before creating a specific mutant peptide for p53 cancer therapy. Further, not all mutations are contained in regions of the protein known to bind to MHC molecules, and therefore would not elicit an anti-tumor response (DeLeo, 1998).

The limitations described above have stimulated the search for antigenic epitopes in wild-type p53 sequences common to the vast majority of tumor derived p53 proteins. Wild-type p53 peptide-specific cytotoxic T lymphocytes have been produced from human and murine responding lymphocytes, some of which recognized p53-overexpressing tumors *in vitro* and *in vivo* (Theobald, *et al.*, 1995; Ropke *et al.*, 1996; Nijman *et al.*, 1994; U.S. Patent 5,747,469, specifically incorporated herein by reference in its entirety). However, since the presentation of antigens is MHC class I restricted, only certain peptides can successfully be administered in certain patients, due to the polymorphic nature of the MHC class I peptide binding site. Further, it is not practical to identify all possible p53 peptides binding to a particular individuals repertoire of MHC molecules. Additionally, a peptide vaccine that does bind to a patient's class I MHC may not be sufficiently presented by MHC class II, the molecules crucial in the induction of $CD4^+$ T cell immune responses.

Researchers have to attempted to identify multiple p53 epitopes, which should permit more effective immune responses against tumor cells expressing multiple p53 genes with mutations at different sites. This could be accomplished by immunizing cells with intact wild-type p53 to take advantage of the overexpression of the whole p53 polypeptide in most human tumors. The dendritic cell (DC) is the cell type best suited for vaccine antigen delivery (described further in section B), as they are the most potent antigen presenting cells, effective in the stimulation of both primary and secondary immune responses (Steinman, 1991; Celluzzi and Falo, 1997). It is contemplated in the present invention that the transduction of dendritic cells with

wild-type p53 protein, using a viral expression construct, will elicit a potent antitumor immune response specific for a variety of cells expressing different mutant p53 proteins. Further, since most mutations of p53 are single-base missense mutations, the approach of the present invention overcomes the limitations of identifying the site of the p53 mutation and subsequent preparation of a customized mutant peptide for immunotherapy. Thus, the method of the present invention provides the basis for a simple and novel approach to immunotherapy based cancer treatment.

Wild-type p53 is recognized as an important growth regulator in many cell types. Missense mutations are common for the p53 gene and are essential for the transforming ability of the oncogene. A single genetic change prompted by point mutations can create carcinogenic p53. Unlike other oncogenes, however, p53 point mutations are known to occur in at least 30 distinct codons, often creating dominant alleles that produce shifts in cell phenotype without a reduction to homozygosity. Additionally, many of these dominant negative alleles appear to be tolerated in the organism and passed on in the germ line. Various mutant alleles appear to range from minimally dysfunctional to strongly penetrant, dominant negative alleles (Weinberg, 1991).

Another inhibitor of cellular proliferation is p16. The major transitions of the eukaryotic cell cycle are triggered by cyclin-dependent kinases, or CDK's. One CDK, cyclin-dependent kinase 4 (CDK4), regulates progression through the G_1. The activity of this enzyme may be to phosphorylate Rb at late G_1. The activity of CDK4 is controlled by an activating subunit, D-type cyclin, and by an inhibitory subunit, the $p16^{INK4}$ has been biochemically characterized as a protein that specifically binds to and inhibits CDK4, and thus may regulate Rb phosphorylation (Serrano *et al.*, 1993; Serrano *et al.*, 1995). Since the $p16^{INK4}$ protein is a CDK4 inhibitor (Serrano, 1993), deletion of this gene may increase the activity of CDK4, resulting in hyperphosphorylation of the Rb protein. p16 also is known to regulate the function of CDK6.

$p16^{INK4}$ belongs to a newly described class of CDK-inhibitory proteins that also includes $p16^{B}$, $p21^{WAF1}$, and $p27^{KIP1}$. The $p16^{INK4}$ gene maps to 9p21, a chromosome region frequently deleted in many tumor types. Homozygous deletions and mutations of the $p16^{INK4}$ gene are frequent in human tumor cell lines. This evidence suggests that the $p16^{INK4}$ gene is a tumor suppressor gene. This interpretation has been challenged, however, by the observation that the frequency of the $p16^{INK4}$ gene alterations is much lower in primary uncultured tumors than in cultured cell lines (Caldas *et al.*, 1994; Cheng *et al.*, 1994; Hussussian *et al.*, 1994; Kamb *et al.*, 1994; Kamb *et al.*, 1994; Mori *et al.,* 1994; Okamoto *et al.*, 1994; Nobori *et al.*, 1995; Orlow *et al.*, 1994; Arap *et al.*, 1995). Restoration of wild-type $p16^{INK4}$ function by transfection with a plasmid expression vector reduced colony formation by some human cancer cell lines (Okamoto, 1994; Arap, 1995).

C-CAM is expressed in virtually all epithelial cells (Odin and Obrink, 1987). C-CAM, with an apparent molecular weight of 105 kD, was originally isolated from the plasma membrane of the rat hepatocyte by its reaction with specific antibodies that neutralize cell aggregation (Obrink, 1991). Recent studies indicate that, structurally, C-CAM belongs to the immunoglobulin (Ig) superfamily and its sequence is highly homologous to carcinoembryonic antigen (CEA) (Lin and Guidotti, 1989). Using a baculovirus expression system, Cheung *et al.* (1993) demonstrated that the first Ig domain of C-CAM is critical for cell adhesive activity.

Cell adhesion molecules, or CAM's are known to be involved in a complex network of molecular interactions that regulate organ development and cell differentiation (Edelman, 1985). Recent data indicate that aberrant expression of CAM's maybe involved in the tumorigenesis of several neoplasms; for example, decreased expression of E-cadherin, which is predominantly expressed in epithelial cells, is associated with the progression of several kinds of neoplasms (Edelman and Crossin, 1991; Frixen *et al.*, 1991; Bussemakers *et al.*, 1992; Matsura *et al.*, 1992; Umbas *et al.*, 1992). Also, Giancotti and Ruoslahti (1990) demonstrated that increasing expression of $\alpha_5\beta_1$ integrin by gene transfer can reduce tumorigenicity of

Chinese hamster ovary cells *in vivo*. C-CAM now has been shown to suppress tumors growth *in vitro* and *in vivo*.

Other tumor suppressors that may be employed according to the present invention include RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, zac1, p73, VHL, MMAC1, FCC and MCC (see Table 1).

3. REGULATORS OF PROGRAMMED CELL DEATH

Apoptosis, or programmed cell death, is an essential occurring process for normal embryonic development, maintaining homeostasis in adult tissues, and suppressing carcinogenesis (Kerr *et al.*, 1972). The Bcl-2 family of proteins and ICE-like proteases have been demonstrated to be important regulators and effectors of apoptosis in other systems. The Bcl-2 protein, discovered in association with follicular lymphoma, plays a prominent role in controlling apoptosis and enhancing cell survival in response to diverse apoptotic stimuli (Bakhshi *et al.*, 1985; Cleary and Sklar, 1985; Cleary *et al.*, 1986; Tsujimoto *et al.*, 1985; Tsujimoto and Croce, 1986). The evolutionarily conserved Bcl-2 protein now is recognized to be a member of a family of related proteins which can be categorized as death agonists or death antagonists.

Subsequent to its discovery, it was shown that Bcl-2 acts to suppress cell death triggered by a variety of stimuli. Also, it now is apparent that there is a family of Bcl-2 cell death regulatory proteins which share in common structural and sequence homologies. These different family members have been shown to either possess similar functions to Bcl-2 (*e.g.*, Bcl_{XL}, Bcl_{W}, Mcl-1, A1, Bfl-1) or counteract Bcl-2 function and promote cell death (*e.g.*, Bax, Bak, Bik, Bim, Bid, Bad, Harakiri).

TABLE 1

ONCOGENES

Gene	*Source*	*Human Disease*	*Function*
Growth Factors[1]			FGF family member
HST/KS	Transfection		
INT-2	MMTV promoter insertion		FGF family member
INTI/WNTI	MMTV promoter insertion		Factor-like
SIS	Simian sarcoma virus		PDGF B
Receptor Tyrosine Kinases[1,2]			
ERBB/HER	Avian erythroblastosis virus; ALV promoter insertion; amplified human tumors	Amplified, deleted squamous cell cancer; glioblastoma	EGF/TGF-α/ amphiregulin/ hetacellulin receptor
ERBB-2/NEU/HER-2	Transfected from rat glioblatoms	Amplified breast, ovarian, gastric cancers	Regulated by NDF/ heregulin and EGF-related factors
FMS	SM feline sarcoma virus		CSF-1 receptor
KIT	HZ feline sarcoma virus		MGF/Steel receptor hematopoieis
TRK	Transfection from human colon cancer		NGF (nerve growth factor) receptor
MET	Transfection from human osteosarcoma		Scatter factor/HGF receptor
RET	Translocations and point mutations	Sporadic thyroid cancer; familial medullary thyroid cancer; multiple endocrine neoplasias 2A and 2B	Orphan receptor Tyr kinase

TABLE 1 (CONT'D)

ROS	URII avian sarcoma virus		Orphan receptor Tyr kinase
PDGF receptor	Translocation	Chronic myclomonocytic leukemia	TEL(ETS-like transcription factor)/ PDGF receptor gene fusion
TGF-β receptor		Colon carcinoma mismatch mutation target	

NONRECEPTOR TYROSINE KINASES[1]

ABI.	Abelson Mul.V	Chronic myelogenous leukemia translocation with BCR	Interact with RB, RNA polymerase, CRK, CBL
FPS/FES	Avian Fujinami SV;GA FeSV		
LCK	Mul.V (murine leukemia virus) promoter insertion		Src family; T cell signaling; interacts CD4/CD8 T cells
SRC	Avian Rous sarcoma virus		Membrane-associated Tyr kinase with signaling function; activated by receptor kinases
YES	Avian Y73 virus		Src family; signaling

SER/THR PROTEIN KINASES[1]

AKT	AKT8 murine retrovirus	Regulated by PI(3)K?; regulate 70-kd S6 k?
MOS	Maloney murine SV	GVBD; cystostatic factor; MAP kinase kinase

TABLE 1 (CONT'D)

PIM-1	Promoter insertion mouse		
RAF/MIL	3611 murine SV; MH2 avian SV		Signaling in RAS pathway
MISCELLANEOUS CELL SURFACE[1]			
APC	Tumor suppressor	Colon cancer	Interacts with catenins
DCC	Tumor suppressor	Colon cancer	CAM domains
E-cadherin	Candidate tumor suppressor	Breast cancer	Extracellular homotypic binding; intracellular interacts with catenins
PTC/NBCCS	Tumor suppressor and *Drosophilia* homology	Nevoid basal cell cancer syndrome (Gorline syndrome)	12 transmembrane domain; signals through Gli homogue CI to antagonize hedgehog pathway
TAN-1 Notch homologue	Translocation	T-ALI.	Signaling?
MISCELLANEOUS SIGNALING[1,3]			
BCL-2	Translocation	B-cell lymphoma	Apoptosis
CBL	Mu Cas NS-1 V		Tyrosine-phosphorylated RING finger interact Abl
CRK	CT1010 ASV		Adapted SH2/SH3 interact Abl
DPC4	Tumor suppressor	Pancreatic cancer	TGF-β-related signaling pathway
MAS	Transfection and tumorigenicity		Possible angiotensin receptor
NCK			Adaptor SH2/SH3
GUANINE NUCLEOTIDE EXCHANGERS AND BINDING PROTEINS[3,4]			
BCR		Translocated with ABL in CML	Exchanger; protein kinase
DBL	Transfection		Exchanger

TABLE 1 (CONT'D)

GSP			
NF-1	Hereditary tumor suppressor	Tumor suppressor neurofibromatosis	RAS GAP
OST	Transfection		Exchanger
Harvey-Kirsten, N-*RAS*	HaRat SV; Ki RaSV; Balb-MoMuSV; transfection	Point mutations in many human tumors	Signal cascade
VAV	Transfection		S112/S113; exchanger
NUCLEAR PROTEINS AND TRANSCRIPTION FACTORS[1,5-9]			
BRCA1	Heritable suppressor	Mammary cancer/ovarian cancer	Localization unsettled
BRCA2	Heritable suppressor	Mammary cancer	Function unknown
ERBA	Avian erythroblastosis virus		thyroid hormone receptor (transcription)
ETS	Avian E26 virus		DNA binding
EVI1	MuLV promotor insertion	AML	Transcription factor 1
FOS	FBI/FBR murine osteosarcoma viruses		transcription factor with c-JUN
GLI	Amplified glioma	Glioma	Zinc finger; cubitus interruptus homologue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog
HMGG/LIM	Translocation *t*(3:12) *t*(12:15)	Lipoma	Gene fusions high mobility group HMGI-C (XT-hook) and transcription factor LIM or acidic domain
JUN	ASV-17		Transcription factor AP-1 with FOS

Claims:

1. A method for treating a human subject with a hyperproliferative disease comprising the steps of:

   i) identifying a subject with a hyperproliferative disease characterized by alteration or increased expression of a self gene product in at least some of the hyperproliferative cells in said subject; and

   ii) intradermally administering to said subject an expression construct comprising a self gene under the control of a promoter operable in eukaryotic dendritic cells, wherein the dendritic cells are infected by said expression construct,

   whereby said self gene product is expressed by dendritic cells and presented to immune effector cells, thereby stimulating an anti-self gene product response.

2. The method of claim 1, wherein the administration step further comprises:

   i) obtaining a dendritic cell from the subject;

   ii) infecting the dendritic cell *ex vivo* with the expression construct; and

iii) administering said infected dendritic cell to the subject.

3. The method of claim 1, wherein the administration step further comprises:

i) obtaining a cell from said subject;

ii) culturing said cell in the presence of one or more cytokines or growth factors that induce said cell to differentiate into a dendritic cell;

iii) infecting said dendritic cell with an expression construct comprising a self gene under the control of a promoter operable in eukaryotic dendritic cells; and

iv) administering said infected dendritic cell to said subject.

4. The method of claim 3, wherein said obtained cell is a stem cell, a monocyte or an undifferentiated dendritic cell.

5. The method of any of the claims 1 to 3, wherein said self-gene product is an oncogene.

6. The method of claim 5, wherein said oncogene is selected from the group consisting of tumor suppressors, tumor associated genes, growth factors, growth-factor receptors, signal transducers, hormones, cell cycle regulators, nu-

clear factors, transcription factors and apoptic factors.

7. The method of claim 6, wherein said tumor suppressor is selected from the group consisting of Rb, p53, p16, p19, p21, p73, DCC, APC, NF-1, NF-2, PTEN, FHIT, C-CAM, E-cadherin, MEN-I, MEN-II, ZAC1, VHL, FCC, MCC, PMS1, PMS2, MLH-1, MSH-2, DPC4, BRCA1, BRCA2 and WT-1.

8. The method of claim 6, wherein said growth-factor receptor is selected from the group consisting of FMS, ERBB/HER, ERBB-2/NEU/HER-2, ERBA, TGF-ß receptor. PDGF receptor, MET, KIT and TRK.

9. The method of claim 6, wherein said signal transducer is selected from the group consisting of SRC, ABI, RAS, AKT/PKB, RSK-1, RSK-2, RSK-3, RSK-B, PRAD, LCK and ATM.

10. The method of claim 6, wherein said transcription factor or nuclear factor is selected from the group consisting of JUN, FOS, MYC, BRCA1, BRCA2, ERBA, ETS, EVII, MYB, HMGI-C, HMGI/LIM, SKI, VHL, WT1, CEBP-α, NFKB, IKB, GL1 and REL.

11. The method of claim 6, wherein said growth factor is selected from the group consisting of SIS, HAST, INT-1/WT1 and INT-2.

12. The method of claim 6, wherein said apoptic factor is selected from the group consisting of Bax, Bak, Bim, Bik, Bid, Bad, Bcl-2, Harakiri and ICE proteases.

13. The method of claim 6, wherein said tumor associated gene is selected from the group consisting of CEA, mucin, MAGE and GAGE.

14. The method of claim 7, wherein said tumor suppressor product is p53.

15. The method of any of the claims 1 to 3, wherein said expression construct is a viral vector.

16. The method of claim 15, wherein said viral vector is an adenoviral vector, a retroviral vector, a vaccinia viral vector, an adeno-associated viral vector, a polyoma viral vector, an alphavirus vector, or a herpesviral vector.

17. The method of claim 16, wherein said viral vector is an adenoviral vector.

18. The method of claim 17, wherein said adenoviral vector is replication-defective.

19. The method of claim 18, wherein the replication defect is a deletion in the E1 region of the virus.

20. The method of claim 19, wherein the deletion maps to the E1B region of the virus.

21. The method of claim 20, wherein the deletion encompasses the entire E1B region of the virus.

22. The method of claim 21, wherein the deletion encompasses the entire E1 region of the virus.

23. The method of any of the claims 1 to 3, wherein said promotor is selected from the group consisting of CMV IE, human or murine dectin-1, human or murine dectin-2, human CD11c, mammalian F4/80 and human or murine MHC class II.

24. The method of claim 23, wherein said promotor is CMV IE.

25. The method of any claims 1 to 3, wherein said expression vector further comprises a polyadenylation signal.

26. The method of any of the claims 1 to 3, wherein said hyperproliferative disease is cancer.

27. The method of claim 26, wherein said cancer is selected from the group consisting of lung, head, neck, breast, pancreatic, prostate, renal, bone, testicular, cervical, gastrointestinal, lymphoma, brain, colon, skin and bladder.

28. The method of any of the claims 1 to 3, wherein said hyperproliferative disease is selected from the group consisting of RA, IBD, OA, leiomyomas, adenomas, lipomas, hemangiomas, fibromas, melanomas, restenosis, preneoplastic lesions in the lung and psoriasis.

29. The method of any of the claims 1 to 3, wherein said expression construct or cell is administered via injection.

30. The method of claim 29, further comprising multiple injections.

31. The method of claim 29, wherein the injection is performed local to a hyperproliferative or tumor site.

32. The method of claim 29, wherein the injection is performed regional to a hyperproliferative or tumor site.

33. The method of claim 29, wherein the injection is performed distal to a hyperproliferative or tumor site.

34. The method of any of the claims 1 to 3, wherein intradermal administration is via continuous infusion.

35. The method of any of the claims 1 to 3, wherein said immune effector cells are CTLs.

36. The method of any of the claims 1 to 3, further comprising administering to said subject at least a first cytokine.

37. The method of claim 36, further comprising administering to said subject a second cytokine, different from said first cytokine.

38. The method of claim 36, wherein said cytokine is selected from the group consisting of GM-CSF, IL-4, C-KIT, Steel factor, TGF-ß, TNF-α and FLT3 ligand.

39. The method of claim 36, wherein said cytokine is administered as a gene encoded by said expression construct.

1/5

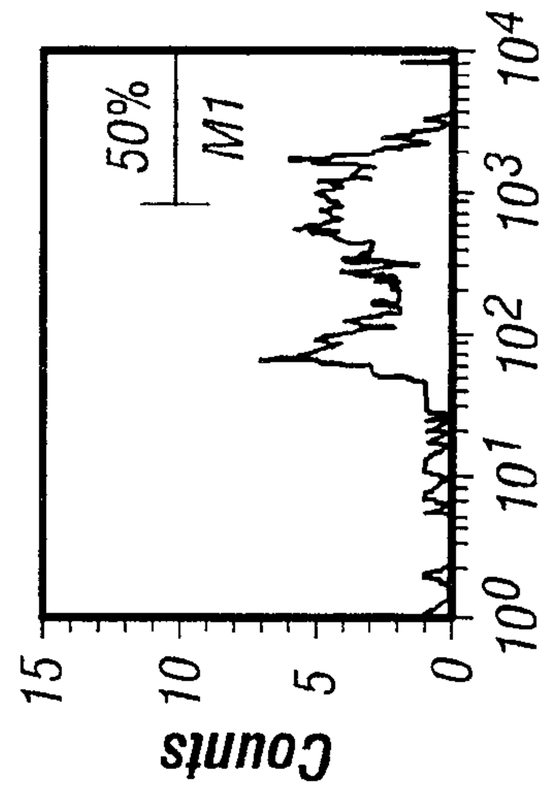


FIG. 1C

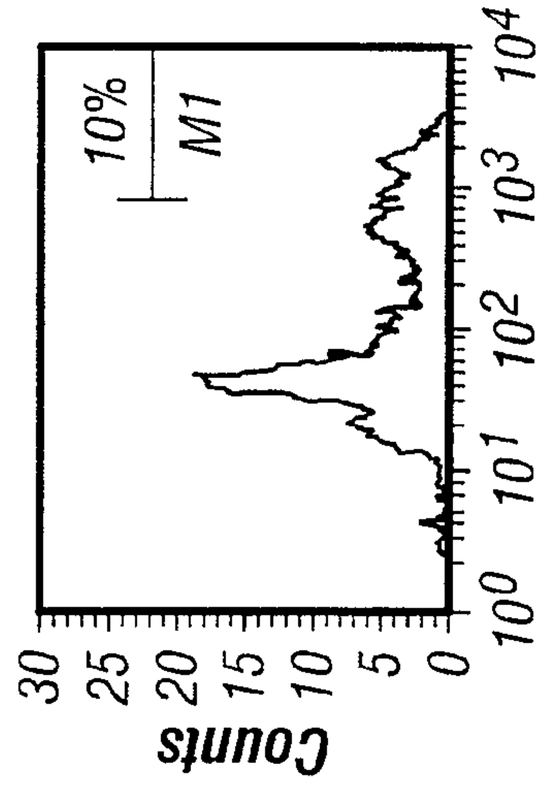


FIG. 1B

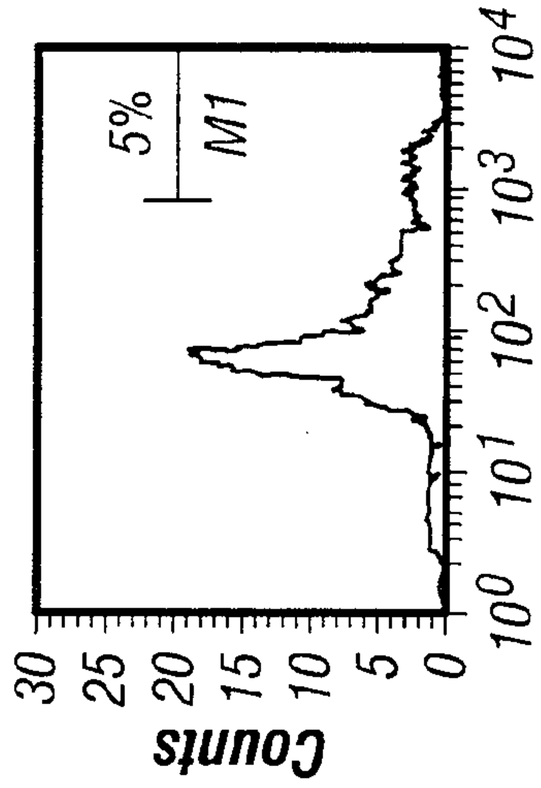
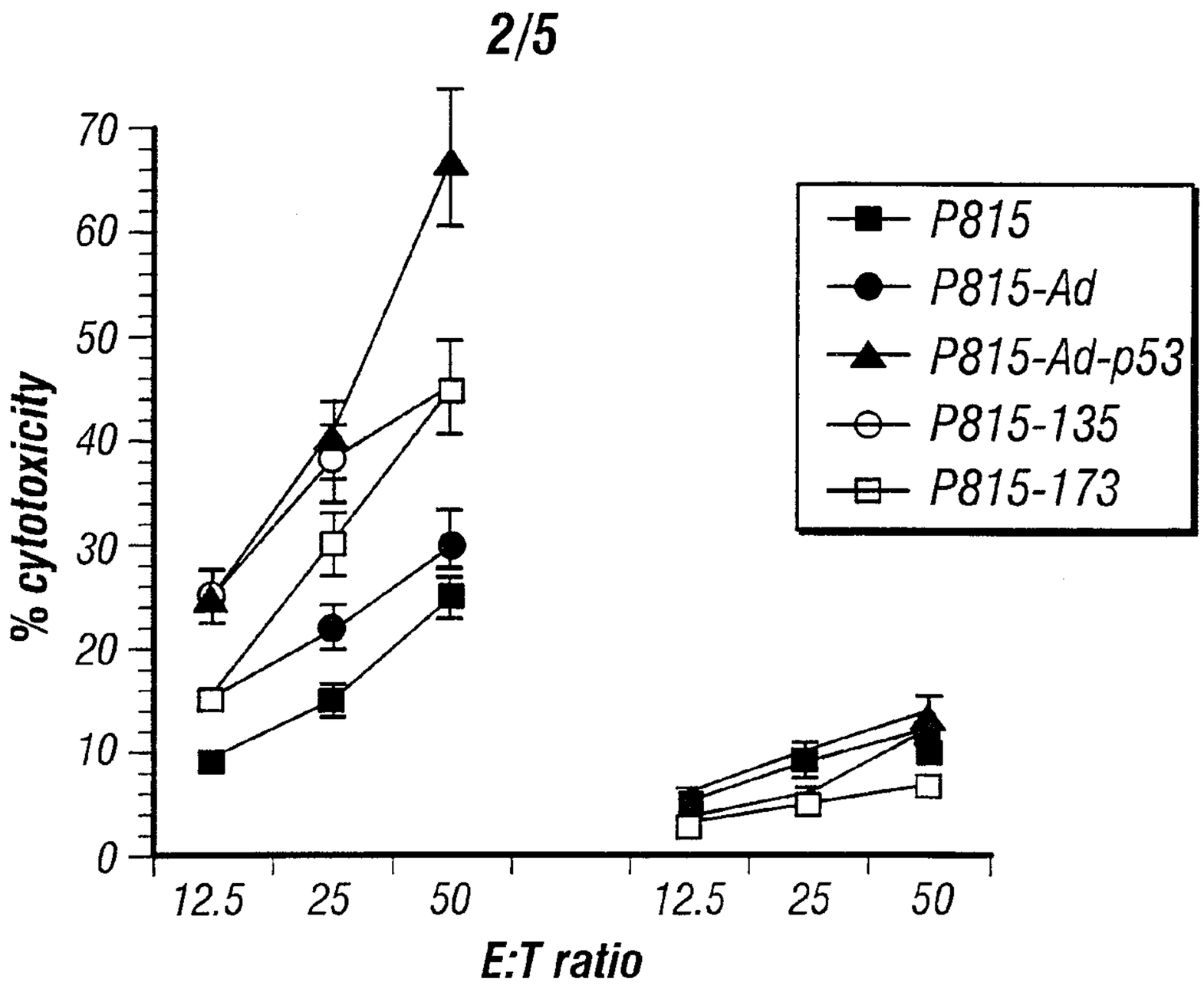


FIG. 1A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60
50
40
30
20
10
0
% cytotoxicity
12.5
25
50
12.5
25
50
E:T ratio
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P815-Ad
P815-Ad-p53
P815-135
P815-173

FIG. 2A

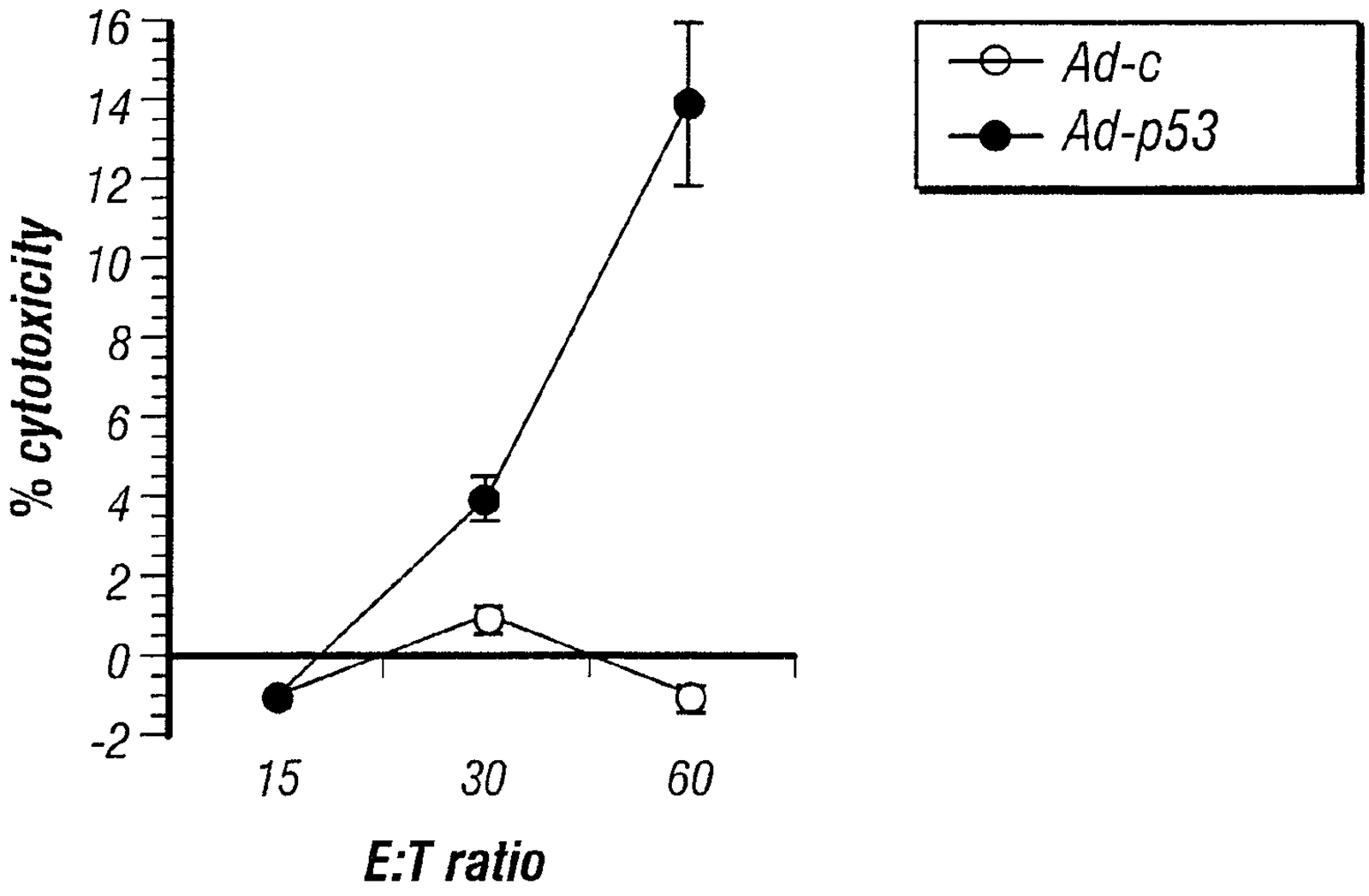
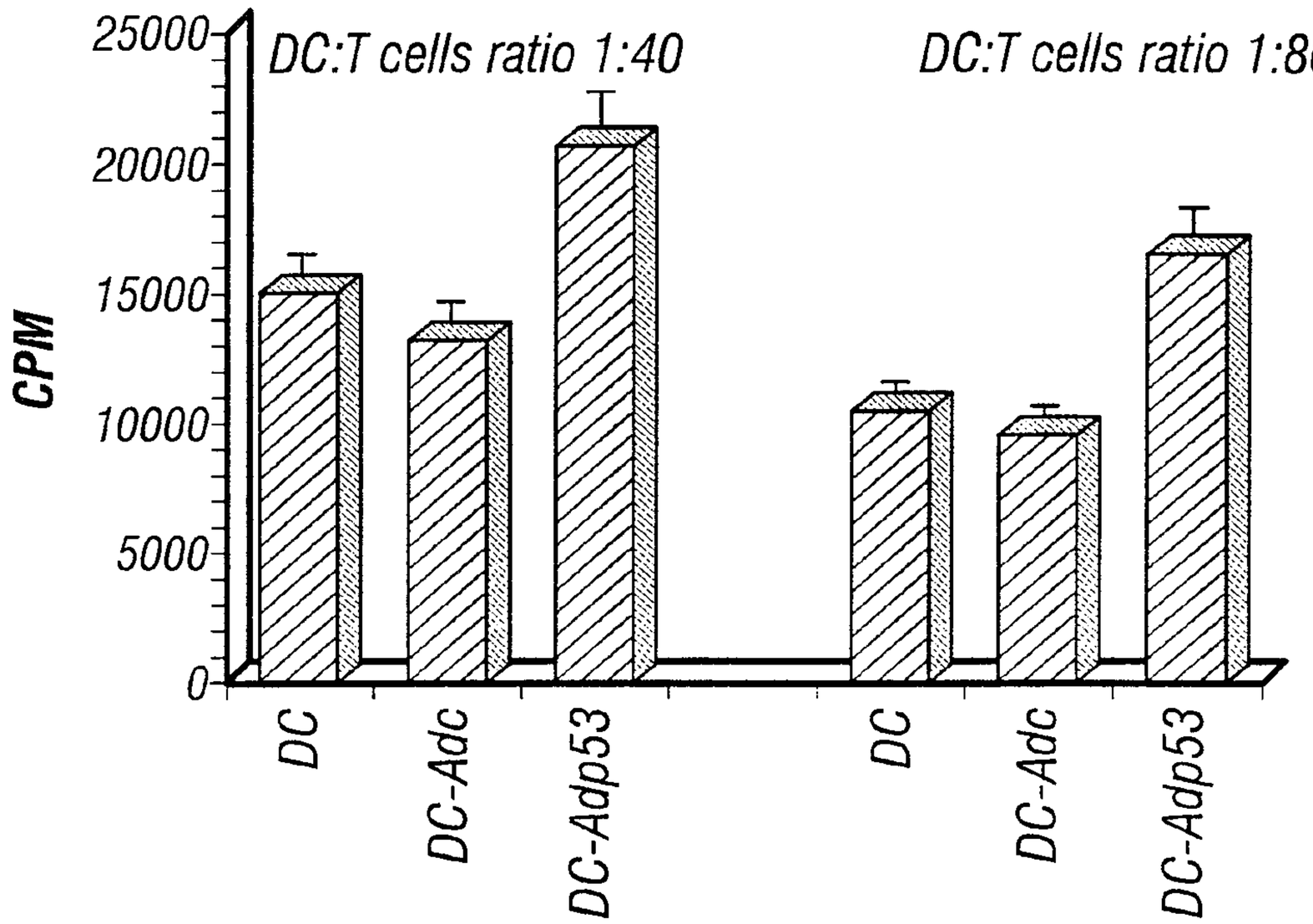

16
14
12
10
8
6
4
2
0
-2
% cytotoxicity
15
30
60
E:T ratio
Ad-c
Ad-p53

FIG. 2B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DC:T cells ratio 1:80
CPM
25000
20000
15000
10000
5000
0
DC
DC-Adc
DC-Adp53
DC
DC-Adc
DC-Adp53

FIG. 2C

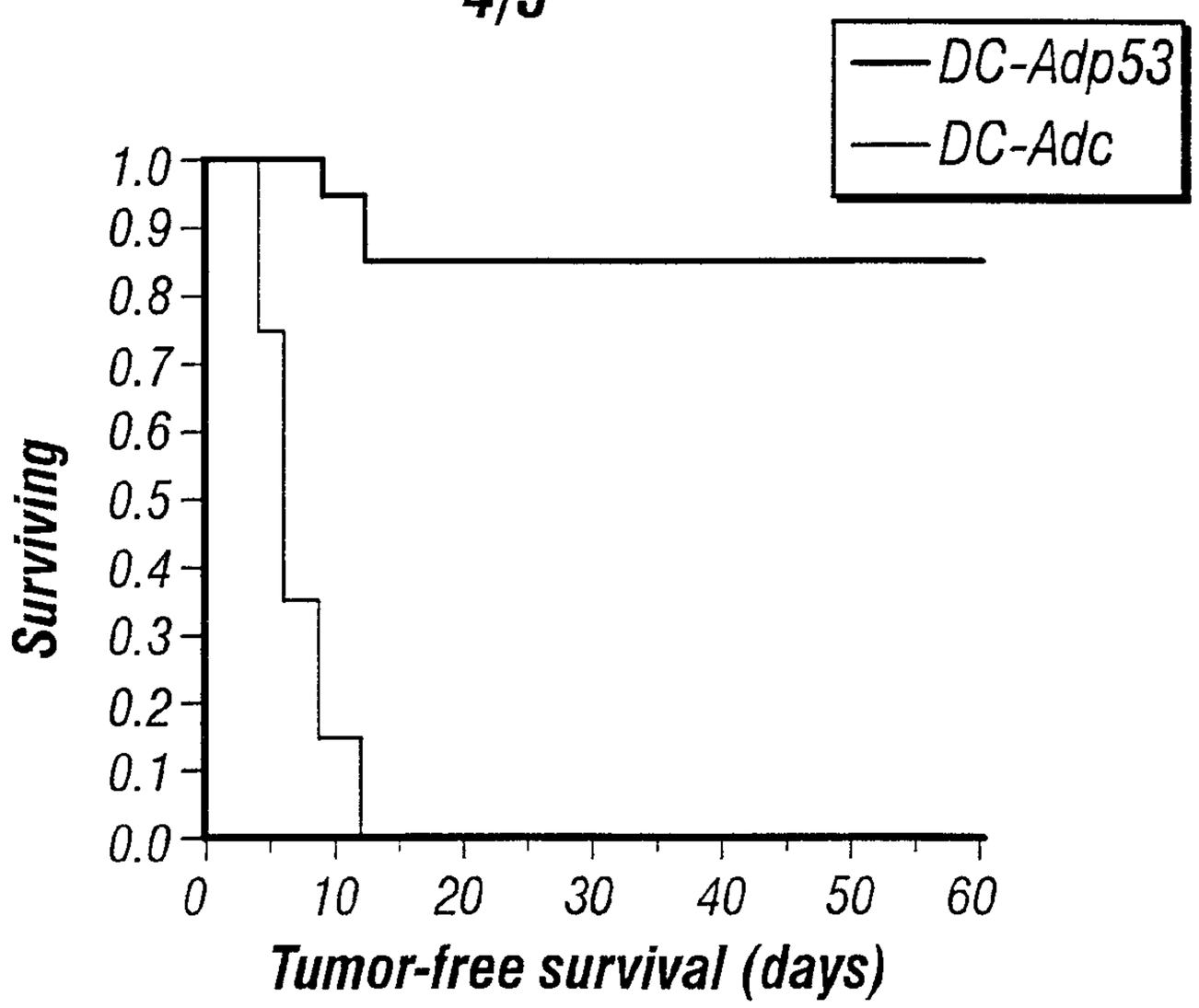
DC-Adp53
DC-Adc
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Surviving
0
10
20
30
40
50
60
Tumor-free survival (days)

FIG. 3A

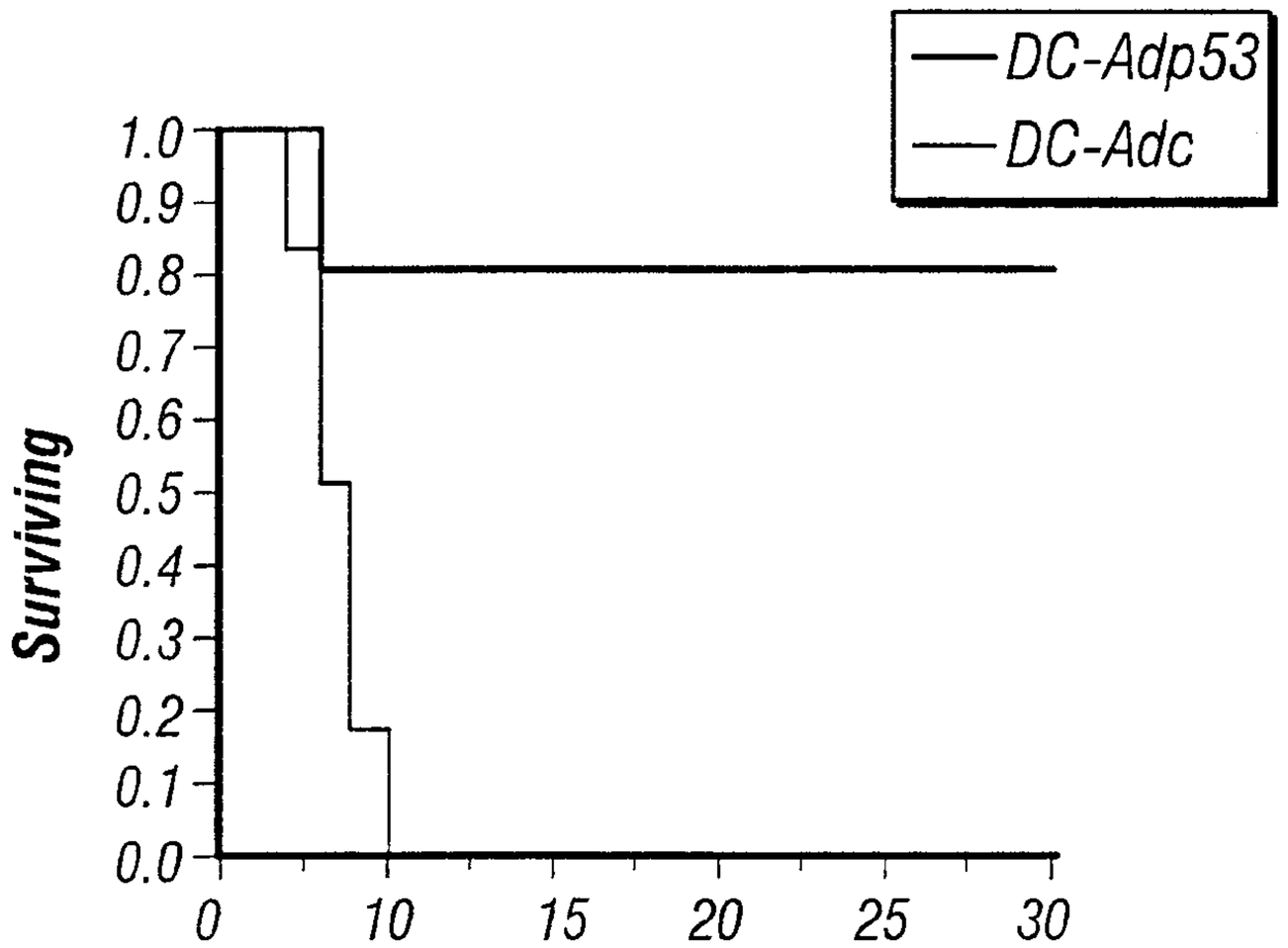
DC-Adp53
DC-Adc
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Surviving
0
10
15
20
25
30
Tumor-free survival (days)

FIG. 3B

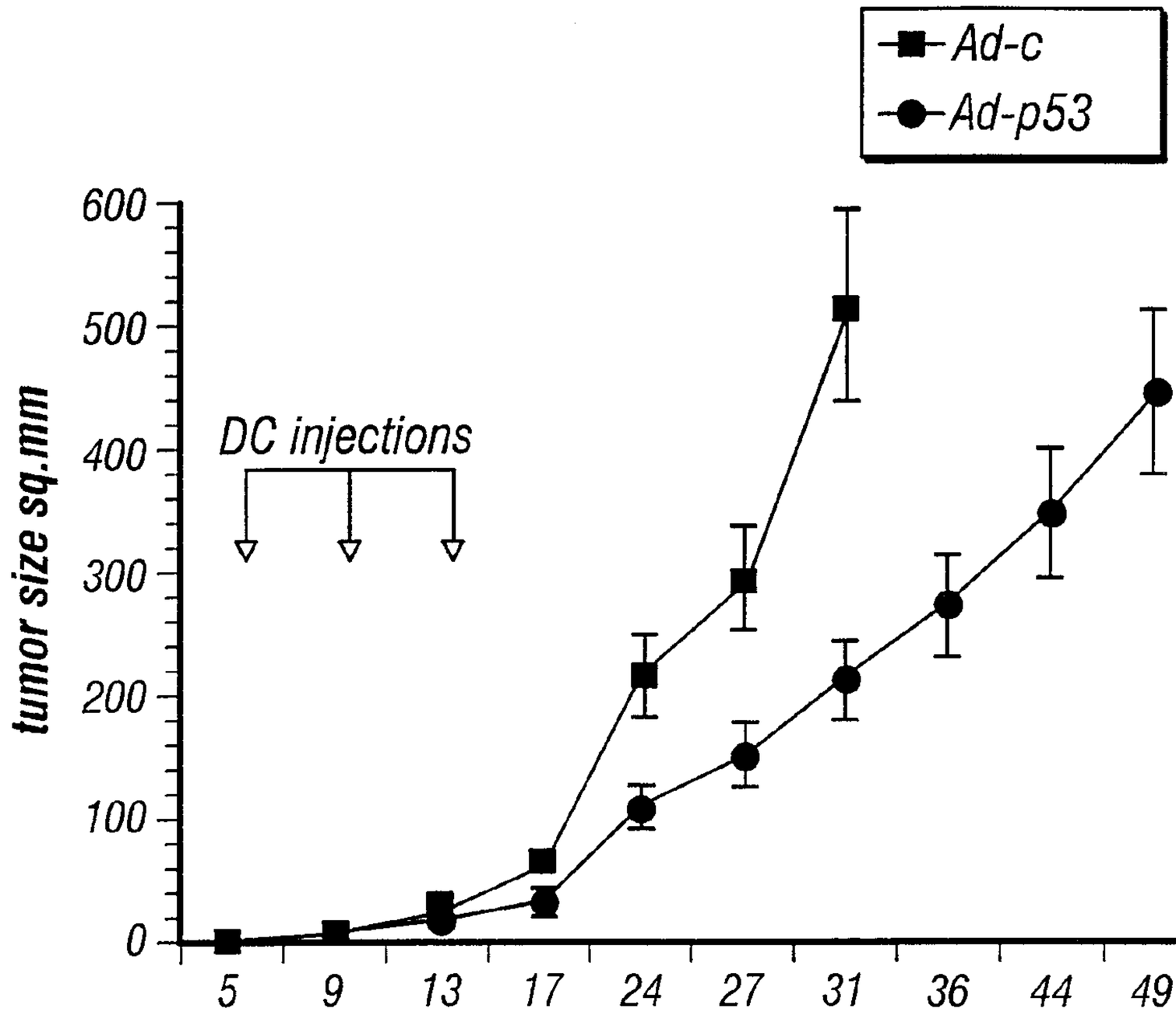
Ad-c
Ad-p53
600
500
400
300
200
100
0
tumor size sq.mm
DC injections
5
9
13
17
24
27
31
36
44
49
Days after tumor injection

FIG. 4