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(54) **ELECTRO-ENDOCYTOTIC THERAPY AS A TREATMENT MODALITY OF CANCER**

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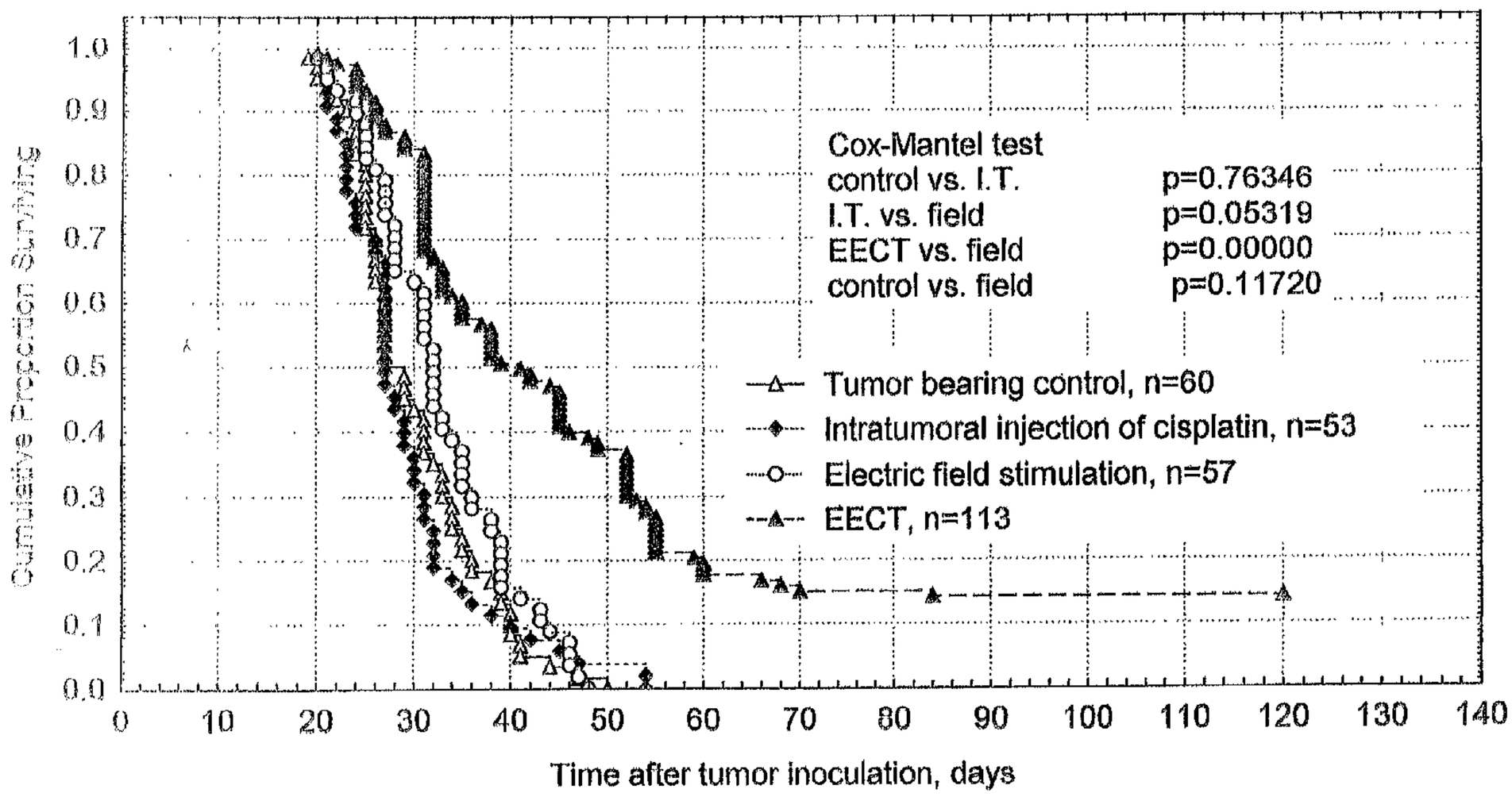
(57) **ABSTRACT**

There is provided a method for introducing a therapeutic agent into a membrane vesicle, cell, or tissue by exposing the membrane vesicle, cell, or tissue to a low electric field in the presence of the therapeutic agent in an extracellular compartment of the membrane vesicle, cell, or tissue. Also provided is therapy including a low electric field alone or in combination with a therapeutic agent.

A method for treating tumors and increasing inflammatory and immunogenic responses by exposing cells to a low electric field in the presence or absence of a therapeutic agent, alone or in combination with an immunostimulatory agent in an extracellular compartment of the cell is also provided.

Cumulative Proportion Surviving (Kaplan-Meier) of C57BL/6 mice bearing B16-F10.9 melanoma following EECT with cisplatin.



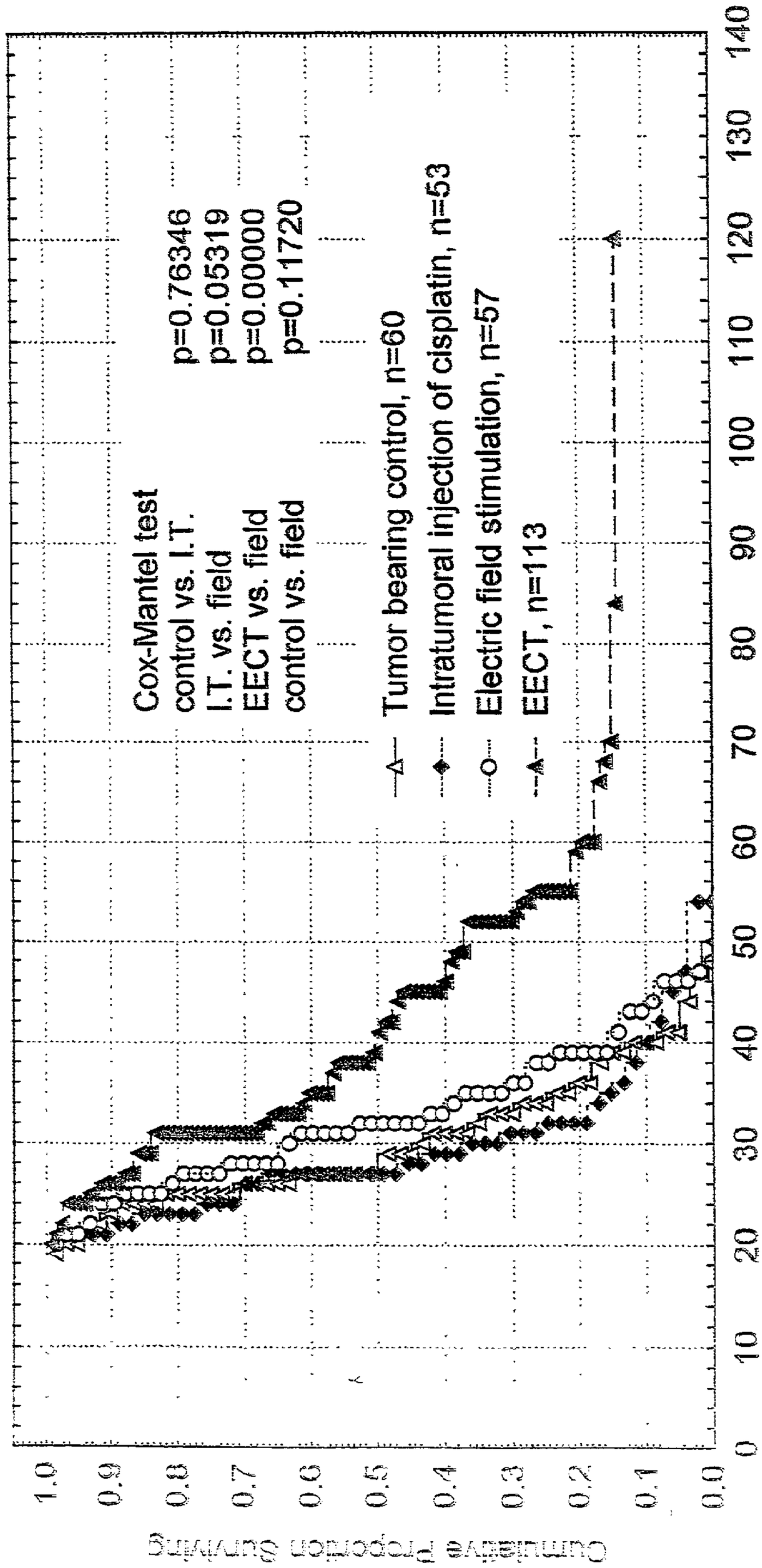

Cumulative Proportion Surviving (Kaplan-Meier) of C57BL/6 mice bearing
B16-F10.9 melanoma following EECT with cisplatin.
Cox-Mantel test
control vs. I.T. p=0.76346
I.T. vs. field p=0.05319
EECT vs. field p=0.00000
control vs. field p=0.11720
Tumor bearing control, n=60
Intratumoral injection of cisplatin, n=57
Electric field stimulation, n=113
EECT, n=113
Time after tumor inoculation, days
Cumulative Proportion Surviving
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
10
20
30
40
50
60
70
80
90
100
110
120
130
140

Fig. 1

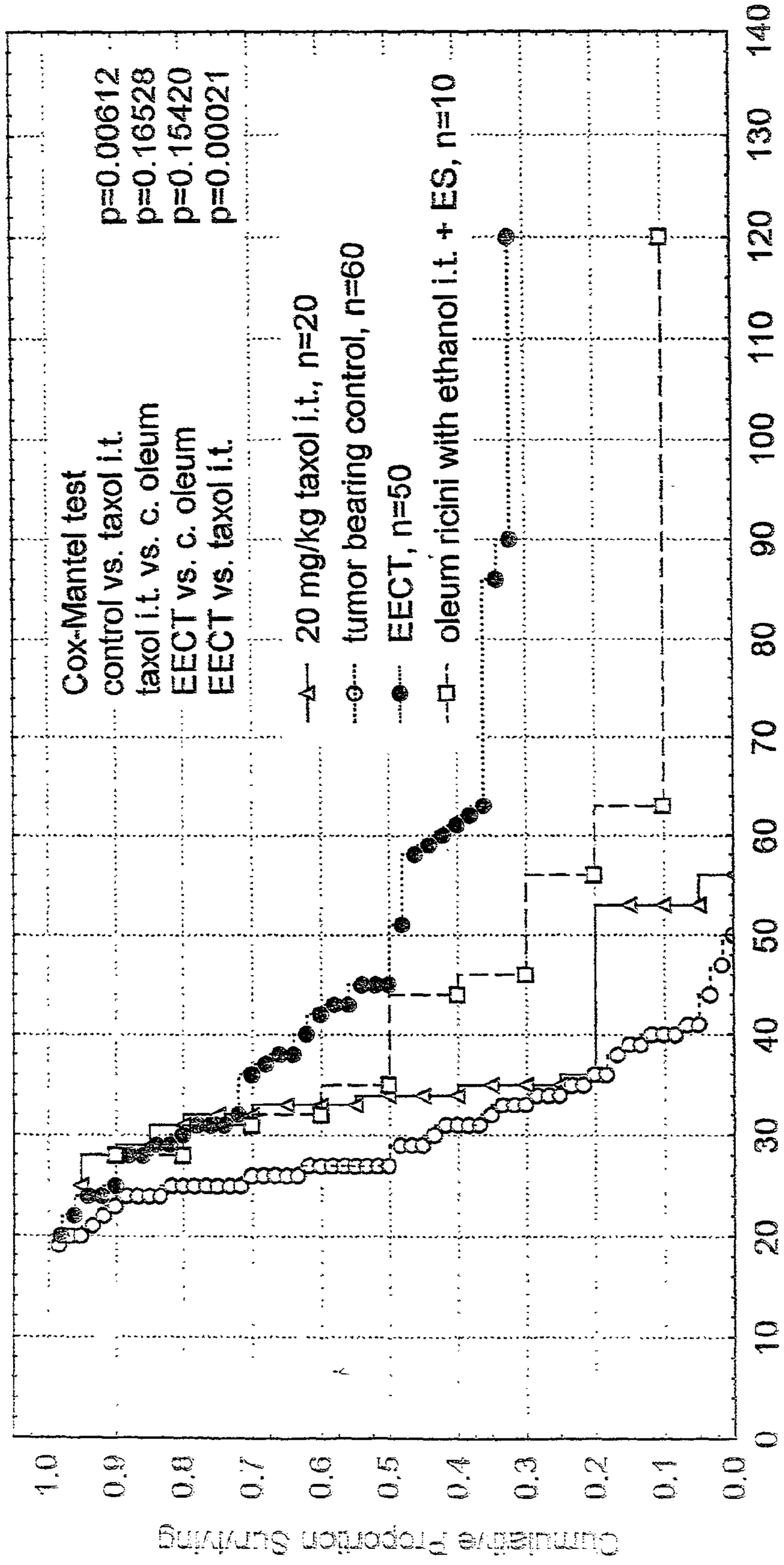

Cumulative Proportion Surviving (Kaplan-Meier) of C57BL/6 mice bearing B16-F10.9 melanoma following EECT with taxol.
Cox-Mantel test
control vs. taxol i.t. p=0.00612
taxol i.t. vs. c. oleum p=0.16528
EECT vs. c. oleum p=0.15420
EECT vs. taxol i.t. p=0.00021
20 mg/kg taxol i.t., n=20
tumor bearing control, n=60
EECT, n=50
oleum ricini with ethanol i.t. + ES, n=10
Time after tumor inoculation, days
Cumulative Proportion Surviving
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Fig. 2

Fig. 3

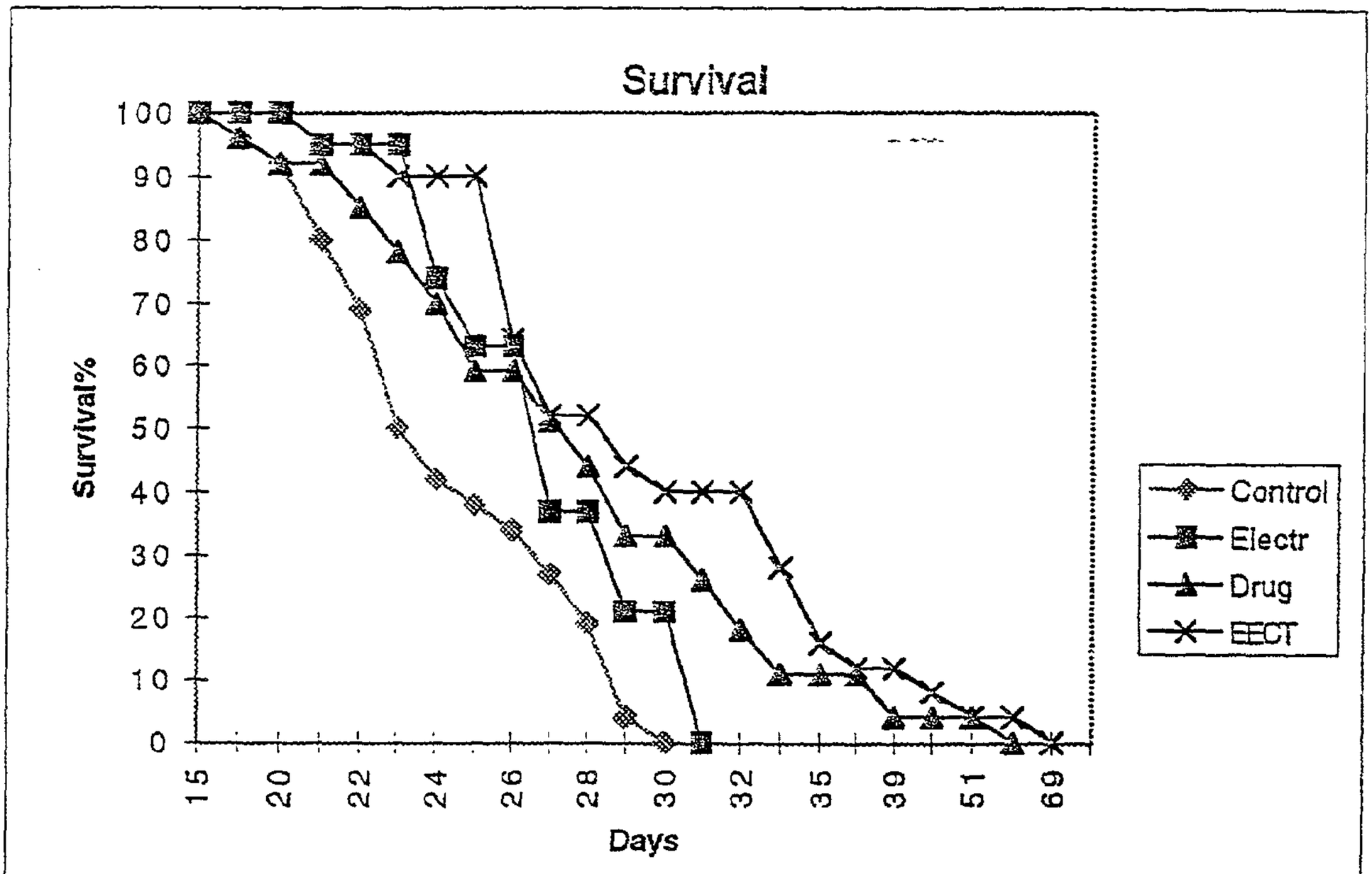


Fig.3 Control: non treated. Electr.: treated with electric field. Drug: treated with 150mg/kg 5FU intratumorally. EECT: treated with 150mg/kg 5FU and electric field.

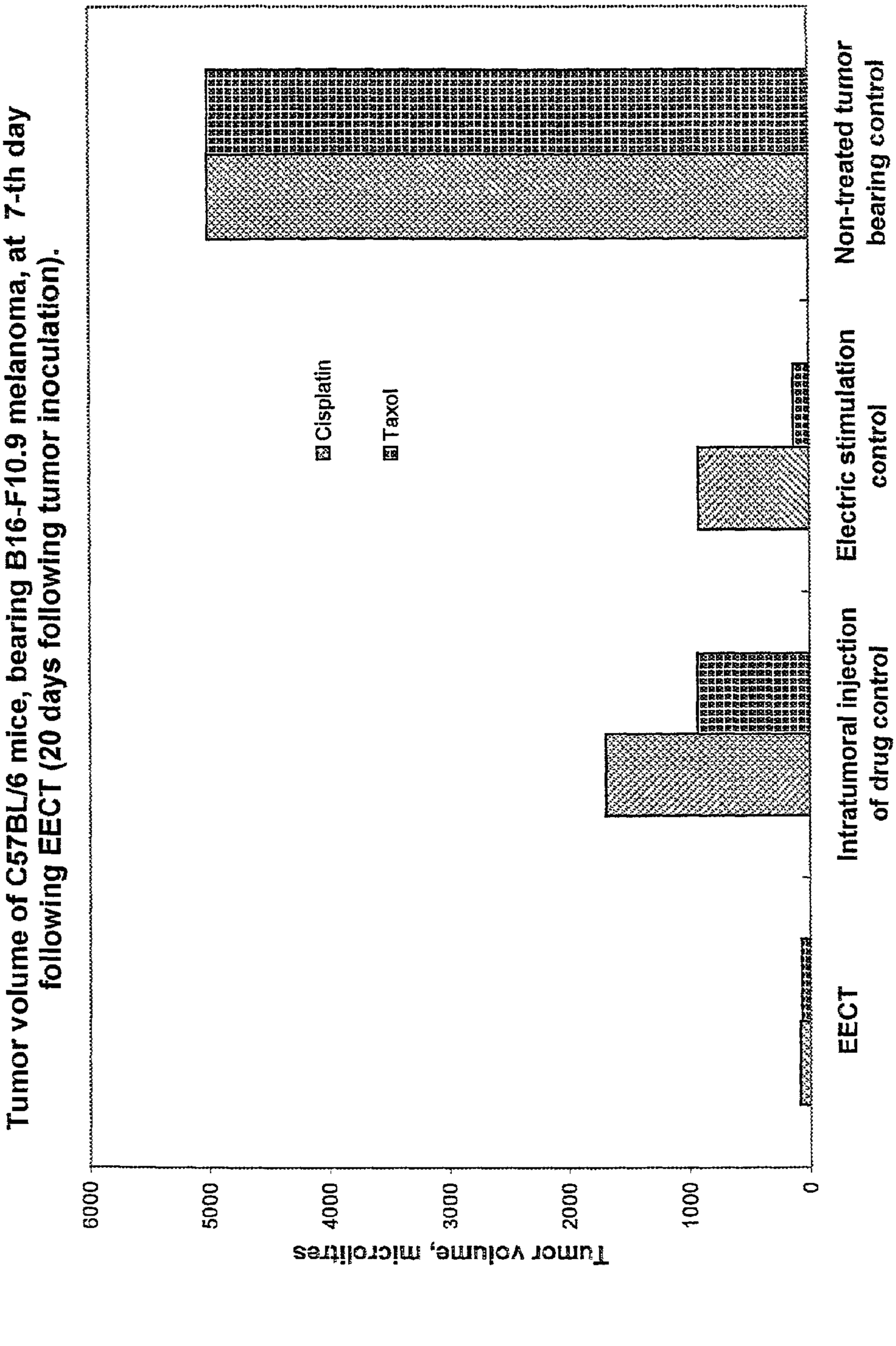

Tumor volume of C57BL/6 mice, bearing B16-F10.9 melanoma, at 7-th day following EECT (20 days following tumor inoculation).
Tumor volume, microlitres
6000
5000
4000
3000
2000
1000
0
EECT
Intratumoral injection of drug control
Electric stimulation control
Non-treated tumor bearing control
Cisplatin
Taxol

Fig. 4

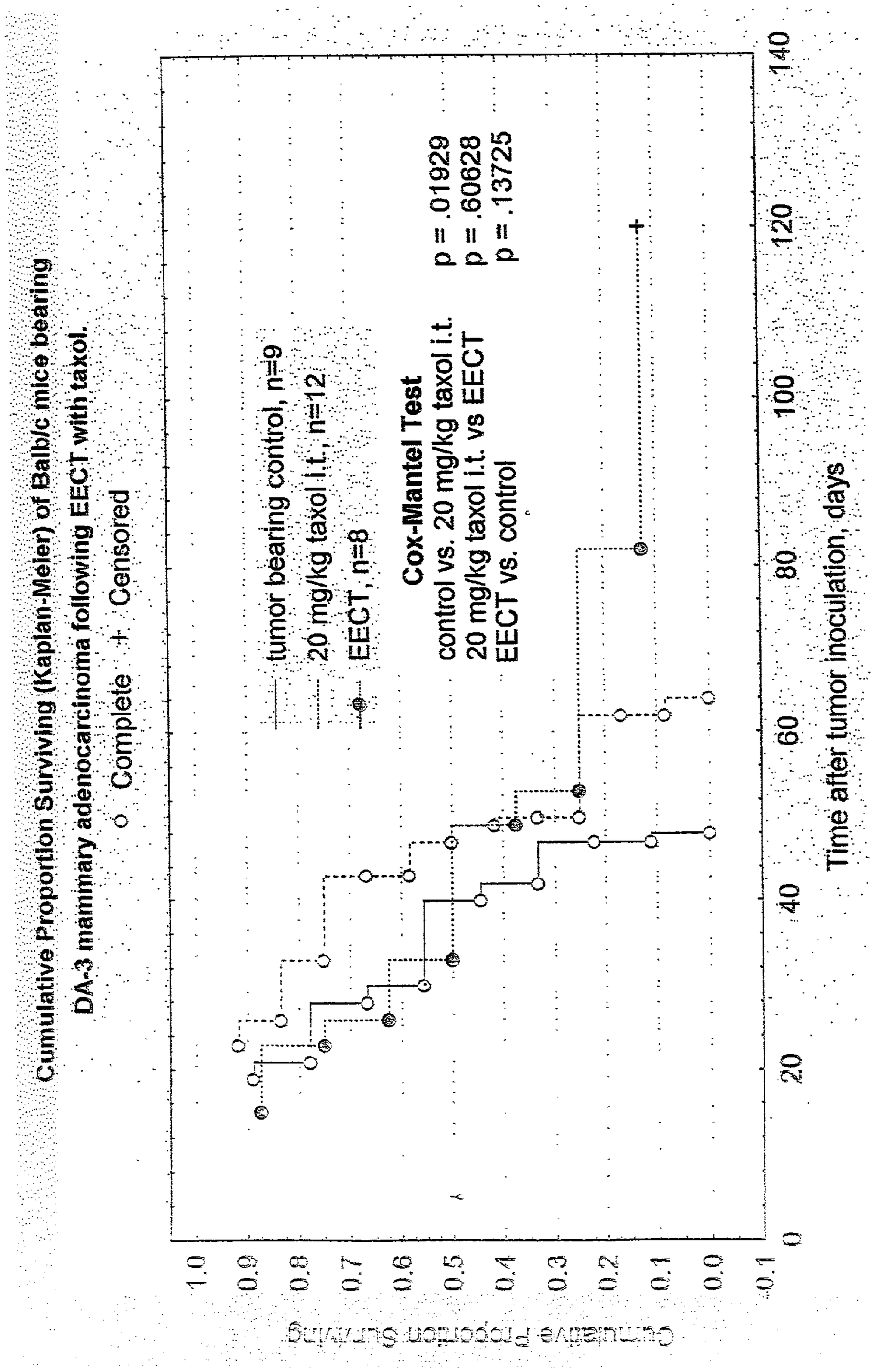
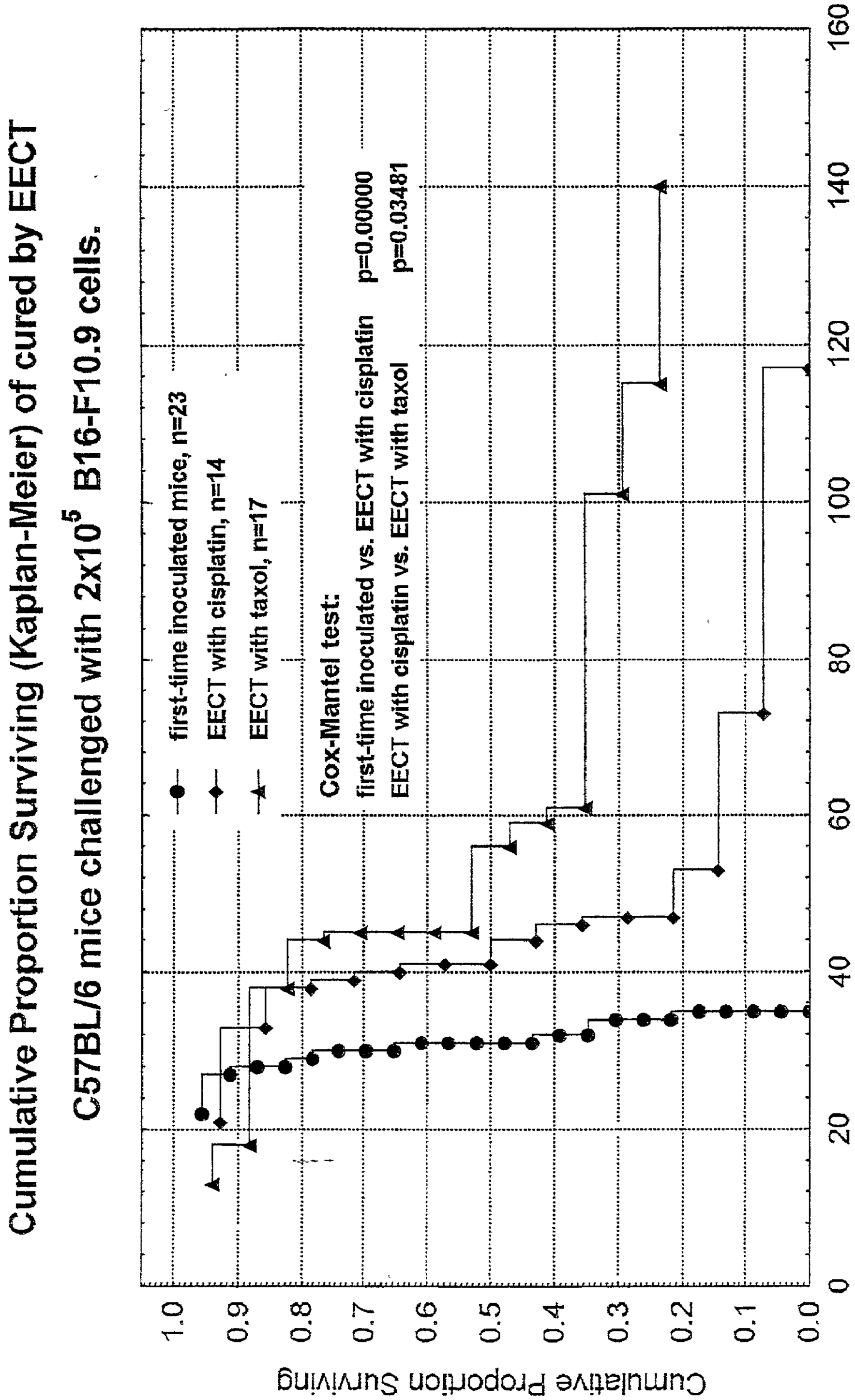

Cumulative Proportion Surviving (Kaplan-Meier) of Balb/c mice bearing
DA-3 mammary adenocarcinoma following EECT with taxol.
Complete + Censored
tumor bearing control, n=9
20 mg/kg taxol i.t., n=12
EECT, n=8
Cox-Mantel Test
control vs. 20 mg/kg taxol i.t. p = .01929
20 mg/kg taxol i.t. vs EECT p = .60628
EECT vs. control p = .13725
Cumulative Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
20
40
60
80
100
120
140
Time after tumor inoculation, days

Fig. 5

Fig. 6


Cumulative Proportion Surviving (Kaplan-Meier) of cured mice challenged with 2x10^5 B16-F10.9 cells.
C57BL/6 mice challenged with 2x10^5 B16-F10.9 cells.
first-time inoculated mice, n=23
EECT with cisplatin, n=14
EECT with taxol, n=17
Cox-Mantel test:
first-time inoculated vs. EECT with cisplatin p=0.00000
EECT with cisplatin vs. EECT with taxol p=0.03481
Cumulative Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
20
40
60
80
100
120
140
160
Time after challenge giving, days

ELECTRO-ENDOCYTOTIC THERAPY AS A TREATMENT MODALITY OF CANCER

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates to a system for exposing cells to low electric fields in the presence or absence of therapeutic agents. More specifically, the present invention relates to a pulsed low voltage system capable of stimulation of endocytosis and of macromolecular transfer.

[0003] 2. Description of Related Art

[0004] In order to introduce molecules into cells, the permeability barrier of the cell membrane must be overcome. Present day research has created two categories of approach for loading cells with otherwise membrane impermeable molecules.

[0005] In a first method, low molecular weight molecules are incorporated into cells based either of two methods, one method uses the employment of lipophilic molecules which are modified into hydrophilic molecules following their penetration into the cytosol by intracellular enzymes, such as esterases. The esterases act on, for example, the pH indicator 2', 7"-bis (2-carboxyethyl)-5(6)-carboxyfluorescein, an acetoxy methyl ester. Permeability change can be induced by adenosine tri-phosphate via formation of small pores in the membrane.

[0006] The second method relates to the incorporation of high molecular weight molecules and is based on several approaches. One approach is the use of chemicals such as detergents, polyethyleneglycol, and lipofectin. Additional approaches include liposome-cell fusion, electroporation, and cell bombardment by particles coated with molecules.

[0007] One application requiring the introduction of molecules into cells is the field of gene therapy. In this application, macromolecules, generally DNA, must be introduced into the cell. The cells must be transfected with the DNA leading to transformed viable cells.

[0008] While significant progress has been made in manipulating cellular genes, the application to gene therapy has been limited and the field of gene therapy is still not commercially developed. One major limiting factor to apply gene therapy effectively is the need to achieve a very high number of transformed cells. In most cases following transfection, transformation does not occur and neither further selection nor multiplication of cells can be achieved. Thus, the efficiency of transfection resulting in transformation is the rate limiting step for applications of gene therapy.

[0009] One successful approach in gene therapy has been to use a gene, spliced into a retrovirus, as a vector to introduce the gene into the target cell. However, the disadvantage of this strategy is that it involves the multiplication of target cells in vitro. Other limitations of this approach are the limited size of the gene which can be carried by the vector and still be implanted and the procedures needed to express only the target gene without the additional expression of the viral genes. Another limitation is that this procedure is limited to DNA and does not accommodate other molecular species that could, for example, lead to the intracellular employment of exogenous enzymes, such as restriction enzymes, for gene manipulations.

[0010] Fusing liposomes is another approach. In this approach the liposomes are loaded with the appropriate genes and are fused with the target cells.

[0011] An alternative procedure for the introduction of genes into cells is based on exposing cells to exogenous DNA in high electric fields, the procedure is known as electroporation. Electroporation is generally defined as the formation of hydrophilic pores through an electrical process wherein larger pores allow higher permeability. Electroporation utilizes short, high voltage electrical pulses to produce a transient high permeability state (reversible electrical breakdown, REB) which occurs at the beginning of the high permeability state. REB is a decrease in the electrical resistance of a tissue which is caused by brief exposure to an abnormally high transtissue potential.

[0012] U.S. Pat. No. 5,019,034 to Weaver discloses the use of a high voltage, short duration electrical pulse on the tissue surface to produce electroporation of molecules into cells of the tissue. However, this and other existing methods suffer from at least one of the following problems:

[0013] (1) There is low efficiency of loading or transfection of high molecular weight molecules (i.e., macromolecules);

[0014] (2) The demonstrated loading of macromolecules appears limited to DNA; there is a lack of loading of high molecular weight proteins, enzymes of the molecular weight of 250 kD and higher, as disclosed in the literature; and

[0015] (3) Many of the extensively used methods involve steps for transfection that have a high rate of cell killings.

[0016] The present invention provides an alternative method to the above, which not only enables the incorporation into cells of macromolecules without destruction of the cells, but also provides an efficient introduction of the molecules. The method can be accomplished in vivo or in vitro.

[0017] In relation to potential diseases in which such treatment is valuable, cancer is second only to heart disease as a cause of death. The leading cause of cancer death is the growth of metastases, and in the majority of patients, by the time of diagnosis of primary malignant neoplasms, metastases have spread to regional or distant sites, but only in the minority of these patients metastases can be clinically detected (for review see Fidler and Balch, 1987) or effectively treated. It would therefore be useful to develop a method and therapy for the efficient removal of the primary tumor mass and prevention of secondary tumor growth, eradication of metastatic cells, and reconstitution of the immune response.

SUMMARY OF THE INVENTION

[0018] According to the present invention, there is provided a method for treating cancer by one of the two methods. The first includes introducing a chemotherapeutic agent into a membrane vesicle, cell or tissue by exposing the membrane vesicle, cell, or tissue to a low electric field in the presence of the chemotherapeutic agent in an extracellular compartment of the membrane vesicle, cell, or tissue. Also provided is therapy utilizing a low electric field and a

chemotherapeutic agent or the tumor can be exposed to a low electric field in the absence of externally added therapeutic agent.

[0019] A method for treating tumors and increasing inflammatory and immunologic responses by exposing cells to a low electric field in the presence and absence of a therapeutic agent and in the presence or absence of immunomodulatory agent in an extracellular compartment of the cell is also provided.

[0020] Finally, a method for treating tumors and increasing inflammatory response including the step of exposing cells to a low electric field is also provided.

DESCRIPTION OF THE DRAWINGS

[0021] Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

[0022] **FIG. 1** is a graph showing the cumulative proportion of surviving C57BL/6 mice bearing B16-F10.9 melanoma following EECT with cisplatin;

[0023] **FIG. 2** is a graph showing the cumulative proportion of surviving C57BL/6 mice bearing B16-F10.9 melanoma following EECT with taxol;

[0024] **FIG. 3** is a graph showing the survival percentages of mice utilizing various treatments;

[0025] **FIG. 4** is a graph showing the tumor volume of C57BL/6 mice, bearing B16-F10.9 melanoma at seventh day following EECT (20 days following tumor inoculation);

[0026] **FIG. 5** is a graph showing the cumulative proportion of surviving Balb/c mice bearing DA-3 mammary adenocarcinoma following EECT with taxol; and

[0027] **FIG. 6** is a graph showing the cumulative proportion of surviving C57BL/6 mice cured by EECT challenged with 2×10^5 B16-F10.9 cells.

DETAILED DESCRIPTION OF THE INVENTION

[0028] Generally, the present invention provides a method and apparatus for the introduction of a therapeutic agent into a cell while maintaining the function of the therapeutic agent. The method is accomplished by the general steps of preparing either a suspension or an adherent growth of cells, introducing a therapeutic agent into the cells, and applying a train of unipolar or alternating low voltage pulses to the suspension or adherent layer to incorporate the molecules into the cell. The exposure to unipolar or alternating train or series of trains of voltage pulses leads to electrophoretic movement of the charged proteins into the cell membrane, thereby leading to membrane destabilization resulting in endocytosis. This induces uptake of therapeutic molecules/macromolecules into the cell.

[0029] By "extracellular compartment" as used herein it is meant any of the space located outside of the cell membrane.

[0030] Also provided by the present invention, is a method for treating cancer cells by applying a low electric field to the cancer cells. This electric field induces endocytosis in the cancer cells thereby leading to cell death. The treatment can also include applying the low electric field in the absence of a therapeutic agent. This treatment functions because the endocytosis incorporates cell surface receptors into the cytosol. Once these receptors are incorporated into the cell, this down regulates the cell's response, eventually leading to cell death.

[0031] Cell membrane destabilization causes penetration of therapeutic molecules and macromolecules into the cytosolic compartment of the cell via an endocytic process. The process leads to increased vesicle formation with the therapeutic molecules and macromolecules trapped inside the vesicle. Alternatively or additionally the therapeutic macromolecules penetrate the cells via diffusion through the induced structural defects in the lipid bilayer.

[0032] More specifically, a series of pulses, such as a single train or multiple trains of unipolar low-voltage direct current (D.C.) or alternating current (A.C.) pulses, is applied to an environment containing the cells and therapeutic agents. The term "series" is used to designate a single train of voltage pulses or a number of continuous repetitions of the train of voltage pulses. These low voltage pulses can be unipolar, bipolar or alternating bipolar. This step of the process is a critical difference from other processes, such as electroporation.

[0033] In vitro, for cells growing in suspension, the cells are suspended in a medium of low conductance in order to limit electric heating effects and/or electrolytic reactions at the electrode—medium interface. Such medium can consist of 300 mM sucrose or mannitol in the presence of a small amount of a buffer (e.g. 1 to 3 mM Hepes) of a pK in the pH range of 7.0 to 8.0. In some cases, 5% glycerol can be added to the medium in order to enhance incorporation. Following the exposure to the electric field, the cells are washed with PBS or growth medium and finally, the medium is replaced with fresh growth medium in the presence of 5%-10% FCS (fetal calf serum) or bovine calf serum.

[0034] The train or series of trains of unipolar voltage pulses is applied by the application of two electrodes to the suspension. The electrodes can be made from different metal with the preference of using inert and non-polarizable electrodes (e.g., platinum or Ag/AgCl electrodes, correspondingly).

[0035] In vitro, for cells growing while attached or adhered to a surface such as a petri dish or culture flask, the cells are adhered to a surface by growth in a suitable growth medium. The growth medium is then removed and replaced with a suitable incubation medium such as BGJ media supplemented with 10% FCS. An alternating current field (60 V/cm at 30 kHz) is then applied to the cells. Following exposure to the field, the cells are washed with PBS or medium and finally replaced with fresh grown medium. Unlike the treatment of suspended cells, the incorporation of therapeutic macromolecules is carried out in the presence of a very conductive medium. That is, therapeutic macromolecules are incorporated into cells in the presence of a highly conductive medium.

[0036] The present invention can also be applied to enhance incorporation of therapeutic molecules into adherent cells by utilizing both the method and apparatus of the present invention. For use with adherent cells, there is no

need to change the growth medium of the cells to a low conductivity medium. That is, the same medium used for cell growth can be utilized for incorporating the therapeutic macromolecule.

[0037] The present invention can also be utilized in vivo by application of the molecules to be introduced at the site or area containing the cells which are the target of the introduction. Sites such as skin and internal tissues can be targets either non-invasively or invasively, respectively.

[0038] Therapeutic agents include any molecules capable of incorporation by the present invention, which can be defined over a wide range. This range includes agents known in the art, whether electrically charged or neutral. This can include, but is not limited to, DNA, antisense, enzymes, taxol etc. The endocytotic event is independent of any electrical charge of the agent. Further, the present invention induces increased incorporation of the agent into the cell. Accordingly, the process increases drug uptake and thereby, increases drug potency at a desired cell site. That is, the present invention enables an efficient introduction of therapeutic molecules and macromolecules into living cells in vitro and in vivo. These therapeutic molecules are within an extremely wide range of molecular weight and size. Moreover, transfection with different DNA vectors is possible by use of the present inventive method either by itself or in combination with other methods of transfection, thereby enhancing the effectiveness of those methods. Other existing methods of incorporation include incorporation of therapeutic macromolecules based on the use of chemical compounds e.g., DNA transfections based on the use of calcium phosphate precipitation. The therapeutic agents can also include antisense oligonucleotides.

[0039] As stated above, the present invention is based on an electric field induced relative movement of charged or neutral therapeutic molecules, charged therapeutic macromolecules, or charged liposomes toward cells (or the converse) using appropriate electric field parameters which yield an accumulation of the charged entities near the cell membrane. These charged therapeutic agents can either be charged initially or can be neutral agents to which a charge is added. Unlike prior art methods, the present invention does not use either high voltages, such as those used with electroporation, or frequencies which create summation of voltage pulses in a way where the effective pulse is the summation of several pulses, resulting in equilibrating molecules between the extracellular and intracellular compartments. This method differs from electroporation (or the equivalent terms) by the following features:

[0040] 1. The electric parameters used by the present invention to efficiently incorporate therapeutic molecules/macromolecules into cells induce a transmembrane potential difference across the cell membrane which is much lower than the known threshold of transmembrane potential difference which causes electroporation to take place.

[0041] 2. Electroporation and consequent uptake, under specific electric field parameters, were shown to be proportional to the volume of the biological object (e.g. cell, bacteria etc.) which is exposed to the electric field. Under the experimental conditions of the present invention, the uptake by the method of the present invention is independent of the volume of the exposed biological object and can be proportional to the surface area of the object.

[0042] 3. Uptake is not limited by the electrochemical potential difference across the membrane. Under conditions of electroporation, at most, the same concentration of therapeutic molecules/macromolecules in the cytosol and in the external medium can be achieved. Under the experimental conditions, applications have demonstrated up to two orders of magnitude increase of therapeutic molecules/macromolecules concentration in the cytosol as compared with the concentration in the external medium.

[0043] In order to achieve an increased incorporation of the therapeutic molecules into cells, the cell suspension or the cells adhered to a surface in the presence of the chemotherapeutic molecular entity to be incorporated are exposed to an electric field produced by several trains of electrical pulses. The amplitude of the voltage in each train or series of trains of pulses is in the amplitude range of 1 V/cm to 150 V/cm. The frequency range of the pulses of different shapes is from 1 Hz to 50 MHz processing pulse with widths of 20 ns to 20 ms, in the case of unipolar pulses (D.C.), and 5-500 kHz for bipolar pulses of and different shapes. The exact field characteristics to be applied depend upon the charge and the molecular weight of the specific therapeutic molecules to be incorporated.

[0044] The present invention has possible applications in various fields. The present invention can be used to load drugs into cells for slow drug release. In this application, the drugs are the molecules being transferred into the cells via the present invention. Other molecules, such as dyes and tracers, can be loaded into cells for imaging-based diagnosis. In-situ enzymology (loading agents for diagnostics) can also be accomplished. Loading of antibodies for therapy or diagnostics can be carried out.

[0045] The present invention can be used for incorporating therapeutic drugs into specific cell or tissue types, for example loading of drugs into tumor tissue for cancer therapy. Likewise, enzymes can be loaded into cells for specific purposes. Specifically, the present invention enables therapeutic agents to be endocytotically incorporated into the intracellular space without losing any therapeutic functions. This increases the effectiveness of therapeutic agents without necessitating an increase in the amount of the agent being administered. Additionally, multiple therapeutic agents can be administered in a single treatment.

[0046] Another area for use of the present invention, as discussed above, is in the field of genetic engineering and gene therapy. The present invention can be used as a means of transfection, as well as tissue gene therapy. The gene therapy can be used to make a cell more susceptible to treatments including, but not limited to, radiation and chemotherapy.

[0047] For example, an antisense oligonucleotide can be incorporated into a cell, whereby upon incorporation the cell is made luminescent and thus more easily recognized for surgery. Alternatively, the antisense oligonucleotide can be programmed to induce cell death.

[0048] Additionally, exposure of primary tumors to low electric fields, in the presence of extracellular therapeutic agents, increases the incorporation of the agents into the intracellular compartment. This increases tumor cell destruction, and renders the tumoral mass more antigenic

and more accessible to the host's immune response. Activation of inflammatory and immunologic responses facilitates the recognition and elimination of tumor cells at primary and metastatic tumor sites. These responses can be individually or collectively stimulated.

[**0049**] The immunologic response can be augmented by the application of immunostimulatory agents. Such agents can be selected from the group including agents such as cytokines, thymic factors, bacteria derived immunostimulants, and other agents known in the art. These can be combined with other therapeutic agents to effect a combinatorial treatment. That is, the immunostimulator can be induced and is induced during the application of other therapeutics. Hence, augmentation by the application of immunostimulants can be combined with treatment of therapeutic agents. Various combination therapies can also be augmented by increased cellular uptake pursuant to the present invention.

[**0050**] The field of gene therapy can also use the methods of the present invention to activate an immunological or inflammatory response. This occurs, for example, by incorporating genetic material into the target cell. The genetic material through translation, creates an inflammatory or immunological response by causing an antigen or other signal to be displayed on the cells surface.

[**0051**] The above list of utilities is only a sampling of the possibilities for which the present invention can be used. The list is intended to be exemplary and not exhaustive of the uses of the present invention.

[**0052**] The above discussion provides a factual basis for the use of electroendocytotic chemotherapy. The methods used with and the utility of the present invention can be shown by the following non-limiting examples and accompanying figure.

EXAMPLES

Example 1

Electroendocytotic Chemotherapy (EECT) as Treatment Modality of Primary and Secondary Tumors

[**0053**] The Endocytosis-like process is a mechanism of internalization of macromolecules. Endocytosis includes a complex sequence of membrane-linked processes that results in the uptake of extrinsic substances by binding to the cell surface, or incapsulation in the endocytotic formed vesicles, maturation of endocytotic vesicles, and partial transfer to lysosomes (Mellman, 1996).

[**0054**] Exposure of membrane vesicles and cells to low electric fields can generate, among others, electrophoretic lateral mobility of charged proteins and lipids in the plane of the cell membrane (Poo, 1981; Brumfield et al., 1989). Also generated is an induction of a cross membrane potential difference across the membrane (Farkas et al., 1984).

[**0055**] Exposure of cells to low electric fields induces endocytosis-like processes which results in structural changes in the cell membrane, vesiculation phenomenon, and cytoskeleton involvement in incorporation of macromolecules.

[**0056**] The results showed that the exposure of cells in suspension or in a monolayer to trains of pulsed electric DC fields (in the range of 0-100 Vcm^{-1}), and AC fields (peak-to-peak amplitude 0-60 Vcm^{-1}) leads to an efficient uptake of macromolecules possessing molecular weight in the range of 1-2000 kDa. The uptake of the macromolecules into cells, following exposure to pulsed low electric fields, does not involve electric breakdown of the membrane (electroporation). This is supported by the fact that the magnitudes of the applied electric fields are too low to induce permeability changes of the membrane (Rosemberg and Korenstein, 1990; 1997).

[**0057**] Since the efficiency of electroporation-based incorporation of molecules is restricted, primary tumors were exposed to a low electric field, in the presence of a chemotherapeutic agent in the extracellular compartment of the tumor.

[**0058**] This methodology termed electroendocytotic-like chemotherapy (EECT) has several advantages over the alternative ECT method based on electroporation:

[**0059**] (i) It possesses a higher efficiency of incorporation of molecules into cells, both in regard to the final intracellular concentration, and to the molecular size of the incorporated molecule; and

[**0060**] (ii) The application of low electric fields is safer for in vivo and clinical use.

[**0061**] Exposure of primary tumors to low electric fields, in the presence of extracellular therapeutic agents, increases the incorporation of the agents into the intracellular compartment. This increases tumor cell destruction, and renders the tumoral mass more antigenic and more accessible to the host's immune response. Activation of inflammatory and immunologic responses facilitates the recognition and elimination of tumor cells at primary and metastatic tumor sites.

[**0062**] EXPERIMENTAL RESULTS

[**0063**] Experimental System:

[**0064**] An experimental protocol suitable for evaluating clinical relevance, was developed. These experimental models of tumor growth and spontaneous metastases in mice were used in the present application (Ophir et al 1999, Rashid et al 1996).

[**0065**] Tumors

[**0066**] Highly metastatic and weakly immunogenic clones of the tumor cell line B16-F10.9 melanoma were used. Also used were DA-3 mammary adenocarcinoma, a weakly immunogenic tumor that metastasizes in the lung after a long period, in the presence of a primary tumor.

[**0067**] In Vivo Development of Tumors

[**0068**] C57BL/6 mice were injected subcutaneously with 2×10^5 B16-F10.9 tumor cells. BALB/c mice were inoculated subcutaneously (4×10^5) with the metastatic Mammary Adenocarcinoma DA-3 tumor cell line.

[**0069**] EECT Treatment:

[**0070**] C57BL/6 and BALB/c mice were treated by a single EECT treatment when the tumor reached the size of four to five mm in diameter. EECT was performed with a unipolar pulsed field strength of 40 V/cm having a repetition frequency of 500 Hz; a pulse width of 180 μs; and a distance between electrodes of 5 mm. The electric pulse was applied for 10-15 minutes.

[**0071**] To expose tumors to electric fields, stainless Teflon® coated steel needles, soldered at their ends with thin

silver wires, were used as electrodes. The needles penetrated into the tumor with one cathode needle in the middle of the tumor and three anodes around it, 5 mm from the cathode and connected to electric pulse generator.

[**0072**] The doses for intra-tumoral chemotherapy treatment were as follows: for 5-FU, a dose of 75 mg/kg; for taxol, a dose of 20 mg/kg.; and for cisplatin, a dose of 4 mg/kg.

[**0073**] Experimental Parameters:

[**0074**] Effects of EECT on tumor growth in vivo were tested with regard to primary tumor reductions and mean survival time or median.

[**0075**] The statistical significance ($p \leqq 0.05$) of the differences in survival periods between groups was assessed by the two sided Mantel-Cox test.

[**0076**] Results

[**0077**] A. In Vivo Response of C57BL/6 Mice Bearing B16-F10.9 Melanoma to EECT as Compared to Chemotherapy and Electrostimulation Alone:

[**0078**] Mice bearing subcutaneous B16-F10.9 tumors were treated with chemotherapy intratumorally (i.t.) and/or with electric stimulation (ES). The exposure to electric field was carried out three to four minutes after i.t. injection of the cytotoxic drug for a ten minute duration. The mortality of B16-F10.9 melanoma bearing mice (n—number of animals in group) after electroendocytotic-like chemotherapy (EECT) is displayed in FIGS. **1-3**.

[**0079**] Cisplatin was the first anti-cancer drug tested as a candidate for EECT. The results presented in **FIG. 1** clearly show a significant difference in life expectancy of the experimental groups. Mean survival time±standard error (MST±SE) of EECT treated mice was 50.5±5.2 days, while non treated tumor bearing mice had a mean survival time of 27.4±1.5 days. Chemotherapy with cisplatin alone and electrostimulation alone posed a survival time of 30.5±1.7 and 33±1.7 days, respectively. Furthermore, in the EECT group 14% of the animals were cured of the tumor.

[**0080**] A similar pattern was obtained when Taxol was used as the cytotoxic agent, and the results presented in **FIG. 2** demonstrate the potential of EECT. All the animals with tumor died within 50 days absent treatment, and Taxol alone prolonged the survival, but no cured animals were observed. Electrostimulation alone extended the survival and cured 10% of the animals, while EECT cured 32% of the tumor bearing animals.

[**0081**] When 5-fluorouracil was used as the chemotherapeutic agent, no animals were cured by the treatment, but the mean survival time increased from 24±0.7 in the control to 33.7±2.5 in the EECT group ($p=0.0001$). The drug alone also extended the life expectancy but to a smaller extent (28.6±1.48) (**FIG. 3**).

[**0082**] Measurements of the tumor size, seven days after the treatment, revealed that EECT caused the most pronounced inhibition of tumor growth, both when Taxol or Cisplatin were used as the chemotherapeutic agents (**FIG. 4**).

[**0083**] B. In Vivo Response of BALB/c Mice Bearing DA-3 Mammary Carcinoma to EECT as Compared to Chemotherapy and Electrostimulation Alone.

[**0084**] The effect of EECT was also tested on a different tumor. Treatment of mice bearing DA-3 mammary carcinoma revealed that in 12% of the animals the tumor disappeared after EECT while all the animals treated with taxol alone died (**FIG. 5**).

[**0085**] C. Effect of EECT on the Development of an Antitumoral Reaction

[**0086**] It was of interest to determine whether mice, that were tumor free after electroendocytotic chemotherapy, were rendered resistant to a tumorigenic dose of B16-F10.9 melanoma cells. For this purpose, mice cured by EECT were injected with 2×10^5. B16-F10.9 cells subcutaneously 120-180 days after initial tumor inoculation. Challenge of these mice with B16-F10.9 cells shows significant survival extension as compared to the first-time inoculated normal mice. Also, there is significant survival difference, following the challenge, between the groups of the animals treated by EECT with cisplatin and by EECT with taxol. MST±SE of first time inoculated normal mice and challenged mice, previously treated EECT with cisplatin and by EECT with taxol, was 31.30±0.67, 48.57±6.08 and 73.24±11.01 days respectively. Moreover, about 23.5% of the challenged mice, earlier treated by EECT with taxol, did not develop tumor at all.

[**0087**] **FIG. 6** shows the resistance of mice, previously bearing B16-F10.9 melanoma and cured by electroendocytotic chemotherapy with cisplatin and taxol, to subsequent inoculation of B16-F10.9 melanoma cells. Mice cured by EECT were challenged with 2×10^5 B16-F10.9 cells subcutaneously 120-180 days after initial tumor inoculation. The data were plotted using Kaplan-Meir technique.

[**0088**] Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

[**0089**] The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

[**0090**] Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

TABLE

Experiment 1.

Groups	Mean survival	pV
1. Control	24.5 ± 0.68	0.0064
"Electr"	27 ± 0.67	
2. Control	24.5 ± 0.68	0.0074
Drug	28.6 ± 1.68	
3. Control	24.5 ± 0.68	0.0001
EECT	33.7 ± 2.50	
4. "Electr"	27 ± 0.68	0.0066
EECT	33.7 ± 2.50	
5. Drug	28.6 ± 1.48	0.037
EECT	33.7 ± 2.50	

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What is claimed is:

1. A method for inducing endocytosis in cells by exposing the cells to a low electric field thereby inducing processes leading to cell death.

2. The method according to claim 1, wherein said exposing step includes incorporating a therapeutic agent into an intracellular compartment of the cells.

3. The method according to claim 1, further including the steps of preparing a suspension of therapeutic agents to be introduced therein, and applying a train of low voltage pulses to the suspension.

4. A method according to claim 1, further including the steps of introducing the therapeutic agent to an area of a population of cells in vivo and applying the low electric field to the area.

5. A therapy comprising a low electric field.

6. The therapy according to claim 5, wherein said low electric field is a train of unipolar or alternating voltage pulses of different width and wave forms having an amplitude of voltage in the range of 1v/cm to 150v/cm at a frequency range of 1 Hz and 50 MHz.

7. The therapy according to claim 6, wherein said therapy includes a therapeutic agent.

8. The therapy according to claim 7, wherein said therapeutic agent is selected from the group consisting essentially of DNA, antisense and enzymes.

9. A method of treating cells comprising the steps of exposing tumor cells to low electric fields.

10. The method according to claim 9, wherein said exposing step includes endocytotically incorporating the therapeutic agents into an intracellular compartment from an extracellular compartment.

11. A method of activating inflammatory and immuologic responses to a cell by exposing the cells to low electric fields.

12. The method according to claim 11, including the step of augmenting the inflammatory and immunologic response by treating with immunostimulatory agents.

13. A treatment for cancer cells by exposing the cancer cells to a low electric field and inducing endocytosis in the cancer cells.

14. The treatment according to claim 13, wherein said inducing step further includes inducing cell death.

15. The treatment according to claim 13, including the step of combinatorially treating by inducing endocytosis while also augmenting an immunological response.

16. The treatment according to claim 15, further including the step of increasing uptake of both a chemotherapeutic agent and an immunostimulating agent.

17. The treatment according to claim 16, wherein said increasing step further includes increasing the uptake of an agent selected from the group consisting essentially of DNA, antisense and enzymes.

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