



(86) Date de dépôt PCT/PCT Filing Date: 2005/02/28  
(87) Date publication PCT/PCT Publication Date: 2005/09/09  
(85) Entrée phase nationale/National Entry: 2006/08/22  
(86) N° demande PCT/PCT Application No.: CA 2005/000302  
(87) N° publication PCT/PCT Publication No.: 2005/083064  
(30) Priorité/Priority: 2004/02/26 (US60/548,667)

(51) Cl.Int./Int.Cl. *C12N 5/18* (2006.01),  
*A61K 51/10* (2006.01), *A61K 39/395* (2006.01),  
*A61P 35/00* (2006.01), *C07K 16/18* (2006.01),  
*C07K 16/30* (2006.01), *C12N 5/12* (2006.01)

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(54) Titre : ANTICORPS MODIFIANT DES MALADIES CANCEREUSES  
(54) Title: CANCEROUS DISEASE MODIFYING ANTIBODIES

(57) **Abrégé/Abstract:**

The present invention relates to a method for producing patient cancerous disease modifying antibodies using a novel paradigm of screening. By segregating the anti-cancer antibodies using cancer cell cytotoxicity as an end point, the process makes possible the production of anti-cancer antibodies for therapeutic and diagnostic purposes. The antibodies can be used in aid of staging and diagnosis of a cancer, and can be used to treat primary tumors and tumor metastases. The anti-cancer antibodies can be conjugated to toxins, enzymes, radioactive compounds, and hematogenous cells.



## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
9 September 2005 (09.09.2005)

PCT

(10) International Publication Number  
**WO 2005/083064 A1**

(51) International Patent Classification<sup>7</sup>: C12N 5/18, 5/12,  
C07K 16/18, 16/30, A61K 39/395, 51/10, A61P 35/00

(21) International Application Number:  
PCT/CA2005/000302

(22) International Filing Date: 28 February 2005 (28.02.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/548,667 26 February 2004 (26.02.2004) US

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(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ,  
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA,  
ZM, ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO,  
SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,  
GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a  
patent (Rule 4.17(ii)) for all designations
- as to the applicant's entitlement to claim the priority of the  
earlier application (Rule 4.17(iii)) for all designations

**Published:**

- with international search report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: CANCEROUS DISEASE MODIFYING ANTIBODIES

(57) Abstract: The present invention relates to a method for producing patient cancerous disease modifying antibodies using a novel paradigm of screening. By segregating the anti-cancer antibodies using cancer cell cytotoxicity as an end point, the process makes possible the production of anti-cancer antibodies for therapeutic and diagnostic purposes. The antibodies can be used in aid of staging and diagnosis of a cancer, and can be used to treat primary tumors and tumor metastases. The anti-cancer antibodies can be conjugated to toxins, enzymes, radioactive compounds, and hematogenous cells.

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## CANCEROUS DISEASE MODIFYING ANTIBODIES

### FIELD OF THE INVENTION

This invention relates to the isolation and production of cancerous disease modifying antibodies (CDMAB) and to the use of these CDMAB in therapeutic and diagnostic processes, optionally in combination with one or more chemotherapeutic agents. The invention further relates to binding assays, which utilize the CDMAB of the instant invention.

### BACKGROUND OF THE INVENTION

Each individual who presents with cancer is unique and has a cancer that is as different from other cancers as that person's identity. Despite this, current therapy treats all patients with the same type of cancer, at the same stage, in the same way. At least 30 percent of these patients will fail the first line therapy, thus leading to further rounds of treatment and the increased probability of treatment failure, metastases, and ultimately, death. A superior approach to treatment would be the customization of therapy for the particular individual. The only current therapy, which lends itself to customization, is surgery. Chemotherapy and radiation treatment cannot be tailored to the patient, and surgery by itself, in most cases is inadequate for producing cures.

With the advent of monoclonal antibodies, the possibility of developing methods for customized therapy became more realistic since each antibody can be directed to a single epitope. Furthermore, it is possible to produce a combination of antibodies that are directed to the constellation of epitopes that uniquely define a particular individual's tumor.

Having recognized that a significant difference between cancerous and normal cells is that cancerous cells contain antigens that are specific to transformed cells, the scientific community has long held that monoclonal antibodies can be designed to specifically target transformed cells by binding specifically to these cancer antigens; thus giving rise to the belief that monoclonal antibodies can serve as "Magic Bullets" to eliminate cancer cells.

Monoclonal antibodies isolated in accordance with the teachings of the instantly disclosed invention have been shown to modify the cancerous disease process in a

manner which is beneficial to the patient, for example by reducing the tumor burden, and will variously be referred to herein as cancerous disease modifying antibodies (CDMAB) or “anti-cancer” antibodies.

At the present time, the cancer patient usually has few options of treatment.  
5 The regimented approach to cancer therapy has produced improvements in global survival and morbidity rates. However, to the particular individual, these improved statistics do not necessarily correlate with an improvement in their personal situation.

Thus, if a methodology was put forth which enabled the practitioner to treat each tumor independently of other patients in the same cohort, this would permit the  
10 unique approach of tailoring therapy to just that one person. Such a course of therapy would, ideally, increase the rate of cures, and produce better outcomes, thereby satisfying a long-felt need.

Historically, the use of polyclonal antibodies has been used with limited success in the treatment of human cancers. Lymphomas and leukemias have been treated with  
15 human plasma, but there were few prolonged remissions or responses. Furthermore, there was a lack of reproducibility and no additional benefit compared to chemotherapy. Solid tumors such as breast cancers, melanomas and renal cell carcinomas have also been treated with human blood, chimpanzee serum, human plasma and horse serum with correspondingly unpredictable and ineffective results.

20 There have been many clinical trials of monoclonal antibodies for solid tumors. In the 1980s there were at least 4 clinical trials for human breast cancer which produced only 1 responder from at least 47 patients using antibodies against specific antigens or based on tissue selectivity. It was not until 1998 that there was a successful clinical trial using a humanized anti-Her2 antibody in combination with Cisplatin. In this trial  
25 37 patients were accessed for responses of which about a quarter had a partial response rate and another half had minor or stable disease progression.

The clinical trials investigating colorectal cancer involve antibodies against both glycoprotein and glycolipid targets. Antibodies such as 17-1A, which has some specificity for adenocarcinomas, has undergone Phase 2 clinical trials in over 60  
30 patients with only 1 patient having a partial response. In other trials, use of 17-1A produced only 1 complete response and 2 minor responses among 52 patients in protocols using additional cyclophosphamide. Other trials involving 17-1A yielded



results that were similar. The use of a humanized murine monoclonal antibody initially approved for imaging also did not produce tumor regression. To date there has not been an antibody that has been effective for colorectal cancer. Likewise there have been equally poor results for lung cancer, brain cancers, ovarian cancers, pancreatic cancer, prostate cancer, and stomach cancer. There has been some limited success in the use of anti-GD3 monoclonal antibodies for melanoma. Thus, it can be seen that despite successful small animal studies that are a prerequisite for human clinical trials, the antibodies that have been tested thus far have been, for the most part, ineffective.

Prior Patents:

10 U.S. Patent No. 5,750,102 discloses a process wherein cells from a patient's tumor are transfected with MHC genes, which may be cloned from cells or tissue from the patient. These transfected cells are then used to vaccinate the patient.

15 U.S. Patent No. 4,861,581 discloses a process comprising the steps of obtaining monoclonal antibodies that are specific to an internal cellular component of neoplastic and normal cells of the mammal but not to external components, labeling the monoclonal antibody, contacting the labeled antibody with tissue of a mammal that has received therapy to kill neoplastic cells, and determining the effectiveness of therapy by measuring the binding of the labeled antibody to the internal cellular component of the degenerating neoplastic cells. In preparing antibodies directed to human intracellular antigens, the patentee recognizes that malignant cells represent a convenient source of such antigens.

20 U.S. Patent No. 5,171,665 provides a novel antibody and method for its production. Specifically, the patent teaches formation of a monoclonal antibody which has the property of binding strongly to a protein antigen associated with human tumors, e.g. those of the colon and lung, while binding to normal cells to a much lesser degree.

25 U.S. Patent No. 5,484,596 provides a method of cancer therapy comprising surgically removing tumor tissue from a human cancer patient, treating the tumor tissue to obtain tumor cells, irradiating the tumor cells to be viable but non-tumorigenic, and using these cells to prepare a vaccine for the patient capable of inhibiting recurrence of the primary tumor while simultaneously inhibiting metastases. The patent teaches the development of monoclonal antibodies, which are reactive with surface antigens of

30

tumor cells. As set forth at col. 4, lines 45 et seq., the patentees utilize autochthonous tumor cells in the development of monoclonal antibodies expressing active specific immunotherapy in human neoplasia.

U.S. Patent No. 5,693,763 teaches a glycoprotein antigen characteristic of human carcinomas is not dependent upon the epithelial tissue of origin.

U.S. Patent No. 5,783,186 is drawn to anti-Her2 antibodies, which induce apoptosis in Her2 expressing cells, hybridoma cell lines producing the antibodies, methods of treating cancer using the antibodies and pharmaceutical compositions including said antibodies.

U.S. Patent No. 5,849,876 describes new hybridoma cell lines for the production of monoclonal antibodies to mucin antigens purified from tumor and non-tumor tissue sources.

U.S. Patent No. 5,869,268 is drawn to a method for generating a human lymphocyte producing an antibody specific to a desired antigen, a method for producing a monoclonal antibody, as well as monoclonal antibodies produced by the method. The patent is particularly drawn to the production of an anti-HD human monoclonal antibody useful for the diagnosis and treatment of cancers.

U.S. Patent No. 5,869,045 relates to antibodies, antibody fragments, antibody conjugates and single chain immunotoxins reactive with human carcinoma cells. The mechanism by which these antibodies function is two-fold, in that the molecules are reactive with cell membrane antigens present on the surface of human carcinomas, and further in that the antibodies have the ability to internalize within the carcinoma cells, subsequent to binding, making them especially useful for forming antibody-drug and antibody-toxin conjugates. In their unmodified form the antibodies also manifest cytotoxic properties at specific concentrations.

U.S. Patent No. 5,780,033 discloses the use of autoantibodies for tumor therapy and prophylaxis. However, this antibody is an anti-nuclear autoantibody from an aged mammal. In this case, the autoantibody is said to be one type of natural antibody found in the immune system. Because the autoantibody comes from "an aged mammal", there is no requirement that the autoantibody actually comes from the patient being treated. In addition the patent discloses natural and monoclonal anti-nuclear autoantibody from



an aged mammal, and a hybridoma cell line producing a monoclonal anti-nuclear autoantibody.

### SUMMARY OF THE INVENTION

The instant inventors have previously been awarded U.S. Patent 6,180,357,  
5 entitled "Individualized Patient Specific Anti-Cancer Antibodies" directed to a process for selecting individually customized anti-cancer antibodies, which are useful in treating a cancerous disease.

This application utilizes, in part, the method for producing patient specific anti-cancer antibodies as taught in the '357 patent for isolating hybridoma cell lines which  
10 encode for cancerous disease modifying monoclonal antibodies. These antibodies can be made specifically for one tumor and thus make possible the customization of cancer therapy. Within the context of this application, anti-cancer antibodies having either cell killing (cytotoxic) or cell-growth inhibiting (cytostatic) properties will hereafter be referred to as cytotoxic. These antibodies can be used in aid of staging and diagnosis of  
15 a cancer, and can be used to treat tumor metastases.

The prospect of individualized anti-cancer treatment will bring about a change in the way a patient is managed. A likely clinical scenario is that a tumor sample is obtained at the time of presentation, and banked. From this sample, the tumor can be  
20 typed from a panel of pre-existing cancerous disease modifying antibodies. The patient will be conventionally staged but the available antibodies can be of use in further staging the patient. The patient can be treated immediately with the existing antibodies, and a panel of antibodies specific to the tumor can be produced either using the methods outlined herein or through the use of phage display libraries in conjunction with the screening methods herein disclosed. All the antibodies generated will be  
25 added to the library of anti-cancer antibodies since there is a possibility that other tumors can bear some of the same epitopes as the one that is being treated. The antibodies produced according to this method may be useful to treat cancerous disease in any number of patients who have cancers that bind to these antibodies.

In addition to anti-cancer antibodies, the patient can elect to receive the  
30 currently recommended therapies as part of a multi-modal regimen of treatment. The fact that the antibodies isolated via the present methodology are relatively non-toxic to

non-cancerous cells allows for combinations of antibodies at high doses to be used, either alone, or in conjunction with conventional therapy. The high therapeutic index will also permit re-treatment on a short time scale that should decrease the likelihood of emergence of treatment resistant cells.

5 Furthermore, it is within the purview of this invention to conjugate standard chemotherapeutic modalities, e.g. radionuclides, with the CDMAB of the instant invention, thereby focusing the use of said chemotherapeutics.

If the patient is refractory to the initial course of therapy or metastases develop, the process of generating specific antibodies to the tumor can be repeated for re-  
10 treatment. Furthermore, the anti-cancer antibodies can be conjugated to red blood cells obtained from that patient and re-infused for treatment of metastases. There have been few effective treatments for metastatic cancer and metastases usually portend a poor outcome resulting in death. However, metastatic cancers are usually well vascularized and the delivery of anti-cancer antibodies by red blood cells can have the effect of  
15 concentrating the antibodies at the site of the tumor. Even prior to metastases, most cancer cells are dependent on the host's blood supply for their survival and anti-cancer antibodies conjugated to red blood cells can be effective against *in situ* tumors as well. Alternatively, the antibodies may be conjugated to other hematogenous cells, e.g. lymphocytes, macrophages, monocytes, natural killer cells, etc.

20 There are five classes of antibodies and each is associated with a function that is conferred by its heavy chain. It is generally thought that cancer cell killing by naked antibodies are mediated either through antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). For example murine IgM and IgG2a antibodies can activate human complement by binding the C-1 component of the  
25 complement system thereby activating the classical pathway of complement activation, which can lead to tumor lysis. For human antibodies the most effective complement-activating antibodies are generally IgM and IgG1. Murine antibodies of the IgG2a and IgG3 isotype are effective at recruiting cytotoxic cells that have Fc receptors which will lead to cell killing by monocytes, macrophages, granulocytes and certain lymphocytes.  
30 Human antibodies of both the IgG1 and IgG3 isotype mediate ADCC.

Another possible mechanism of antibody-mediated cancer killing may be through the use of antibodies that function to catalyze the hydrolysis of various



chemical bonds in the cell membrane and its associated glycoproteins or glycolipids, so-called catalytic antibodies.

There are two additional mechanisms of antibody-mediated cancer cell killing which are more widely accepted. The first is the use of antibodies as a vaccine to  
5 induce the body to produce an immune response against the putative cancer antigen that resides on the tumor cell. The second is the use of antibodies to target growth receptors and interfere with their function or to down regulate that receptor so that its function is effectively lost.

The clinical utility of a cancer drug is based on the benefit of the drug under an  
10 acceptable risk profile to the patient. In cancer therapy survival has generally been the most sought after benefit, however there are a number of other well-recognized benefits in addition to prolonging life. These other benefits, where treatment does not adversely affect survival, include symptom palliation, protection against adverse events, prolongation in time to recurrence or disease-free survival, and prolongation in time to  
15 progression. These criteria are generally accepted and regulatory bodies such as the U.S. Food and Drug Administration (F.D.A.) approve drugs that produce these benefits (Hirschfeld et al. *Critical Reviews in Oncology/Hematology* 42:137-143 2002). In addition to these criteria it is well recognized that there are other endpoints that may presage these types of benefits. In part, the accelerated approval process granted by the  
20 U.S. F.D.A. acknowledges that there are surrogates that will likely predict patient benefit. As of year-end (2003), there have been sixteen drugs approved under this process, and of these, four have gone on to full approval, i.e., follow-up studies have demonstrated direct patient benefit as predicted by surrogate endpoints. One important endpoint for determining drug effects in solid tumors is the assessment of tumor burden  
25 by measuring response to treatment (Therasse et al. *Journal of the National Cancer Institute* 92(3):205-216 2000). The clinical criteria (RECIST criteria) for such evaluation have been promulgated by Response Evaluation Criteria in Solid Tumors Working Group, a group of international experts in cancer. Drugs with a demonstrated effect on tumor burden, as shown by objective responses according to RECIST criteria,  
30 in comparison to the appropriate control group tend to, ultimately, produce direct patient benefit. In the pre-clinical setting tumor burden is generally more straightforward to assess and document. In that pre-clinical studies can be translated to

the clinical setting, drugs that produce prolonged survival in pre-clinical models have the greatest anticipated clinical utility. Analogous to producing positive responses to clinical treatment, drugs that reduce tumor burden in the pre-clinical setting may also have significant direct impact on the disease. Although prolongation of survival is the most sought after clinical outcome from cancer drug treatment, there are other benefits that have clinical utility and it is clear that tumor burden reduction can also lead to direct benefits and have clinical impact (Eckhardt et al. Developmental Therapeutics: Successes and Failures of Clinical Trial Designs of Targeted Compounds; ASCO Educational Book, 39<sup>th</sup> Annual Meeting, 2003, pages 209-219).

Accordingly, it is an objective of the invention to utilize a method for producing CDMAB from cells derived from a particular individual which are cytotoxic with respect to cancer cells while simultaneously being relatively non-toxic to non-cancerous cells, in order to isolate hybridoma cell lines and the corresponding isolated monoclonal antibodies and antigen binding fragments thereof for which said hybridoma cell lines are encoded.

It is an additional objective of the invention to teach CDMAB and antigen binding fragments thereof.

It is a further objective of the instant invention to produce CDMAB whose cytotoxicity is mediated through antibody dependent cellular toxicity.

It is yet an additional objective of the instant invention to produce CDMAB whose cytotoxicity is mediated through complement dependent cellular toxicity.

It is still a further objective of the instant invention to produce CDMAB whose cytotoxicity is a function of their ability to catalyze hydrolysis of cellular chemical bonds.

A still further objective of the instant invention is to produce CDMAB, which are useful in a binding assay for diagnosis, prognosis, and monitoring of cancer.

Other objects and advantages of this invention will become apparent from the following description wherein, by way of illustration and example, certain embodiments of this invention are set forth.



## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 includes representative FACS histograms of AR36A36.11.1 and anti-EGFR antibodies directed against several cancer and non-cancer cell lines.

Figure 2 compares the percentage cytotoxicity of the hybridoma supernatants against cell lines PC-3, LnCap and CCD-27sk versus binding levels;

Figure 3 is a comparison of cytotoxicity of AR36A36.11.1 versus positive and negative controls;

Figure 4 represents binding of AR36A36.11.1 versus anti-EGFR control and tabulated the mean fluorescence intensity fold increase above isotype control. Results are presented qualitatively as: between 1.5 to 5 (+); 5 to 25 (++); 25 to 50 (+++); and above 50 (++++);

Figure 5 demonstrates the effect of AR36A36.11.1 on tumor growth in a MB-231 breast cancer model. The vertical lines indicate the period during which the antibody was administered. Data points represent the mean +/- SEM;

Figure 6 demonstrates the effect of AR36A36.11.1 on body weight in a MB-231 breast cancer model. Data points represent the mean +/- SEM;

Figure 7 demonstrates the effect of AR36A36.11.1 on tumor growth in an established MB-231 breast cancer model. The vertical lines indicate the period during which the antibody was administered. Data points represent the mean +/- SEM;

Figure 8 demonstrates the effect of AR36A36.11.1 on body weight in an established MB-231 breast cancer model. Data points represent the mean +/- SEM;

Figure 9 demonstrates the effect of AR36A36.11.1 on tumor growth in a SW1116 colon cancer model. The vertical lines indicate the period during which the antibody was administered. Data points represent the mean +/- SEM;

Figure 10 demonstrates the effect of AR36A36.11.1 on body weight in a SW1116 colon cancer model. Data points represent the mean +/- SEM;

Figure 11 demonstrates the effect of AR36A36.11.1 on tumor growth in a PC-3 colon cancer model. The vertical lines indicate the period during which the antibody was administered. Data points represent the mean +/- SEM;

Figure 12 demonstrates the effect of AR36A36.11.1 on body weight in a PC-3 prostate cancer model. Data points represent the mean +/- SEM.

## DETAILED DESCRIPTION OF THE INVENTION

EXAMPLE 1Hybridoma Production – Hybridoma Cell Line AR36A36.11.1

The hybridoma cell line AR36A36.11.1 was deposited, in accordance with the  
5 Budapest Treaty, with the International Depository Authority of Canada (IDAC),  
Bureau of Microbiology, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba,  
Canada, R3E 3R2, on January 28, 2004, under Accession Number 280104-02. In  
accordance with 37 CFR 1.808, the depositors assure that all restrictions imposed on  
the availability to the public of the deposited materials will be irrevocably removed  
10 upon the granting of a patent.

To produce the hybridoma that produces the anti-cancer antibody  
AR36A36.11.1, a fresh single cell suspension of the PC-3 prostate cancer cell line that  
had been grown as a solid tumor in SCID mice, was prepared in PBS.  
IMMUNEASY™ (Qiagen, Venlo, Netherlands) adjuvant was prepared for use by  
15 gentle mixing. Five to seven week old BALB/c mice were immunized by injecting  
subcutaneously, 2 million cells in 50 microliters of the antigen-adjuvant. Recently  
prepared antigen-adjuvant was used to boost the immunized mice intraperitoneally, 2  
and 5 weeks after the initial immunization, with 2 million cells in 50 microliters. A  
spleen was used for fusion three days after the last immunization. The hybridomas were  
20 prepared by fusing the isolated splenocytes with NSO-1 myeloma partners. The  
supernatants from the fusions were tested for subcloning of the hybridomas.

To determine whether the antibodies secreted by the hybridoma cells are of the  
IgG or IgM isotype, an ELISA assay was employed. 100 microliters/well of goat anti-  
mouse IgG + IgM (H+L) at a concentration of 2.4 micrograms/mL in coating buffer  
25 (0.1M carbonate/bicarbonate buffer, pH 9.2-9.6) at 4°C was added to the ELISA plates  
overnight. The plates were washed thrice in washing buffer (PBS + 0.05% Tween).  
100 microliters/well blocking buffer (5% milk in wash buffer) was added to the plate  
for 1 hr. at room temperature and then washed thrice in washing buffer. 100  
microliters/well of hybridoma supernatant was added and the plate incubated for 1 hr.  
30 at room temperature. The plates were washed thrice with washing buffer and 1/100,000  
dilution of either goat anti-mouse IgG or IgM horseradish peroxidase conjugate (diluted  
in PBS containing 5% milk), 100 microliters/well, was added. After incubating the



plate for 1 hr. at room temperature the plate was washed thrice with washing buffer. 100 microliters/well of TMB solution was incubated for 1-3 minutes at room temperature. The color reaction was terminated by adding 100 microliters/well 2M H<sub>2</sub>SO<sub>4</sub> and the plate was read at 450 nm with a Perkin-Elmer HTS7000 plate reader. As indicated in Figure 2, the AR36A36.11.1 hybridoma secreted primarily antibodies of the IgG isotype.

After one round of limiting dilution hybridoma supernatants were tested for antibodies that bound to target cells in a cell ELISA assay. Two human prostate cancer cell lines and 1 human normal skin cell line were tested: PC-3, LnCap and CCD-27sk respectively. The plated cells were fixed prior to use. The plates were washed thrice with PBS containing MgCl<sub>2</sub> and CaCl<sub>2</sub> at room temperature. 100 microliters of 2% paraformaldehyde diluted in PBS was added to each well for 10 minutes at room temperature and then discarded. The plates were again washed with PBS containing MgCl<sub>2</sub> and CaCl<sub>2</sub> three times at room temperature. Blocking was done with 100 microliters/well of 5% milk in wash buffer (PBS + 0.05% Tween) for 1 hr at room temperature. The plates were washed thrice with wash buffer and the hybridoma supernatant was added at 100 microliters/well for 1 hr at room temperature. The plates were washed 3 times with wash buffer and 100 microliters/well of 1/25,000 dilution of goat anti-mouse IgG or IgM antibody conjugated to horseradish peroxidase (diluted in PBS containing 5% milk) was added. After 1 hr incubation at room temperature the plates were washed 3 times with wash buffer and 100 microliter/well of TMB substrate was incubated for 1-3 minutes at room temperature. The reaction was terminated with 100 microliters/well 2M H<sub>2</sub>SO<sub>4</sub> and the plate read at 450 nm with a Perkin-Elmer HTS7000 plate reader. The results as tabulated in Figure 2 were expressed as the number of folds above background compared to an in-house IgG isotype control that has previously been shown not to bind to the cell lines tested. The antibodies from the hybridoma AR36A36.11.1 showed binding to the prostate cancer cell line PC-3 and weaker binding to another prostate cancer cell line LnCap. AR36A36.11.1 also showed detectable binding to the normal skin cell line.

In conjunction with testing for antibody binding the cytotoxic effect of the hybridoma supernatants were tested in the same cell lines: PC-3, LnCap and CCD-27sk. The Live/Dead cytotoxicity assay was obtained from Molecular Probes (Eu,

OR). The assays were performed according to the manufacturer's instructions with the changes outlined below. Cells were plated before the assay at the predetermined appropriate density. After 2 days, 100 µl of supernatant from the hybridoma microtitre plates were transferred to the cell plates and incubated in a 5 percent CO<sub>2</sub> incubator for 5 days. The wells that served as the positive controls were aspirated until empty and 100 µl of sodium azide (NaN<sub>3</sub>) or cycloheximide was added. After 5 days of treatment, the plates were then emptied by inverting and blotting dry. Room temperature DPBS (Dulbecco's phosphate buffered saline) containing MgCl<sub>2</sub> and CaCl<sub>2</sub> was dispensed into each well from a multichannel squeeze bottle, tapped 3 times, emptied by inversion and then blotted dry. 50 µl of the fluorescent calcein dye diluted in DPBS containing MgCl<sub>2</sub> and CaCl<sub>2</sub> was added to each well and incubated at 37°C in a 5% CO<sub>2</sub> incubator for 30 minutes. The plates were read in a Perkin-Elmer HTS7000 fluorescence plate reader and the data was analyzed in Microsoft Excel. The results were tabulated in Figure 2. The AR36A36.11.1 hybridoma produced specific cytotoxicity of 17 percent in PC-3 cells, which was 46 and 25 percent of the cytotoxicity obtained with the positive controls sodium azide and cycloheximide respectively. The AR36A36.11.1 hybridoma also produced specific cytotoxicity of 25 percent in LnCap cells, which was 36 and 69 percent of the cytotoxicity obtained with the positive controls sodium azide and cycloheximide respectively. As tabulated in Figure 2, despite binding to CCD-27sk, AR36A36.11.1 did not produce significant cytotoxicity in this normal cell line. The known non-specific cytotoxic agents cycloheximide and NaN<sub>3</sub> generally produced cytotoxicity as expected.

## EXAMPLE 2

### Antibody Production:

AR36A36.11.1 monoclonal antibody was produced by culturing the hybridoma in CL-1000 flasks (BD Biosciences, Oakville, ON) with collections and reseeded occurring twice/week. Standard antibody purification procedures with Protein G Sepharose 4 Fast Flow (Amersham Biosciences, Baie d'Urfé, QC) were followed. It is within the scope of this invention to utilize monoclonal antibodies that are humanized, chimerized or murine.



AR36A36.11.1 was compared to a number of both positive (anti-EGFR (C225, IgG1, kappa, 5 µg/mL, Cedarlane, Hornby, ON), cycloheximide (CHX, 0.5 µM, Sigma, Oakville, ON), and NaN<sub>3</sub> (0.1%, Sigma, Oakville, ON)) and negative (G155-178 (anti-TNP, IgG2a, kappa, 20 µg/mL, BD Biosciences, Oakville, ON), and IgG Buffer (3%)) controls in a cytotoxicity assay (Figure 3). Breast (MDA-MB-231 (MB-231), NCI-MCF-7 (MCF-7)), colon (DLD-1, Lovo, SW1116), ovarian (OVCAR-3), pancreatic (BxPC-3), and prostate (PC-3, LnCap, DU-145) cancer, and non-cancer skin (CCD-27sk), and lung (Hs888.Lu) cell lines were tested (all from the ATCC, Manassas, VA). The Live/Dead cytotoxicity assay was obtained from Molecular Probes (Eugene, OR). The assays were performed according to the manufacturer's instructions with the changes outlined below. Cells were plated before the assay at the predetermined appropriate density. After 2 days, 100 µl of purified antibody or controls were diluted into media, and then transferred to the cell plates and incubated in a 5 percent CO<sub>2</sub> incubator for 5 days. The plates were then emptied by inverting and blotted dry. Room temperature DPBS containing MgCl<sub>2</sub> and CaCl<sub>2</sub> was dispensed into each well from a multichannel squeeze bottle, tapped 3 times, emptied by inversion and then blotted dry. 50 µl of the fluorescent calcein dye diluted in DPBS containing MgCl<sub>2</sub> and CaCl<sub>2</sub> was added to each well and incubated at 37°C in a 5 percent CO<sub>2</sub> incubator for 30 minutes. The plates were read in a Perkin-Elmer HTS7000 fluorescence plate reader and the data was analyzed in Microsoft Excel and the results were tabulated in Figure 3. The data was represented as an average of four experiments tested in triplicate and is presented qualitatively in the following fashion: 3/4 to 4/4 experiments with >15% cytotoxicity above background (++++), 2/4 experiments with >15% cytotoxicity above background (+++), at least 2/4 experiments with 10-15% cytotoxicity above background (++) , at least 2/4 experiments with 8-10% cytotoxicity above background (+), 7% cytotoxicity above background (+/-). Unmarked cells in Figure 3 represented inconsistent or effects less than the threshold cytotoxicity. The AR36A36.11.1 antibody produced cytotoxicity in the LnCap prostate cancer cell line relative to both isotype and buffer negative controls; cytotoxicity on LnCap cells was above that observed with the well-characterized anti-EGFR antibody. Importantly, AR36A36.11.1 did not produce cytotoxicity against non-cancer cell lines such as CCD-27sk or Hs888.Lu, indicating

that the antibody was specific for cancer cells. It should be noted that the anti-EGFR antibody produced cytotoxicity in CCD-27sk cells since this epidermal cell line would be expected to express epidermal growth factor receptors. The chemical cytotoxic agents induced their expected non-specific cytotoxicity.

5 Binding of AR36A36.11.1 to the above-mentioned panel of cancer and normal cell lines was assessed by flow cytometry (FACS). Cells were prepared for FACS by initially washing the cell monolayer with DPBS (without  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ). Cell dissociation buffer (INVITROGEN, Burlington, ON) was then used to dislodge the cells from their cell culture plates at 37°C. After centrifugation and collection, the cells  
10 were resuspended in DPBS containing  $\text{MgCl}_2$ ,  $\text{CaCl}_2$  and 2 percent fetal bovine serum at 4°C (staining media) and counted, aliquoted to appropriate cell density, spun down to pellet the cells and resuspended in staining media at 4°C in the presence of test antibodies (AR36A36.11.1) or control antibodies (isotype control, anti-EGFR) at 20  $\mu\text{g}/\text{mL}$  on ice for 30 minutes. Prior to the addition of Alexa Fluor 488-conjugated  
15 secondary antibody the cells were washed once with staining media. The Alexa Fluor 488-conjugated antibody in staining media was then added for 30 minutes. The cells were then washed for the final time and resuspended in fixing media (staining media containing 1.5% paraformaldehyde). Flow cytometric acquisition of the cells was assessed by running samples on a FACScan using the CellQuest software (BD  
20 Biosciences, Oakville, ON). The forward (FSC) and side scatter (SSC) of the cells were set by adjusting the voltage and amplitude gains on the FSC and SSC detectors. The detectors for the fluorescence (FITC) channel was adjusted by running cells stained only with Alexa Fluor 488-conjugated secondary antibody such that cells had a uniform peak with a median fluorescent intensity of approximately 1-5 units. For each sample,  
25 approximately 10,000 stained fixed cells were acquired for analysis and the results are presented in Figure 4.

Figure 4 tabulated the mean fluorescence intensity fold increase above isotype control and is presented qualitatively as: between 1.5 to 5 (+); 5 to 25 (++); 25 to 50 (+++); and above 50 (++++). Representative histograms of AR36A36.11.1 antibodies  
30 were compiled for Figure 1. AR36A36.11.1 showed binding to all cell lines tested. These data have shown AR36A36.11.1 exhibited functional specificity in that although there was clear binding to a number of cancer types, there was only associated



cytotoxicity with LnCap prostate cancer cells. By contrast, the anti-EGFR antibody displayed a higher correlation between binding and cytotoxicity with one such example being the non-cancer epidermis derived cell line, CCD-27sk.

### EXAMPLE 3

#### 5 *In Vivo* MDA-MB-231 Tumor Experiments

With reference to Figures 5 and 6, 4 to 8 week old female SCID mice were implanted with 5 million human breast cancer cells (MB-231) in 100 microlitres saline injected subcutaneously in the scruff of the neck. The mice were randomly divided into 2 treatment groups of 5. On the day after implantation, 20 mg/kg of AR36A36.11.1 test  
10 antibody or an isotype control antibody (known not to bind MB-231 cells) was administered intraperitoneally at a volume of 300 microliters after dilution from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl and 20 mM Na<sub>2</sub>HPO<sub>4</sub>. The antibodies were then administered once per week for a period of 7 weeks in the same fashion. Tumor growth was measured about every  
15 seventh day with calipers for up to 8 weeks or until individual animals reached the Canadian Council for Animal Care (CCAC) end-points. Body weights of the animals were recorded for the duration of the study. At the end of the study all animals were euthanised according to CCAC guidelines.

AR36A36.11.1 completely prevented tumor growth and reduced tumor burden  
20 in a preventative *in vivo* model of human breast cancer. On day 56 post-implantation, 6 days after the last treatment dose, the mean tumor volume in the AR36A36.11.1 treated group was 0 percent of the tumor volume in the isotype control-treated group (p=0.0002, t-test, Figure 5).

There were no clinical signs of toxicity throughout the study. Body weight  
25 measured at weekly intervals was a surrogate for well-being and failure to thrive. Figure 6 shows that there was no significant difference in body weight between the groups at the end of the treatment period (p=0.0676, t-test). Therefore AR36A36.11.1 was well-tolerated and decreased the tumor burden in a breast cancer xenograft model.

#### EXAMPLE 4

##### *In Vivo* MB-231 Established Tumor Experiments

With reference to Figures 7 and 8, 5 to 6 week old female SCID mice were implanted with 5 million MB-231 human breast cancer cells in 100 microlitres saline injected subcutaneously in the scruff of the neck. Tumor growth was measured with calipers every week. When the majority of the cohort reached an average tumor volume of around 100 mm<sup>3</sup> (range 60-140 mm<sup>3</sup>) at 59 days post-implantation, 10 mice were randomly assigned into each of 2 treatment groups. 20 mg/kg of AR36A36.11.1 test antibody or buffer control was administered intraperitoneally at a volume of 300 microliters after dilution from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl and 20 mM Na<sub>2</sub>HPO<sub>4</sub>. The antibodies were then administered 3 times per week for 10 doses in total in the same fashion until day 81 post-implantation. Tumor growth was measured about every seventh day with calipers until day 90 post-implantation or until individual animals reached the CCAC end-points. Body weights of the animals were recorded for the duration of the study. At the end of the study all animals were euthanised according to CCAC guidelines.

AR36A36.11.1 prevented tumor growth and reduced tumor burden in this established *in vivo* model of human breast cancer. On day 83 post-implantation, 2 days after the last treatment dose, the mean tumor volume in the AR36A36.11.1 treated group was 46% percent of the tumor volume in the buffer control-treated group (p=0.0038, t-test, Figure 7). This corresponds to a mean T/C of 32%.

There were no clinical signs of toxicity throughout the study. Body weight measured at weekly intervals was a surrogate for well-being and failure to thrive. Figure 8 shows that there was no significant difference in body weight between the groups at the end of the treatment period (p=0.6493, t-test).

In summary, AR36A36.11.1 is well-tolerated and significantly more effective than the buffer control in suppressing tumor growth in an established tumor xenograft model of breast cancer in SCID mice. Over the 3-week treatment period, AR36A36.11.1 achieved an endpoint of mean T/C tumor volumes of less than 50% relative to control. Treatment benefits were observed in a well-recognized model of human cancer disease suggesting pharmacologic and pharmaceutical benefits of this antibody for therapy in other mammals, including man.



## EXAMPLE 5

### *In Vivo* SW1116 Tumor Experiments

With reference to Figures 9 and 10, 4 to 8 week old female SCID mice were implanted with 5 million human colon cancer cells (SW1116) in 100 microlitres saline injected subcutaneously in the scruff of the neck. The mice were randomly divided into 2 treatment groups of 5. On the day after implantation, 20 mg/kg of AR36A36.11.1 test antibody or buffer control was administered intraperitoneally at a volume of 300 microliters after dilution from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl and 20 mM Na<sub>2</sub>HPO<sub>4</sub>. The antibodies were then administered once per week for a period of 7 weeks in the same fashion. Tumor growth was measured about every seventh day with calipers for up to 8 weeks or until individual animals reached the Canadian Council for Animal Care (CCAC) end-points. Body weights of the animals were recorded for the duration of the study. At the end of the study all animals were euthanised according to CCAC guidelines.

AR36A36.11.1 prevented tumor growth and reduced tumor burden in a preventative *in vivo* model of human colon cancer. On day 55 post-implantation, 5 days after the last treatment dose, the mean tumor volume in the AR36A36.11.1 treated group was 51 percent of the tumor volume in the buffer control-treated group (p=0.0055, t-test, Figure 9).

There were no clinical signs of toxicity throughout the study. Body weight measured at weekly intervals was a surrogate for well-being and failure to thrive. Figure 10 shows that there was no significant difference in body weight between the groups at the end of the treatment period (p=0.4409, t-test). Therefore AR36A36.11.1 was well-tolerated and decreased the tumor burden in a colon cancer xenograft model.

## EXAMPLE 6

### *In Vivo* PC-3 Tumor Experiments

With reference to Figures 11 and 12, 4 to 8 week old male SCID mice were implanted with 1 million human prostate cancer cells (PC-3) in 100 microlitres saline injected subcutaneously in the scruff of the neck. The mice were randomly divided into 2 treatment groups of 5. On the day after implantation, 20 mg/kg of AR36A36.11.1 test antibody or buffer control was administered intraperitoneally at a volume of 300

microliters after dilution from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl and 20 mM Na<sub>2</sub>HPO<sub>4</sub>. The antibodies were then administered once per week for the duration of the study in the same fashion. Tumor growth was measured about every seventh day with calipers. The study was  
5 completed after 6 injections (41 days), as the animals reached the Canadian Council for Animal Care (CCAC) end-points due to large ulcerated lesions. Body weights of the animals were recorded for the duration of the study. At the end of the study all animals were euthanised according to CCAC guidelines.

AR36A36.11.1 prevented tumor growth and reduced tumor burden in a  
10 preventative *in vivo* model of human prostate cancer. On day 41 post-implantation, 5 days after the last treatment dose, the mean tumor volume in the AR36A36.11.1 treated group was 14 percent of the tumor volume in the buffer control-treated group (p=0.0009, t-test, Figure 11).

In a PC-3 prostate cancer xenograft model, body weight can be used as a  
15 surrogate indicator of disease progression (Wang et al. Int J Cancer, 2003). As shown in Figure 12, by the end of the study (day 41), control animals exhibited a 27% decrease in body weight from the onset of the study. By contrast, the group treated with AR36A36.11.1 had a significantly higher body weight than the control group (p=0.017). Overall, the AR36A36.11.1-treated group lost only 6% of its body weight,  
20 much less than the 27% lost by the buffer control group.

Therefore AR36A36.11.1 was well-tolerated and decreased the tumor burden and cachexia in a prostate cancer xenograft model.

#### Reference

Wang Z, Corey E, Hass GM, et al. Expression of the human cachexia-associated  
25 protein (HCAP) in prostate cancer and in a prostate cancer animal model of cachexia. Int J Cancer. 2003; 105(1):123-9.



## CLAIMS

What is claimed is:

Claim 1. An isolated monoclonal antibody encoded by the clone deposited with the IDAC as Accession Number 280104-02.

Claim 2. The antibody of claim 1, which is humanized.

Claim 3. The antibody or of claim 1, which is chimerized.

Claim 4. The isolated clone deposited with the IDAC as Accession Number 280104-02.

Claim 5. A method for initiating antibody induced cellular cytotoxicity of cancerous cells in a tissue sample selected from a human tumor comprising:

providing a tissue sample from said human tumor;

providing an isolated monoclonal antibody encoded by the clone deposited with the IDAC as Accession Number 280104-02 or a cellular cytotoxicity inducing antigen binding fragment thereof; and

contacting said isolated monoclonal antibody or cellular cytotoxicity inducing antigen binding fragment thereof with said tissue sample.

Claim 6. The method of claim 5 wherein the human tumor tissue sample is obtained from a tumor originating in a tissue selected from the group consisting of colon, ovarian, prostate, pancreatic and breast tissue.

Claim 7. Antigen binding fragments of the isolated monoclonal antibody of claim 1.

Claim 8. Antigen binding fragments of the humanized antibody of claim 2.

Claim 9. Antigen binding fragments of the chimerized antibody of claim 3.

Claim 10. The isolated antibody or antigen binding fragments of any one of claims 1,2,3,7,8 or 9 conjugated with a member selected from the group consisting of cytotoxic moieties, enzymes, radioactive compounds, and hematogenous cells.

Claim 11. A method of treating a human tumor susceptible to antibody induced cellular cytotoxicity in a mammal, wherein said human tumor expresses an antigen which specifically binds to the monoclonal antibody which has the cellular cytotoxicity inducing characteristics of the monoclonal antibody encoded by a clone deposited with the IDAC as Accession Number 280104-02 or a cellular cytotoxicity inducing antigen binding fragment thereof, comprising administering to said mammal said monoclonal antibody or said antigen binding fragment thereof in an amount effective to induce cellular cytotoxicity and thereby reduce said mammal's tumor burden.

Claim 12. The method of claim 11 wherein said monoclonal antibody is conjugated to a cytotoxic moiety.



Claim 13. The method of claim 12 wherein said cytotoxic moiety is a radioactive isotope.

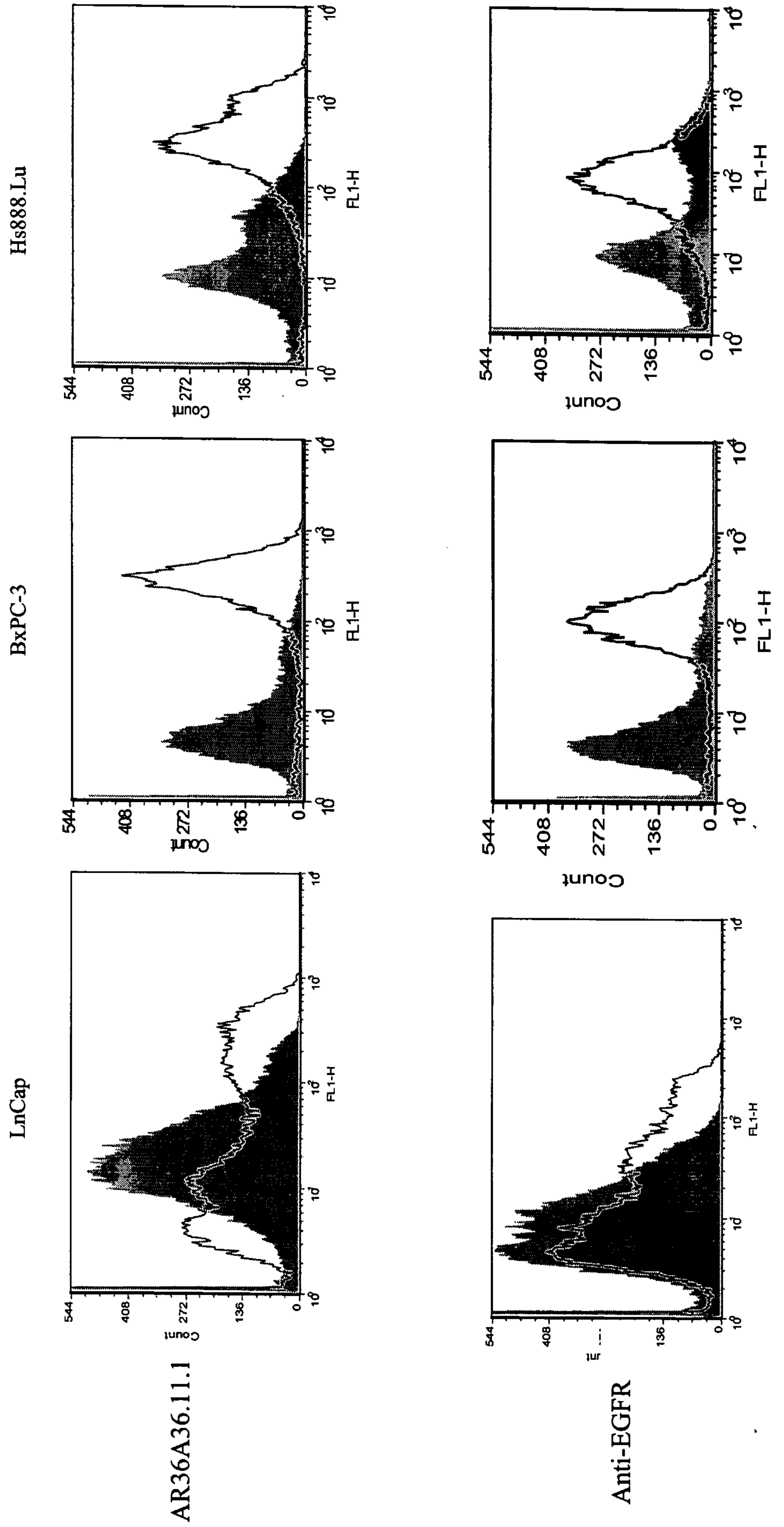
Claim 14. The method of claim 11 wherein said monoclonal antibody activates complement.

Claim 15. The method of claim 11 wherein said monoclonal antibody mediates antibody dependent cellular cytotoxicity.

Claim 16. The method of claim 11 wherein said monoclonal antibody is humanized.

Claim 17. The method of claim 11 wherein said monoclonal antibody is chimerized.

FIGURE 1







**FIGURE 3**

		Table 2															
		COLON		PANCREAS		BREAST		PROSTATE			OVARY		NORMAL				
Cell Line		DLD-1	LoVo	SW1116	BxPC-3	MB-231	MCF-7	PC-3	LnCap	DU-145	OVCAR-3	CCD-27sk	Hs888 Lu				
Negative Controls		AR36A36.11.1							++++								
		G155-178 (IgG2a)	+/-								+						
		IgG buffer (3%)															
Positive Controls		anti-EGFR	+++	++++		+/-			+		+++	++++					
		CHX	+++	++++	++++	+++	+++	++++	+++	+++	++++	++++	++++				





FIGURE 5

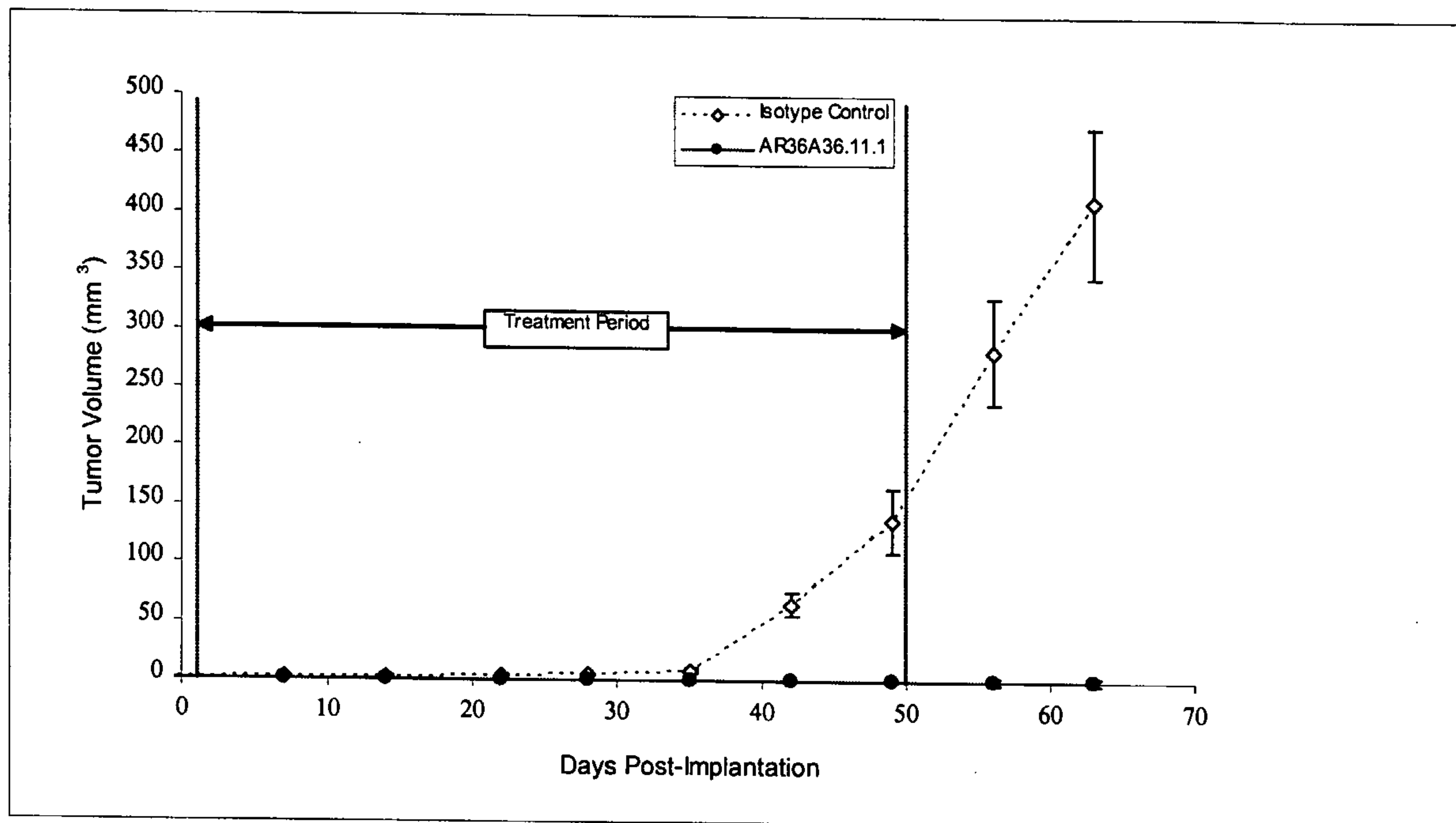


FIGURE 6

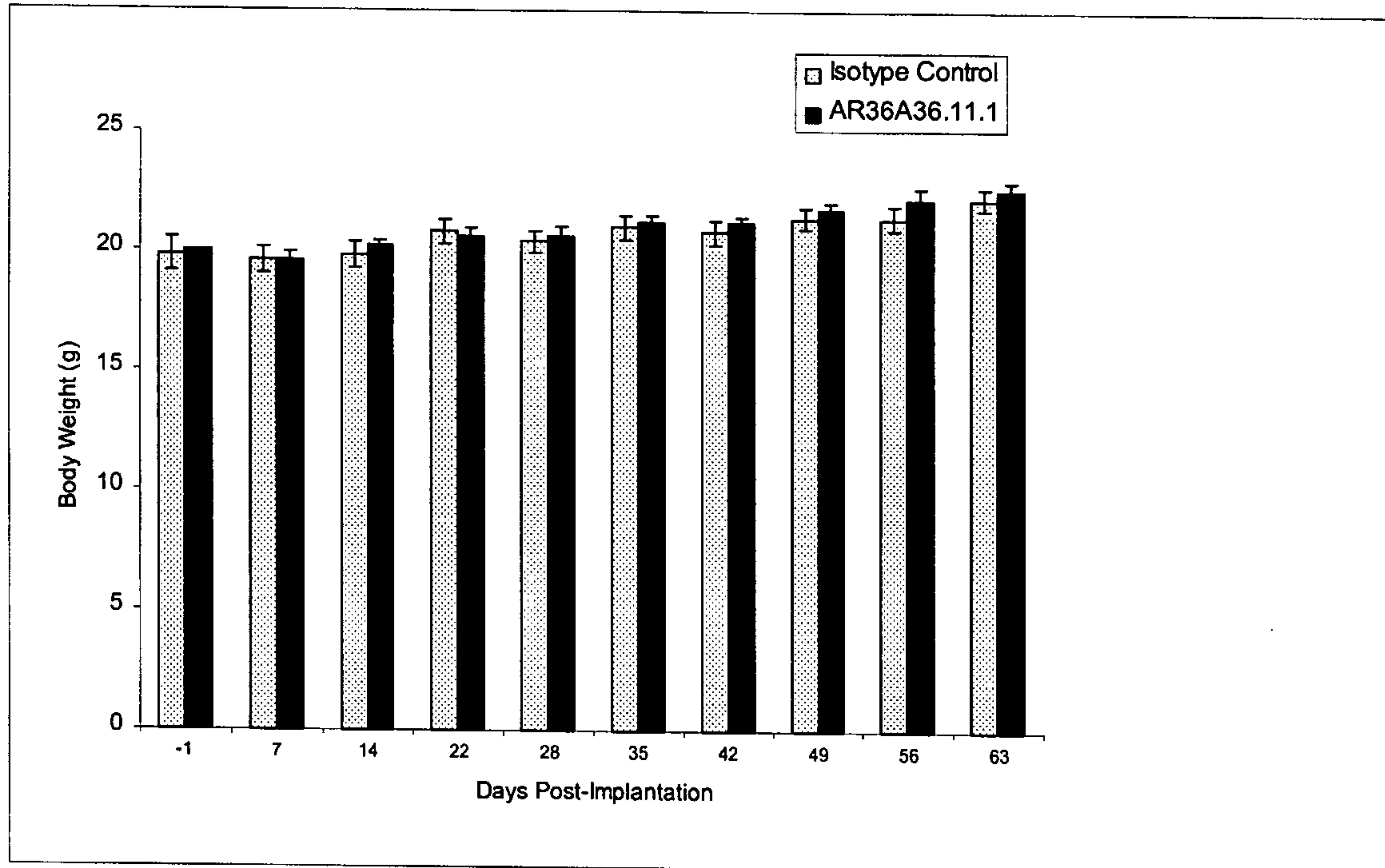




FIGURE 7

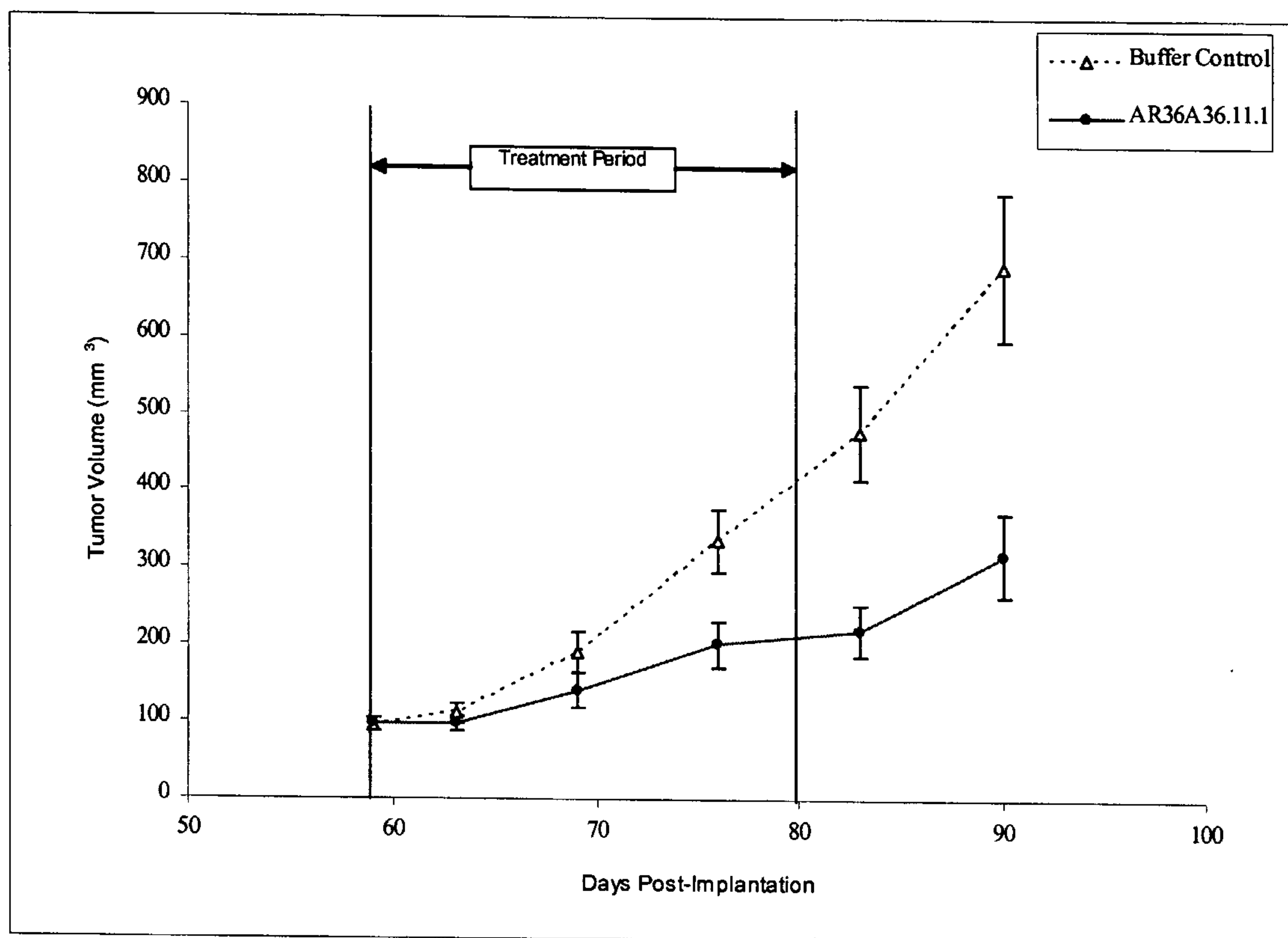


FIGURE 8

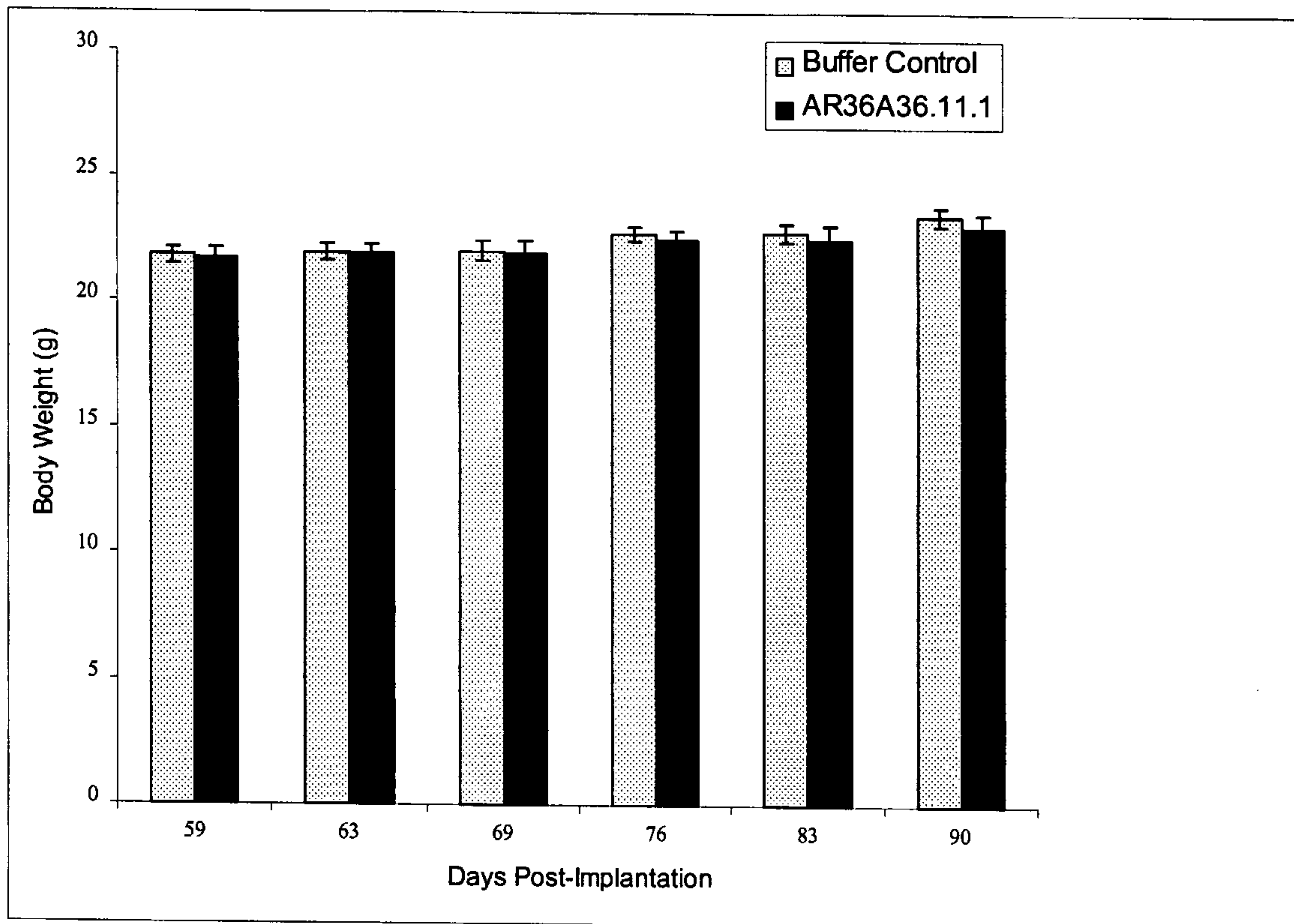


FIGURE 9

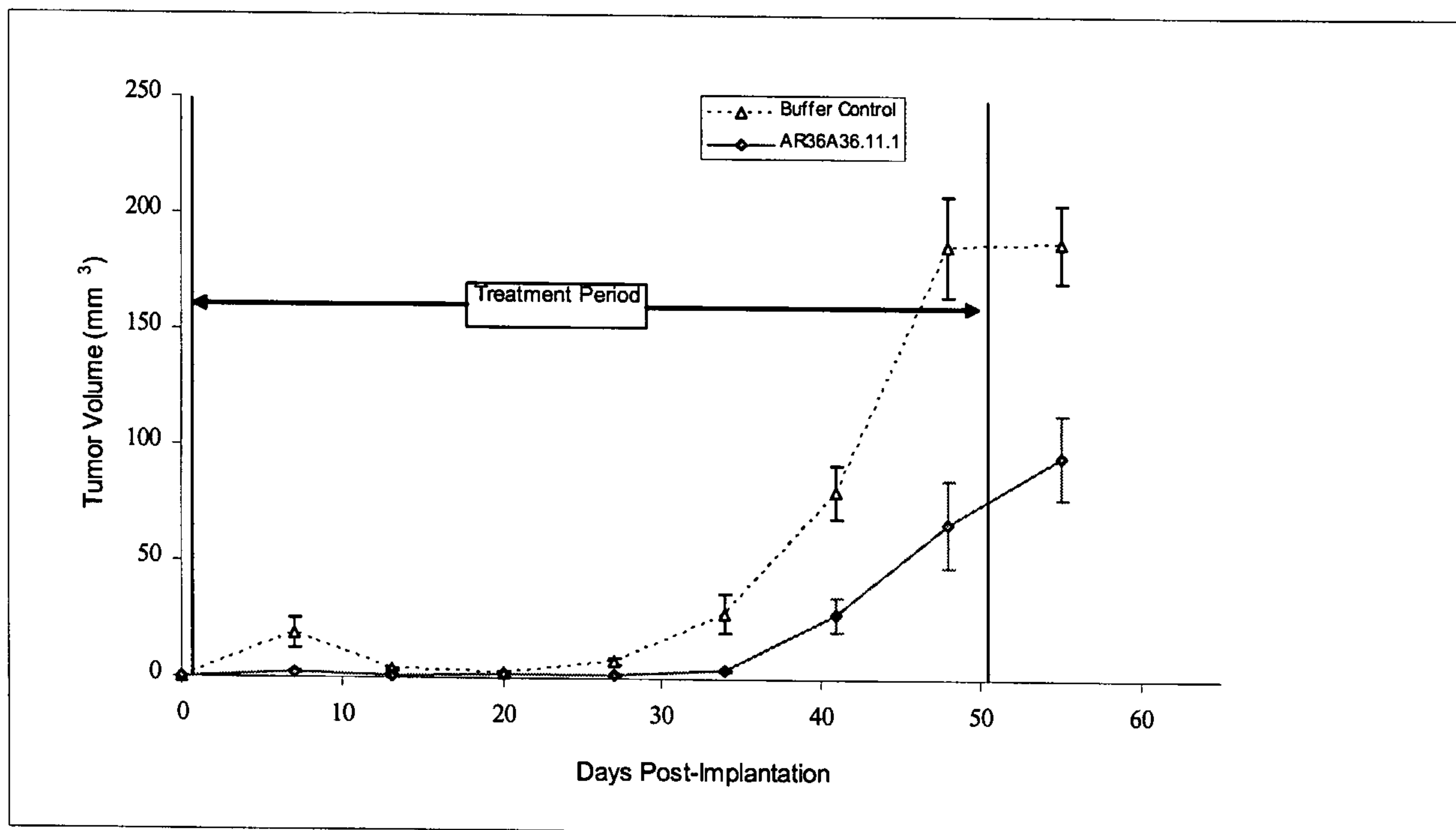




FIGURE 10

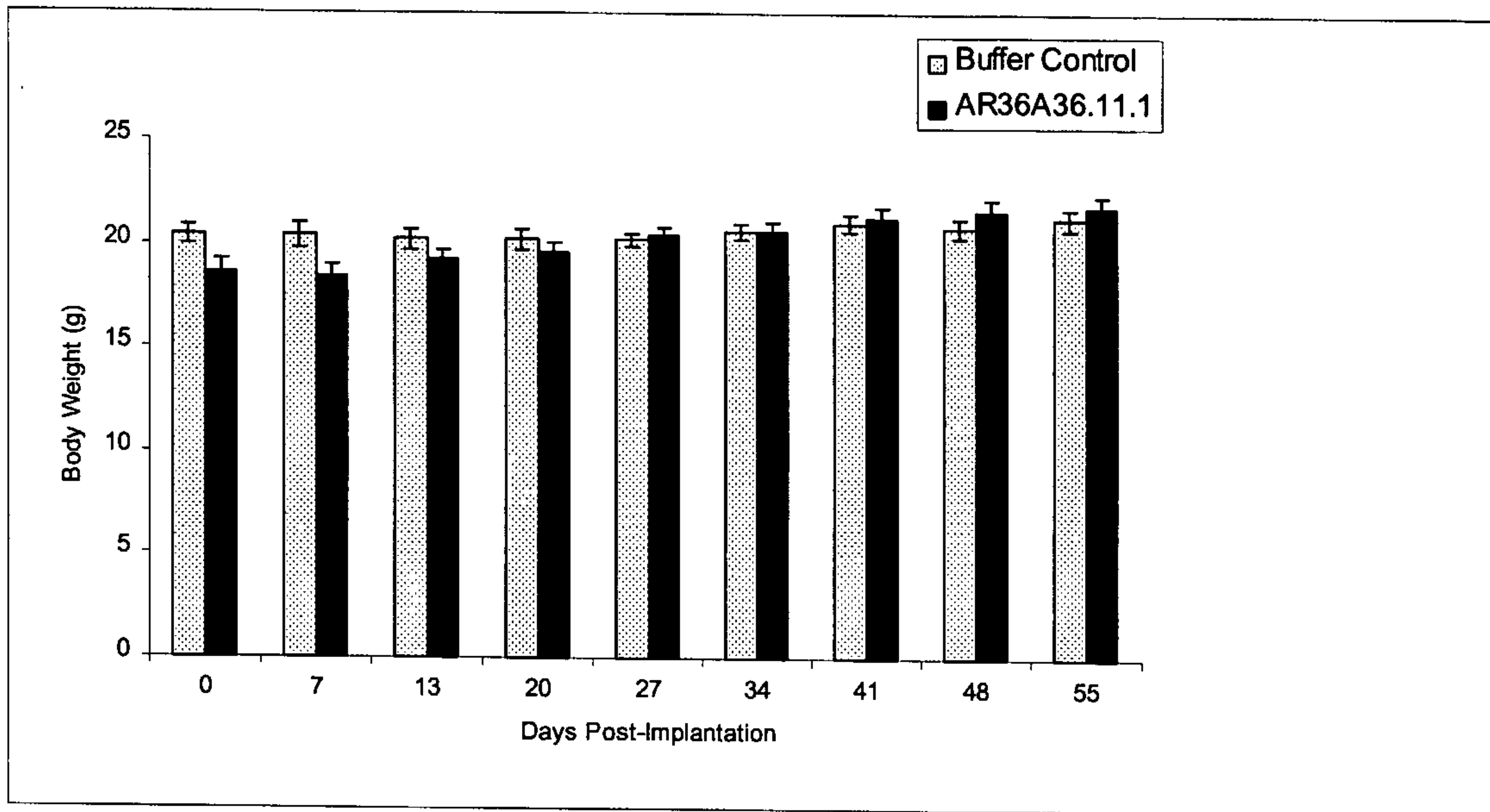


FIGURE 11

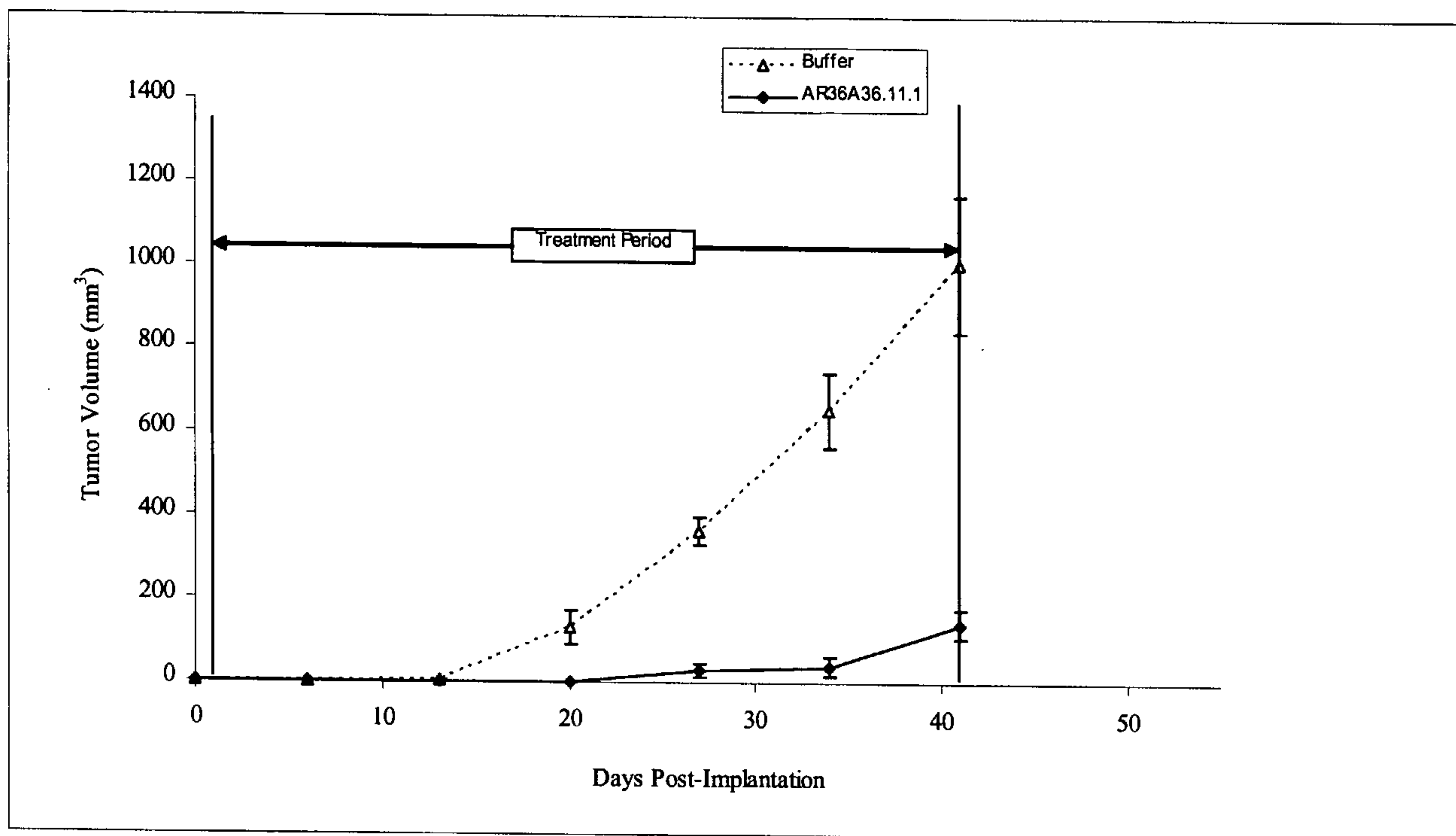


FIGURE 12

