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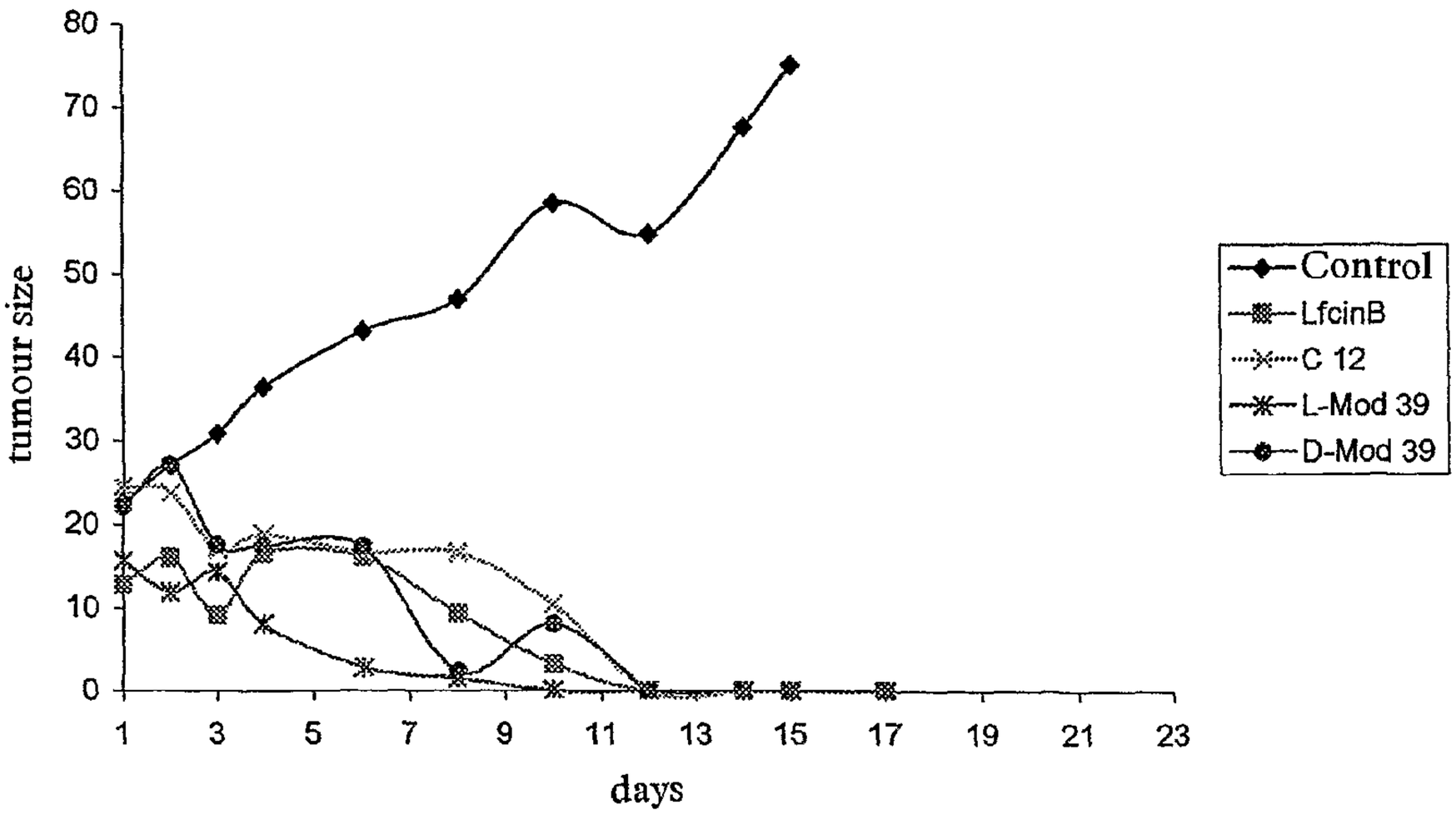
(54) **Titre : INHIBITION DE LA CROISSANCE TUMORALE**
(54) **Title: INHIBITION OF TUMOUR GROWTH**



(57) **Abrégé/Abstract:**
The present invention provides the use of a lytic compound, in particular a lytic peptide, in the manufacture of a medicament for inducing adaptive immunity against tumour growth or establishment in a subject, as well as methods of cancer treatment and vaccination.

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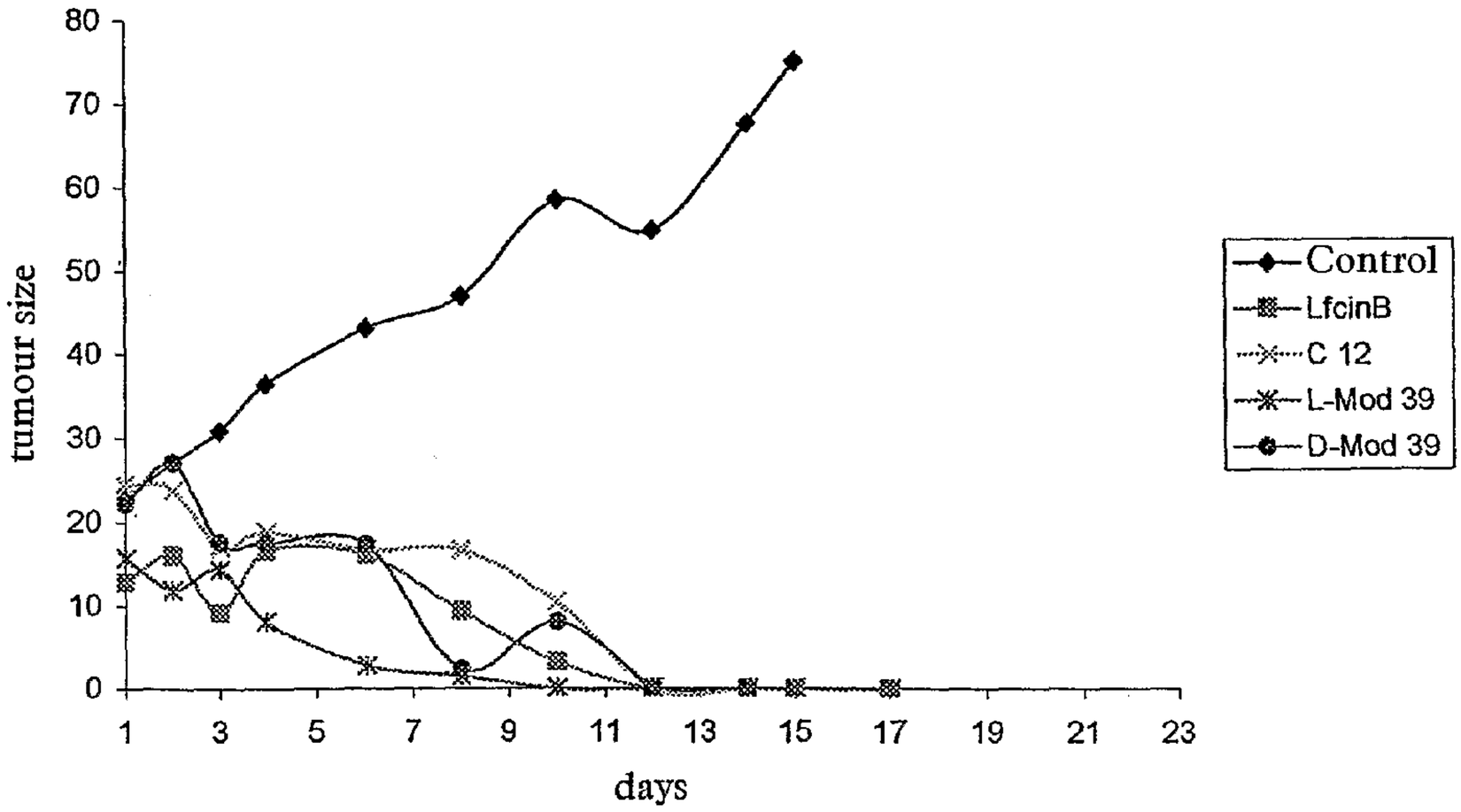

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Inhibition of tumour growth

The present invention relates to a method of treating neoplastic tissue. In particular, it relates to a method of inducing inflammation through the lysis of tumour cells by the use of lytic compounds such as peptides. By triggering an inflammatory response, pathways of the innate and adaptive immune system are activated, facilitating a tumour-specific immune response. Such a triggering of the immune system confers on the subject adaptive immunity against the respective tumour cells thereby inhibiting growth of secondary tumours.

The uncontrolled growth and division of cells may give rise to tumours. Tumours are typically classed as either benign or malignant, based on the criteria of spread and invasion. Malignant tumours are capable of invading and destroying surrounding tissues. Their cells may also spread beyond the original site of the tumour. Benign tumours do not possess these characteristics, but benign tumours may progress to a malignant stage, so it may be useful to treat benign tumours as well as malignant ones. For example, in oral squamous carcinoma neoplasia is not usually treated, but this condition can rapidly progress into a malignant stage where parts or the whole tongue has to be surgically removed. Moreover, benign tumours may still be *per se* undesirable, particularly if they are large and grow adjacent to vital organs, and so treatment of a benign tumour which thereby reduces subsequent similar benign tumours would also be desirable.

The process by which cells from a malignant tumour break away from the primary tumour and spread to other organs in the body by travelling in the bloodstream or lymphatic system is called metastasis. When these cells

reach a new area of the body they may invade tissue and go on dividing and may form a new tumour. Such a new tumour is often referred to as a "secondary tumour".

The growth of secondary tumours usually poses a threat to the health of the subject and there is therefore a need to delevop ways to inhibit the formation or growth of secondary tumours.

Resection of advanced solid tumors is often inefficient in the long term due to the persistence of tumour cells and subsequent growth of previously undectable micrometastasis. Among breast cancer patients this remains a major cause of recurrence and ultimate death. After surgery the disseminated cells often rest in the GO phase of the cell cycle. Such non-proliferating cells are therefore often resistant to chemotherapy. The cure-rates for advanced head and neck cancer patients has not improved significantly in the last decade. Most cases of recurrence in these patients are local or regional, and present in such a way as to make them difficult to access. An example is locally advanced esophageal cancers which are often inoperable with a poor prognosis.

A great hurdle in the search for a way to inhibit growth of secondary tumours is that tumours form from cells which originate from the subject's own body. The immune system struggles to recognise them as abnormal. Recognition of foreign or abnormal cells typically involves the detection of molecules located at the cell surface, antigens. Most tumour cells possess at least one kind of antigen which distinguishes them from normal cells and in many cases the antigens are specific for a particular type of cancer. Some tumour cells may possess a variety of antigens, whilst others may only possess a single type of antigen. The type of antigen, the number

of different antigens and the prominence of the antigens on the cell surface may all influence the chances that the immune system may recognise the tumour cells as abnormal. Many types of tumour possess very few antigens, or only antigens which are poorly recognised by the immune system as foreign and are thus capable of escaping recognition and destruction by the immune system. The type and quanity of antigens possessed by any particular tumour type thus plays a big part in determining how "immunogenic" a tumour is. By "immunogenic" is meant the ability to elict an immune response, so the more immunogenic a tumour is, the more likely it is that it will be recognised and attacked by the immune system.

Various attempts have been made to help the immune system to fight tumours. One early approach involved a general stimulation of the immune system, e.g. through the administration of bacteria (live or killed) to ellicit a general immune response which would also be directed against the tumour. This is also called nonspecific immunity.

More recent approaches aimed at helping the immune system specifically to recognise tumour-specific antigens involve administration of tumour-specific antigens, typically combined with an adjuvant (a substance which is known to cause or enhance an immune response) to the subject. This approach requires the *in vitro* isolation and/or synthesis of antigens, which is costly and time consuming. An alternative approach to reduce recurrence rates of different types of cancer is the use of immunotherapy. Most cancers present several challenges to the use of immunotherapy. Often not all the tumour-specific antigens have been identified, e.g. in breast cancer the known antigens are found in 20-30%

of the total tumours. The use of tumour-specific vaccines have therefore met with limited success.

There remains a strong need for alternative methods for inhibiting the growth or formation of secondary tumours.

The present inventors have surprisingly found that a lytic compound may be used to lyse cells of a first tumour in a patient and thereby inhibit growth of further tumours in said patient. This effect has been demonstrated, as described herein in the Examples, using a lytic peptide. Other lytic compounds are known in the art and Examples include detergents such as Triton X-100 and acids such as HCl.

The use of lytic peptides for the treatment of tumours has been proposed in the art based on their ability to lyse tumour cells (Risso et al., Cell. Immunol. [1998] 107 and WO 01/19852). The finding that such lytic peptides may be used not only to treat a first tumour, but also to inhibit the growth of a second tumour was completely unexpected.

Without wishing to be bound by theory, it is suspected that lysis of the first tumour elicits an inflammatory response. The lysis may cause the exposure of antigens specific for the cancer cell. By "exposure" is meant that the antigen is made available to be recognised as foreign by the immune system.

Thus "exposure" includes making an antigen more readily accessible for the immune system and/or presenting it to the immune system in such a way that it is more likely to be recognised by the immune system, e.g. because it is on a cell fragment, rather than a whole cell. Thus, the term "exposure" includes the release of antigens from an intracellular space but also any other change in

the cell structure which results in an antigen becoming more readily recognisable to the immune system.

The exposed antigen may activate specific B cells and/or T cells of the immune system and cause some of these to mature into memory cells. Memory cells typically have a very long life span and when they encounter the same antigen for a second or further time they are able to respond more readily than virgin B or T cells. This process of generating and maintaining specific memory cells is commonly referred to as an "immunological memory" or "adaptive immunity". Thus, the present inventors have surprisingly found that lytic agents such as peptides may be used to induce an immunological memory against tumours.

The present inventors have demonstrated that by succssful treatment of a tumour with a lytic compound, growth of a second tumour is not observed. In a model experiment, adoptive transfer of spleen (immune) cells from an animal previously successfully treated (cured) with a lytic compound, was shown to confer specific immunity to the naïve acceptor individual. Thus, acceptors which received spleen cells from previously cured mice were able to eliminate implanted tumours, whereas acceptors which received spleen cells from naive mice were unable to eliminate implanted tumours. These results demonstrate that the protective effect is due to the previous successful tumour eradication conferring a long-term, specific immunity against further tumours, in particular further tumours of the same type.

Furthermore, tumours often induce general immune suppression and so the triggering of the immune system observed acording to the present invention is highly advantageous.

Thus in one aspect, the present invention provides a method of inducing adaptive immunity in a subject, which comprises administration of an effective amount of a lytic compound to said subject wherein the lytic compound, through lysis of cells in a first tumour, generates an immune response which inhibits the growth or establishment of a second tumour.

At its simplest, the present invention provides a method of inducing adaptive immunity in a subject, which comprises administration of an effective amount of a lytic compound to said subject.

Adaptive immunity will be understood, in the present context, as immunity against tumour growth or establishment, in particular against tumours which are the same or similar to a tumour which has been directly targetted for lysis by said lytic compound. The lytic compound is therefore designed or selected to lyse tumour cells.

Alternatively viewed, the present invention provides a method of cancer treatment in a subject which comprises administration of an effective amount of a lytic compound to said subject, wherein the lytic compound, through lysis of cells in a first tumour, generates an immune response which inhibits the growth or establishment of a second tumour.

Viewed another way, the inventors have found that lytic compounds may be used in the treatment of a first tumour to generate a vaccine against a second tumour. The vaccine is generated *in situ*, i.e. the antigens which induce an immune response and create an immunological memory are presented to the immune system as a consequence of the lysis of the tumour cells. Such a vaccine where lytic compounds such as peptides are

administered to a subject to generate antigens *in situ* (*in vivo*) represents a radical departure from the prior art, where antigens are typically prepared in the laboratory (i.e. *in vitro*) and are administered to the subject.

Thus in a further aspect the invention provides use of a lytic compound in the manufacture of a medicament for use as a vaccine against tumour growth or development. 'Growth and development' includes establishment of a tumour. The invention also provides a method of vaccinating a subject against tumour growth or development through administration of a lytic compound to said patient, preferably a lytic peptide. Reference to a 'vaccine' and 'vaccinating' both imply a prophylactic effect, thus while there may be beneficial direct treatment of existing tumours, a significant motivation is the prevention or reduction in future tumour establishment, growth or development.

Not wishing to be bound by any particular hypothesis, it is believed that the lytic event induces an inflammatory response that seems to be important in the eradication of the first tumour as well as inducing adaptive immunity protecting against one or more second tumours. This is illustrated by the inventors' findings that they would very often succeed in obtaining full regression of a first tumour in syngenic animal models (with intact immune systems), whereas in nude mice (without a functioning immune system), they have not been able to achieve more than 50% growth inhibition of a first tumour. Hence, it may be sufficient to lyse parts of a first tumour which may promote a directed immune response towards remaining cells of the tumour, as well as inducing a protective antitumour memory against secondary tumours.

Thus adaptive immunity against a tumour is generated in the subject, particularly against tumours which are of the same type or similar to the first, lysed, tumour.

The invention also provides the use of a lytic compound in the manufacture of a medicament for inducing adaptive immunity in a subject. In particular the invention provides the use of a lytic compound in the manufacture of a medicament for inducing adaptive immunity in a subject, wherein the lytic compound, through lysis of cells in a first tumour, generates an immune response which inhibits the growth or establishment of a second tumour.

The invention also provides a lytic compound for use in inducing adaptive immunity in a subject. More particularly the invention provides a lytic compound for use in inducing adaptive immunity in a subject, wherein the lytic compound, through lysis of cells in a first tumour, generates an immune response which inhibits the growth or establishment of a second tumour.

Thus, by a "first tumour" is meant the tumour which has been identified in the subject and which it is intended to treat by causing direct and immediate lysis thereof. The first tumour will typically be a primary tumour, i.e. the first tumour of its kind to develop and/or be identified in the subject. However, the "first tumour" may in fact be a secondary tumour. Such a situation may arise for example where a primary tumour was removed from the subject (surgically or otherwise). Thus by "first tumour" is not necessarily meant the first tumour to develop in the subject; the term "first" is used in relation to the sequence of events of the method of the present invention.

The lytic compounds will typically be administered locally to the first tumour, e.g. injected into the first tumour or in its immediate vicinity, although systemic delivery is also contemplated. Injection solutions may, for example, be produced in a conventional manner, such as by the addition of preservatives such as p-hydroxybenzoates, or stabilisers such as EDTA. The solutions are then filled into injection vials or ampoules.

An objective of the methods and uses of the present invention is to generate an immunological memory and thereby inhibit growth or establishment of a second tumour in a patient who has been subjected to lysis of a first tumour in their body. Inhibition of growth includes regression of the tumour, i.e. when it is reduced in size, preferably to the point where it disappears completely and/or is no longer detectable. Inhibition also includes the prevention of establishment of a second tumour. Thus effective treatments according to the present invention may mean that the patient never develops further detectable tumours after the initial lysis treatment of the first tumour. Inhibition of growth also includes a reduction in the normal rate of tumour growth, slowing or prevention of the establishment of a blood vessel network within the solid tumour.

The term "second tumour" typically refers to secondary tumours, also called metastases, i.e. a tumour which has developed from a cell which has originated from another tumour and has spread to a new site. However, within the scope of the present invention, the term "second tumour" may also include a primary tumour. This situation may arise where two or more tumours co-exist, for example two primary tumours which arose independently, or a primary and a secondary tumour and where a secondary

tumour is treated directly with the lytic peptide to induce an immunological memory against that type of tumour, including the primary tumour.

The term "second" tumour includes literally the second and also any subsequent or further tumours. Thus several secondary tumours may have their growth inhibited according to the present invention. The "second" tumour may also be a tumour that has returned after initial treatment, possibly with conventional therapy (i.e. not necessarily through lysis).

The first tumour and the second tumour preferably have similar immunogenic properties, preferably the first tumour and the second tumour are of the same cancer type. It will be appreciated that within any given tumour not all cells may possess the same phenotype, so the individual cells of a tumour may possess different antigens. This may result in the exposure of a large variety of antigens upon lysis and may provide an immunological memory against a variety of cancer cell types.

Because of the induction of an immunological memory, discussed above, the "second tumour" may not yet exist in the subject or at least not be detectable at the time the lytic compound is administered. Because the primary lytic event has 'primed' the subject and stimulated the immune system it is appropriate to consider that an *in situ* cancer vaccine has been generated.

Throughout the text, any reference to the term "tumour" which is not preceeded by the desingation "first" or "second" is, unless the context clearly suggests otherwise, to be understood to apply both to the first and the second tumour.

References to "lysis" of a first tumour are to be understood to mean lysis of one or more cells of said tumour. Thus lysis of the entire tumour is not required. "Lysis" as used herein includes partial as well as complete lysis of a cell. By partial lysis is meant that the outer cell membrane is sufficiently destabilised to cause cellular components to leak out of the cell and/or to cause fractions of the outer membrane to become detached from the cell. The requirement for antigen presentation does not demand total disintegration of the tumour cells.

Preferably, the tumour is selected from the group consisting of lymphomas, carcinomas and sarcomas, most preferably B-cell lymphoma. Melanomas are also contemplated. In general, the tumours are naturally occurring, pathological tumours; as discussed above, benign tumours may be targetted.

A further preferred application of the present invention is in the treatment of benign tumours, e.g. of oral epithelia. Previously such tumours may not have been treated on first identification, instead subjected to "watch-and-wait". By treating such tumours at an earlier stage the process that might lead to malignant transformation can be stopped. Chemoresistant benign tumours are particularly suitable as targets.

The present invention is not concerned with chemically induced tumours. By "chemically induced tumours" is meant tumours which are deliberately caused to develop by human intervention, typically for research purposes. These are 'unnatural tumours'. An example of a chemically induced tumour is Meth A fibrosarcoma which is induced using methylcholanthrene. Thus all reference herein to tumours should be taken to exclude such chemically induced tumours.

Tumours which arise within a subject as a result of exposure to environmental chemicals without any intention to cause tumour development do not fall within our definition of "chemically induced tumours" and such tumours are thus contemplated by the present invention.

By "environmental chemicals" is meant any chemicals which a subject may naturally come into contact with, such as airborne, water-borne and/or food-borne chemicals which are typically present in low dosis.

The subject may be any human or non-human animal, preferably a mammal, more preferably a human.

By "lytic compound" is meant any compound which is capable of causing animal cells to lyse. Preferably, the lytic compound will have a reasonably high specificity for tumour cells, i.e. it will lyse tumour cells in preference to equivalent healthy cells, to minimize side effects experienced by the subject to which the compounds are administered.

The lytic compound is preferably a peptide. Suitable lytic peptides are known in the art and include for example those described in WO 00/12541, WO 00/12542, WO 01/19852 and WO 01/66147 as well as those described in the following documents: Papo N, Shahar M, Eisenbach L, Shai Y. "A novel lytic peptide composed of DL-amino acids selectively kills cancer cells in culture and in mice". *J Biol Chem* 2003;278(23):21028-23.

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Selsted ME, Novotny MJ, Morris WL, Tang YQ, Smith W, Cullor JS. Indolicidin, a novel bactericidal tridecapeptide amide from neutrophils. *J Biol Chem* 1992;267(7):4292-5.

Lytic peptides are particularly preferred as lytic agents. Typically they have a short half-life, i.e. they generally degrade rapidly after lysing the cells, e.g. due to the releace of proteases and the like from the cells. A short half-life lowers the risk of systemic toxicity and so may be advantageous, but a longer half life may be desirable in some cases. The half-life of peptides may be manipulated, i.e. increased or decreased if desired. For example, the half-life of the peptide may be extended by introducing D- amino acids and/ormodifying the C-terminal and/or N-terminal end.

A further class of preferred lytic compounds are peptidomimetics of known or predicted lytic peptides.

It is now commonplace in the art to replace peptide or protein-based active agents, e.g. therapeutic peptides, with such peptidomimetics having functionally-equivalent activity. Generally such compounds will simply replace the $(-C(R)CONH)-_n$ backbone of the peptide with an

alternative flexible linear backbone, e.g. a $(-C(R)NHCO)-_n$ or $(-C(R)CH_2CH_2)-_n$, or a non-linear backbone (e.g. one based on a string of fused cyclohexane rings). Despite the change in the backbone, the pendant functional groups (the side chains in the peptide original) are presented in a similar fashion allowing the compound to possess similar lytic activity.

Various molecular libraries and combinatorial chemistry techniques exist and are available to facilitate the identification, selection and/or synthesis of such compounds using standard techniques (Kieber-Emons, T. et al. Current Opinion in Biotechnology 1997 8: 435-441).

The peptides will typically be at least 3 amino acids in length, e.g. 4-30, preferably 5-30 amino acids in length, preferably 7-25 amino acids in length and will incorporate one or more, preferably 2-8, more preferably 4-8, positive charges. Preferably the peptides will include groups which are bulky, e.g. 4 or more, more preferably 7 or more, non-hydrogen atoms and lipophilic, these groups are thought to interact with the cell membrane and contribute to lysis, preferably the peptides will have 2-6 of such groups. In a preferred embodiment the lytic peptide contains at least one biphenylalamine (Bip) and/or at least one diphenylalamine (Dip) residue. Further preferred peptides incorporate 1-5, e.g. 2-4, tryptophan residues.

Preferably, the lytic peptide is not a lactoferrin derived peptide, more particularly it is preferably not cyclic LFB (the primary sequence of which is FKCRRWQWRMKKLGAPSITCVRRAF).

The use of esters, amides or cyclic derivatives of peptides or peptidomimetics, in particular of those

peptides mentioned above, is also contemplated by the present invention. The lytic peptide or peptidomimetic (or ester, amide or cyclic derivative thereof) may be used in its free form or e.g. as a conjugate or a salt. The salt will preferably a pharmaceutically acceptable salt, e.g. acetate. In a preferred embodiment, the lytic peptide or peptidomimetic is present as a trifluoroacetate (TFA) salt. Trifluoroacetate is frequently used in chromatographic techniques used to purify peptides after peptide synthesis.

Lytic agents which are not peptides will preferably be delivered intratumorally. Lytic peptides may be delivered in this way but may also be delivered systemically due to their selectivity for tumour cells as compared to healthy cells of the same tissue type. Lytic peptides which are highly selective in this way are preferred. All lytic agents may be targetted to the site of the first tumour in other ways, e.g. using liposome delivery, dextrin-conjugation, or other suitable carrier solutions. Thus systemic delivery is also possible with non-peptide lytic agents.

The lytic compound itself is preferably only weakly immunogenic, more preferably it is not immunogenic at all, i.e. it does not by itself induce an antibody response.

The invention will now be described with reference to the following non-limiting examples in which:

Figure 1 is a graph showing the progress of A20 B-cell lymphoma in Balb/c mice upon treatment of different peptides.

Figure 2 is a graph showing the development of tumours in mice re-inoculated with A20 cells one month after

successful treatment of A20 solid tumours with different peptides.

Figure 3 is a graph showing the effect of re-inoculating A-20 cells in animals that had been successfully treated with Mod 28 or Mod 39.

Figure 4 is a graph showing the effect of re-inoculating A-20 cells in animals that had been successfully treated with C12. The letters a) and b) designate different mice.

Figure 5 is a graph showing the effect of re-inoculating A-20 cells in animals that had been successfully treated with Mod 28 or Mod 39. The positive control shows the growth of A20 cells in mice not pre-treated with peptides.

Figure 6 is a graph showing the primary effect of NDDO1 on C26 colon carcinoma.

Figure 7 is a graph showing the effect of re-innoculating C26 cells in a mouse that had been successfully treated with NDDO1.

Figure 8 is a graph showing adoptive transfer of specific anti-A20 cancer immunity from successfully cured mice (treated with Mod39 lytic peptide) vs. naive, untreated mice. Acceptors that received spleen cells from previously cured mice were able to reject implanted tumours, whereas acceptors that received spleen cells from naive mice were unable to reject the tumour.

Examples

Example 1

a) Syngenic Balb/c mice were used as a model system. The mice were inoculated with cells of A20 B-cell lymphoblast (5x 10^6) though subcutaneous injection. Tumours were allowed to grow to a size of 20-30 mm^2. The mice were randomised into groups of 6-8 and the tumours were treated directly with a peptide selected from Table 1 below. The treatment involved injection of 50 µl of a peptide solution, providing 0.5 mg of peptide once a day for three consecutive days.

Tumour progression was followed by measuring the size of the tumour. As can be seen from Figure 1, the control tumour (untreated) displayed a steady increase in size over 15 days. Treatment of the tumours with the peptides of Table 1 caused a regression in tumour size, leading to an apparently complete disappearance of the tumour.

Table 1: Peptides

Name	Sequence
LfcinB:	H_2N-FKCRRWQWRMKKLGAPSITCVRRAF-COOH
Model 28:	H_2N-KAAKKAAKAbipKKAAKbipKKAA-COOH
Model 39:	H_2N-WKKWdipKKWK-COOH (D and L form)
C12:	H_2N-KAAKKAbipKAAKAbipKKAA-COOH

bip = biphenylalanine

dip = diphenylalanine

b) Mice in which a A20 B-cell lymphoblast tumour had successfully been eradicated as described above were re-inoculated with 5x 10^6 cells of A20 B-cell lymphoma tumour. Untreated mice served as a negative control. No further administration of peptides or any other anti-

tumour agents took place. The results are shown in Figure 2.

When untreated mice were inoculated with tumour cells, significant tumour growth occured. When mice previously treated with cLfinB were re-inoculated with tumour cells, some initial tumour growth occurred, but at day 1 the tumour was significantly smaller than the tumour in the control mice, and no further growth occurred. Some tumour regression was even noted.

Inoculation of mice previously treated with C12 or L-Mod 39 with tumour cells initially resulted in the appearance of a very small tumour, which completely disappeared after 6 or 10 days respectively.

Example 2

The antitumoral activity of three different peptides against A20 B-cell lymphoblast tumours was studied in syngenic Balb/c mice. The peptides were Model 28, Model 39 and C12 as defined in Example 1. Tumour cells (5 x 10^6) were inoculated subcutaneously on the abdomen of the mice and grown into proper size (20-30 mm^2) before peptide treatment. The mice were randomised in groups of 5-6 and the tumours were treated intra-tumorally with 0.5 mg/50 µl peptide once a day for three consecutive days. The tumour size (mean of transversal and longitudinal) was measured with an electronic calliper. Three weeks later, mice that were successfully treated, i.e. showing a full regression of the tumour, received the same number and the same type of tumour cells at similar conditions as described above. The results are presented in Figure 3-5.

Example 3

The antitumoural activity of the peptide, Ad-LFB 14-31 A2,3,6,10,17,F7,R4,K11,L14-NH2, (NDDO1) was tested in a murine C26 colon carcinoma model established in syngenic Balb/c mice. C26 cells (5 x 10^6 cells in 50 µl) cells were inoculated subcutaneously on the abdomen of the mice (3 animals) and grown into proper size (20-30 mm^2) before treatment start. At day 7 the tumours were treated intra-tumourally with 0.5 mg/50 µl peptide once a day for three consecutive days and the progression was followed. In one mouse full tumour regression was obtained (Fig. 6). In this mouse C26 tumour cells (5 x 10^6 cells in 50 µl) were re-inoculated subcutaneously three weeks after peptide treatment. A regression of the tumour after an initial growth was obtained without any further treatment (Fig. 7), suggesting that the mouse had acquired an adaptive immune response.

Example 4

Initial tumour treatment

Syngenic Balb/c mice were selected as a model system to investigate the long-term anti-tumour immunity conferred by an initial treatment with a lytic peptide (H_2N-WKKWdipKKWK-COOH - Mod 39) against A20 B-cell lymphoblastoma. Tumour cells (5 x 10^6) were inoculated subcutaneously on the abdomen of the mice and grown into proper size (20-30 mm^2) before treatment start. The mice were treated intra-tumourally with 0.5 mg/50 µl peptide once a day for three consecutive days and the progression was followed. Mice that were successfully treated were selected as donors (treated donor) for adoptive transfer of spleen cells, 3 weeks after tumour eradication.

Transfer of spleen cells

Day 1 - Naive acceptor mice were subjected to a Total Body Irradiation (TBI) of 500 cGy in preparation for receiving adoptive transfer of immune cells from spleen donors.

Day 2 - Single-cell suspensions of spleenocytes depleted of red blood cells from treated donor mice and naïve mice were prepared as previously described (Ward, B.A. et al., J. Immunology Aughust 1988, vol 141 p1047), except that sterile H_2O was used instead of ammonium chloride to eliminate red blood cells.
Donor spleen cells (approximately 40 million) were injected i.v. into the tail vein of acceptor mice according to the methods described in Bogen B. et al., Eur J Immunology, May 1983, vol 13(5), pages 353-359.

Day 3 - Tumour cells (5×10^6) were inoculated subcutaneously on the abdomen of the mice and growth was followed by measuring tumour size with a calliper.

The results demonstrate that the transfer of immune cells from a previously successfully treated mouse confers immunity towards the same tumour type in the acceptor mice (figure 8 - "Cured" group). In contrast, after transfer of immune cells from naïve donor mice the implanted tumour does not regress (figure 8 - "Naive" group). These results demonstrate that the protective effect is due to the previously successful tumour eradication that confers a long-term, specific immunity against the tumour type.

Examples 1-3 were carried out using the trifluoroacetate (TFA) salt form of the peptides referred to.

CLAIMS

1. Use of a membrane disrupting lytic peptide in the manufacture of a medicament for inhibiting the growth or establishment of a second tumour in a subject, wherein the lytic peptide, through lysis of cells in a first tumour, generates an immunological memory which inhibits the growth or establishment of said second tumour.

2. Use of a membrane disrupting lytic peptide in the manufacture of a medicament for use in generating an *in situ* vaccine against the establishment, growth or development of a second tumour in a subject, wherein the lytic peptide, through lysis of cells in a first tumour, generates an *in situ* vaccine against the establishment, growth or development of said second tumour.

3. A use according to claim **1** or **2**, wherein the peptide is at least 3 amino acids in length, and incorporates one or more positive charges.

4. A use according to claim **3**, wherein the peptide includes groups which are bulky and lipophilic comprising 4 or more non-hydrogen atoms.

5. A use according to claim **4**, wherein the bulky and lipophilic groups have 7 or more non-hydrogen atoms.

6. A use according to claim **5**, wherein the lytic peptide contains at least one of a biphenylalamine (Bip), a diphenylalamine (Dip) residue and 1-5 tryptophan residues.

7. A use according to any one of claims **1** or **3** to **6**, wherein the inhibition of growth is regression of the second tumour.

8. A use according to any one of claims **1** or **3** to **7**, wherein the inhibition of growth includes the prevention of establishment of the second tumour.

9. A use according to any one of claims **1** to **8**, wherein the second tumour is a secondary tumour.

10. A use according to any one of claims **1** to **9**, wherein the first tumour and the second tumour have similar immunogenic properties.

11. A use according to claim **10**, wherein the first tumour and the second tumour are of the same cancer type.

12. A use according to any one of claims **1** to **11**, wherein the first and/or second tumour is selected from the group consisting of lymphomas, carcinomas and sarcomas.

13. A use according to any one of claims **1** to **11**, wherein the first and/or second tumour is a benign tumour.

14. A use according to any one of claims **1** to **13**, wherein the subject is a human.

15. Use of a membrane disrupting lytic peptide for inhibiting the establishment, growth or development of a second tumour in a subject, wherein the lytic peptide, through lysis of cells in a first tumour, generates an immunological memory which inhibits the growth or establishment of said second tumour.

16. Use of a membrane disrupting lytic peptide for generating an *in situ* vaccine against the establishment, growth or development of a second tumour in a subject wherein the lytic peptide, through lysis of cells in a first tumour, generates an *in situ* vaccine against the establishment, growth or development of said second tumour.

17. The use according to claim **15** or **16**, wherein the peptide is at least 3 amino acids in length, and incorporates one or more positive charges.

18. The use according to claim **17**, wherein the peptide includes groups which are bulky and lipophilic.

19. The use according to claim **18**, wherein the bulky and lipophilic groups have 7 or more non-hydrogen atoms.

20. The use according to claim **19**, wherein the lytic peptide contains at least one of a biphenylalamine (Bip), a diphenylalamine (Dip) residue and 1-5 tryptophan residues.

21. The use according to any one of claims **15** or **17** to **20**, wherein the inhibition of growth is regression of the second tumour.

22. The use according to any one of claims **15** or **17** to **21**, wherein the inhibition of growth includes the prevention of establishment of the second tumour.

23. The use according to any one of claims **15** to **22**, wherein the second tumour is a secondary tumour.

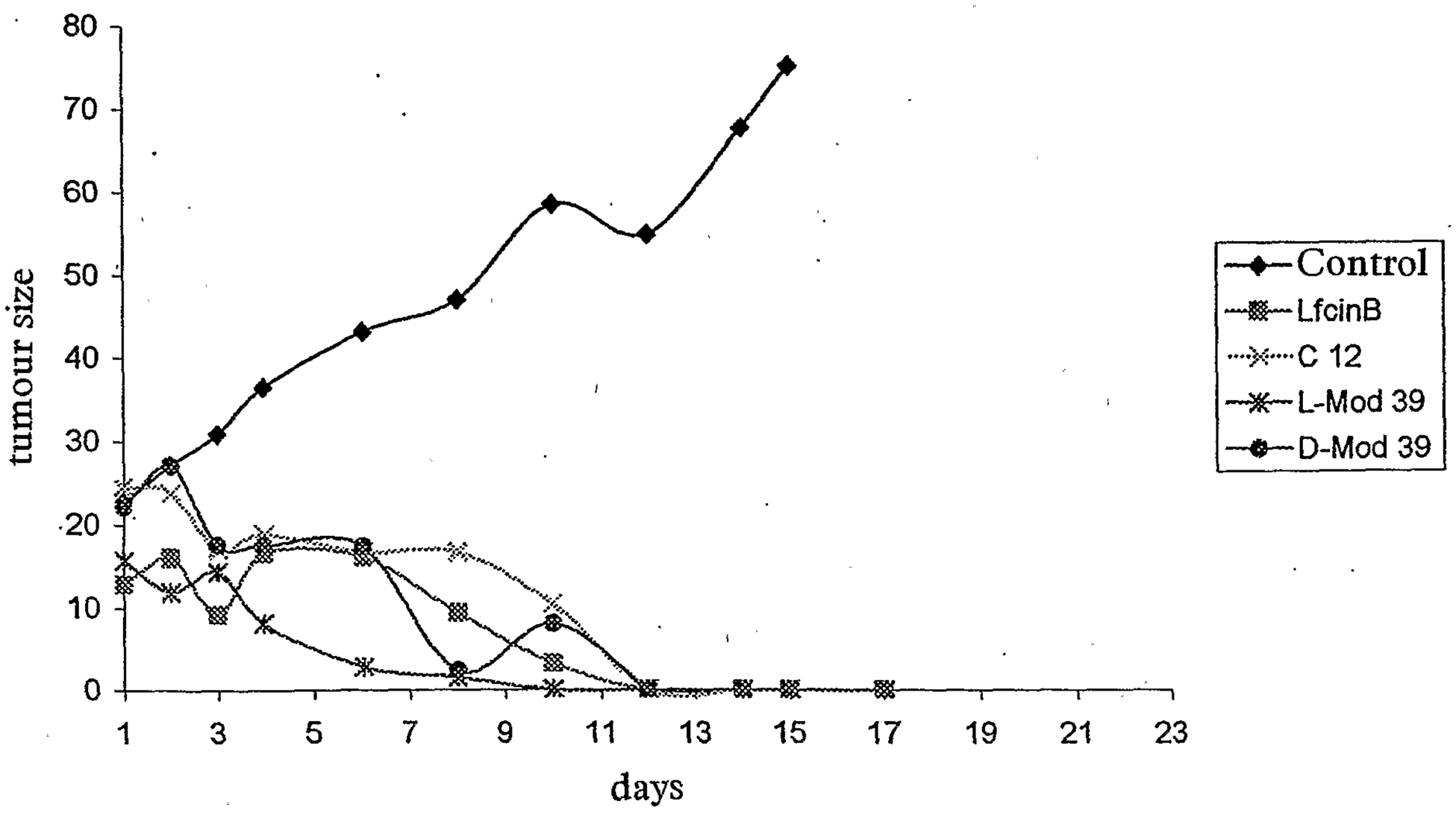
24. The use according to any one of claims **15** to **23**, wherein the first tumour and the second tumour have similar immunogenic properties.

25. The use according to claim **24**, wherein the first tumour and the second tumour are of the same cancer type.

26. The use according to any one of claims **15** to **25**, wherein the first and/or second tumour is selected from the group consisting of lymphomas, carcinomas and sarcomas.

27. The use according to any one of claims **15** to **25**, wherein the first and/or second tumour is a benign tumour.

28. The use according to any one of claims **15** to **27**, wherein the subject is a human.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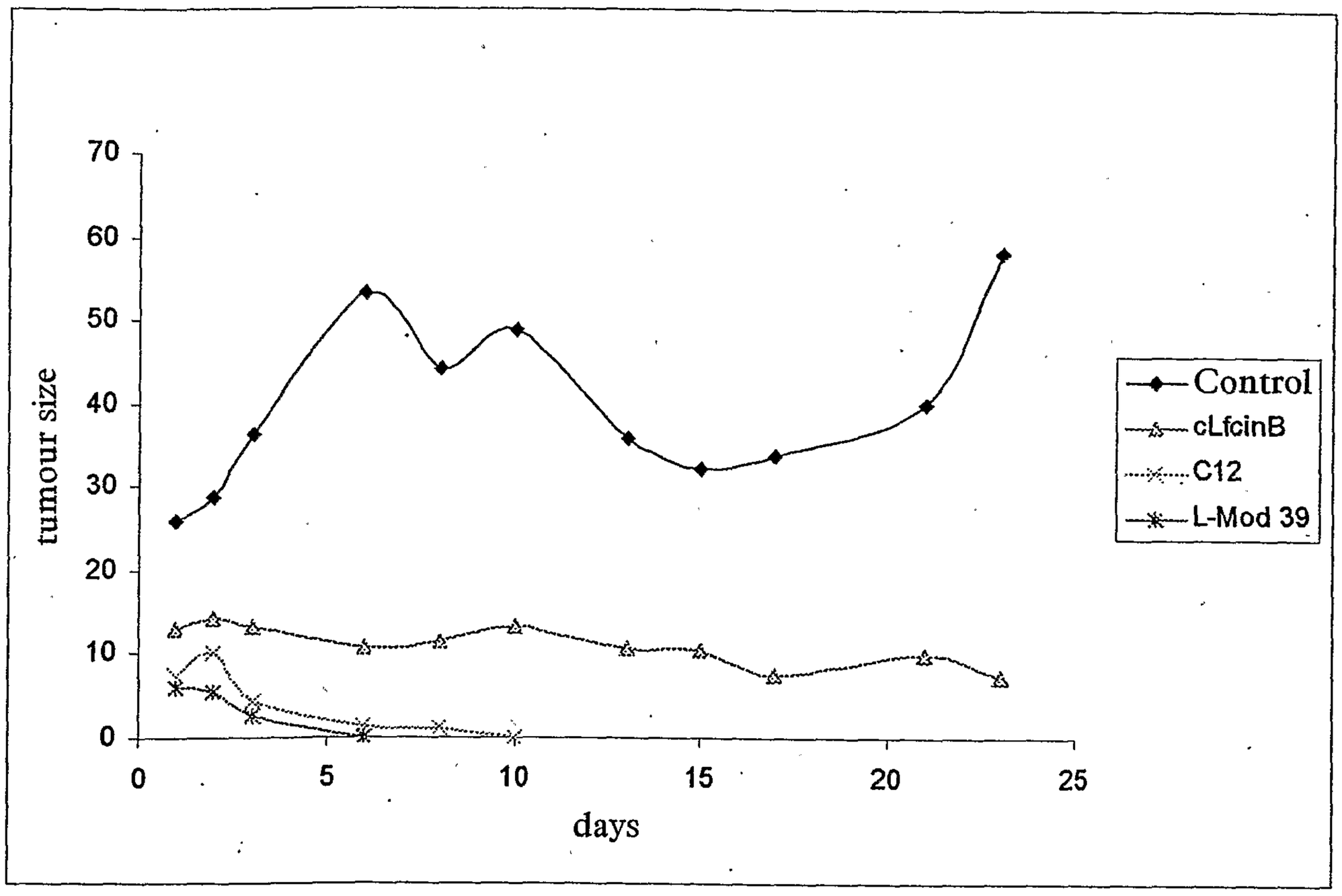
tumour size
1
3
5
7
9
11
13
15
17
19
21
23
days
Control
LfcinB
C 12
L-Mod 39
D-Mod 39

Figure 1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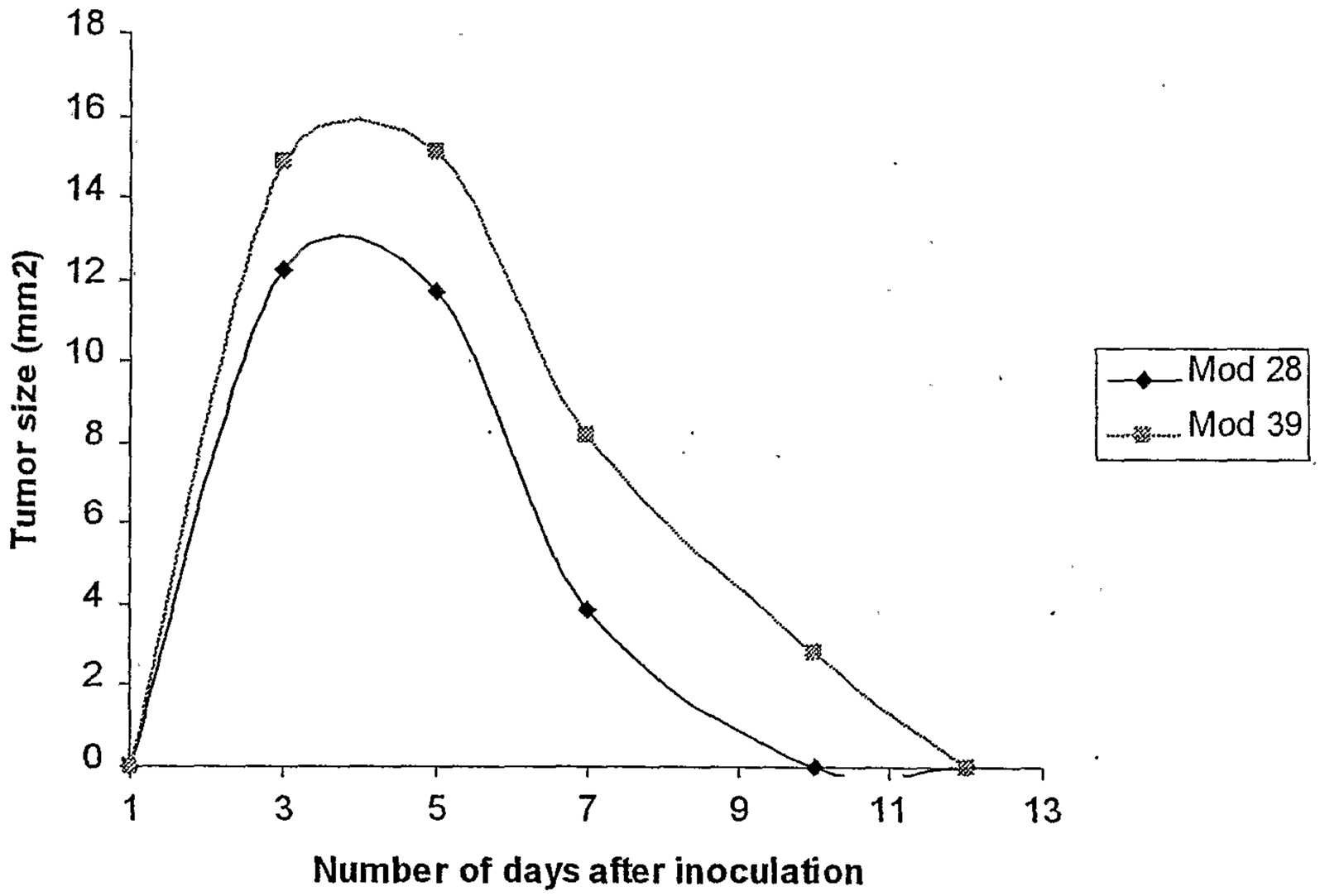
25
days
Control
cLfcinB
C12
L-Mod 39

Figure 2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16
14
12
10
8
6
4
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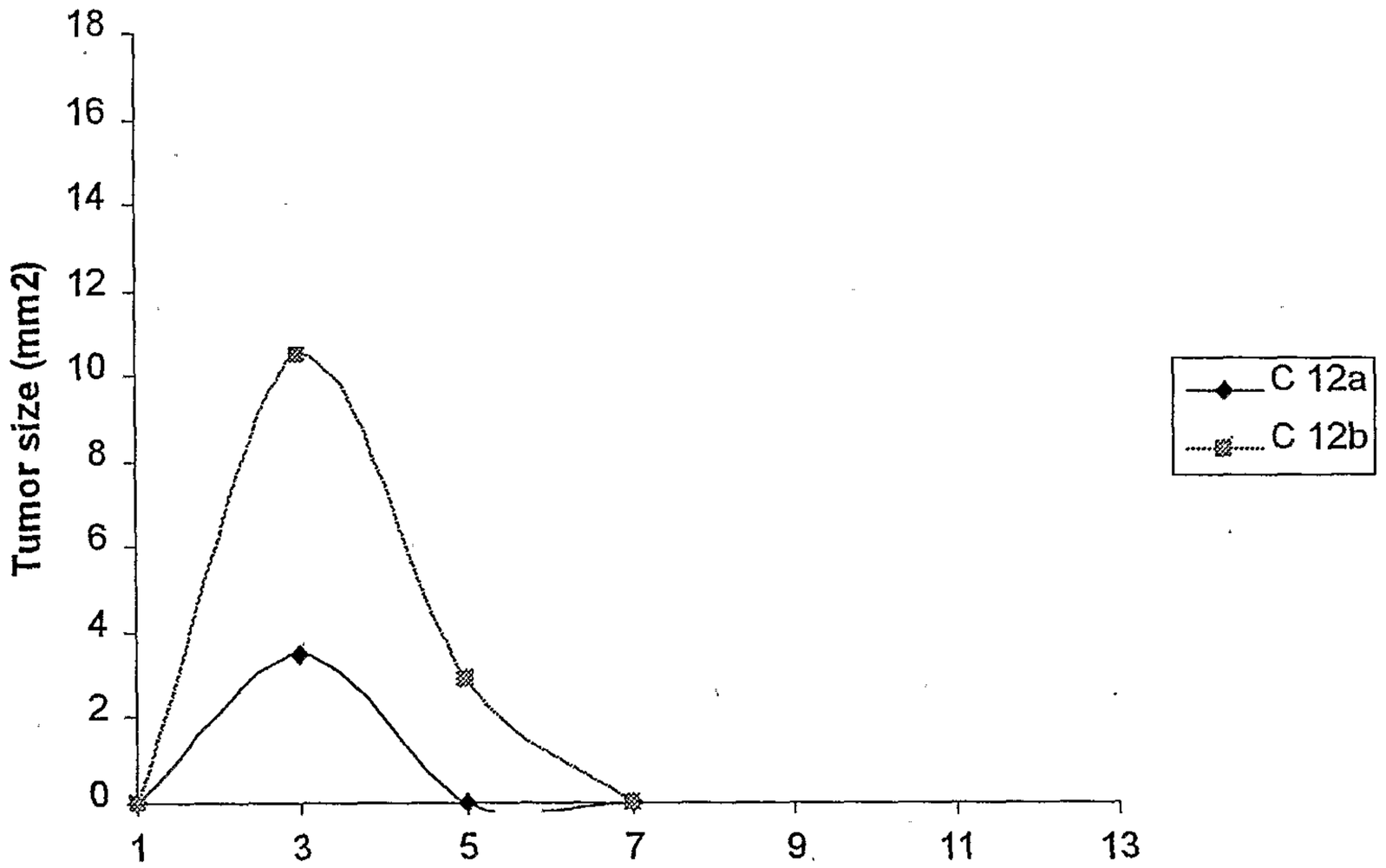
1
3
5
7
9
11
13
Number of days after inoculation
Mod 28
Mod 39

Figure 3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6
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Tumor size (mm2)
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3
5
7
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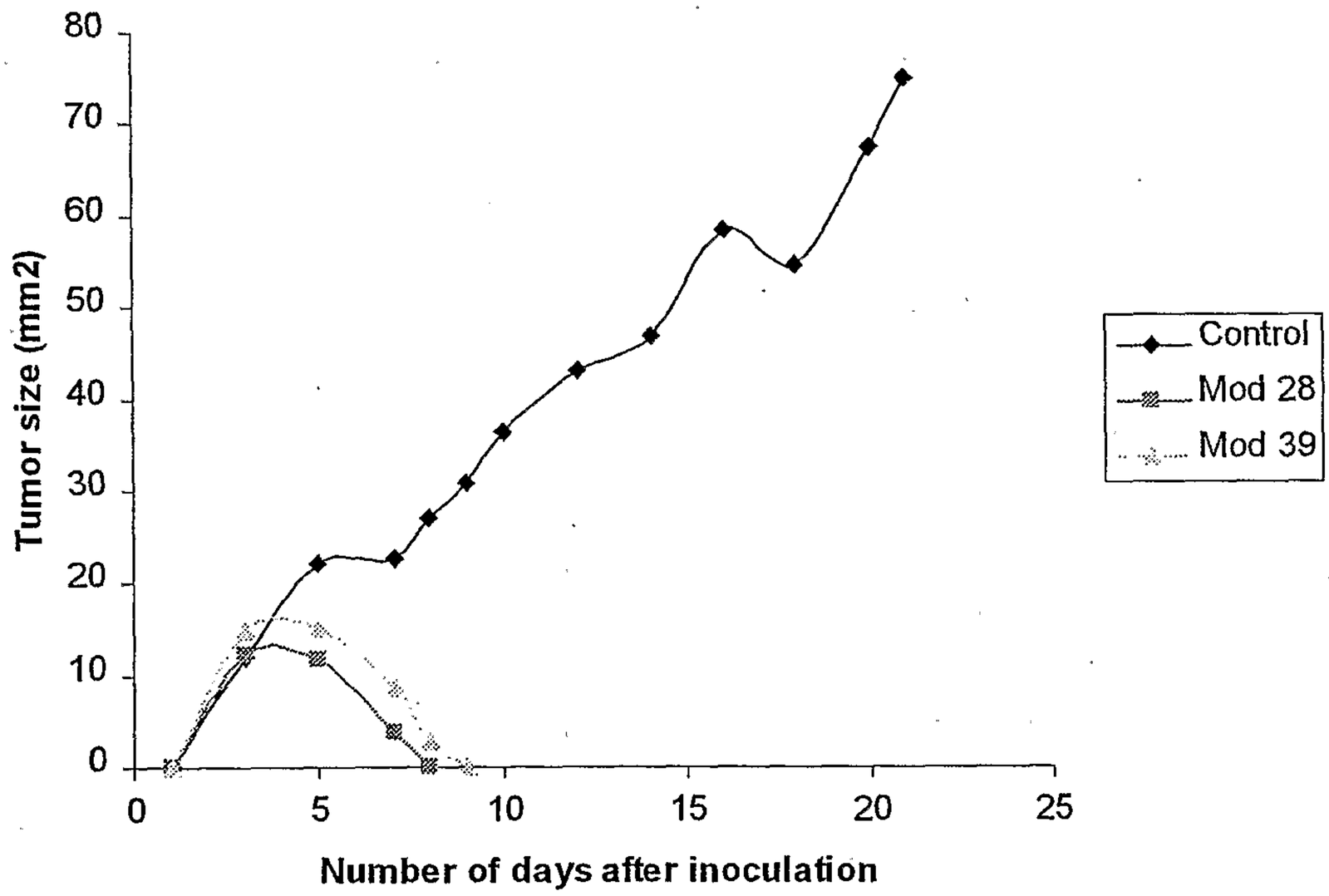
11
13
Number of days after inoculation
C 12a
C 12b

Figure 4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60
50
40
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20
10
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Tumor size (mm2)
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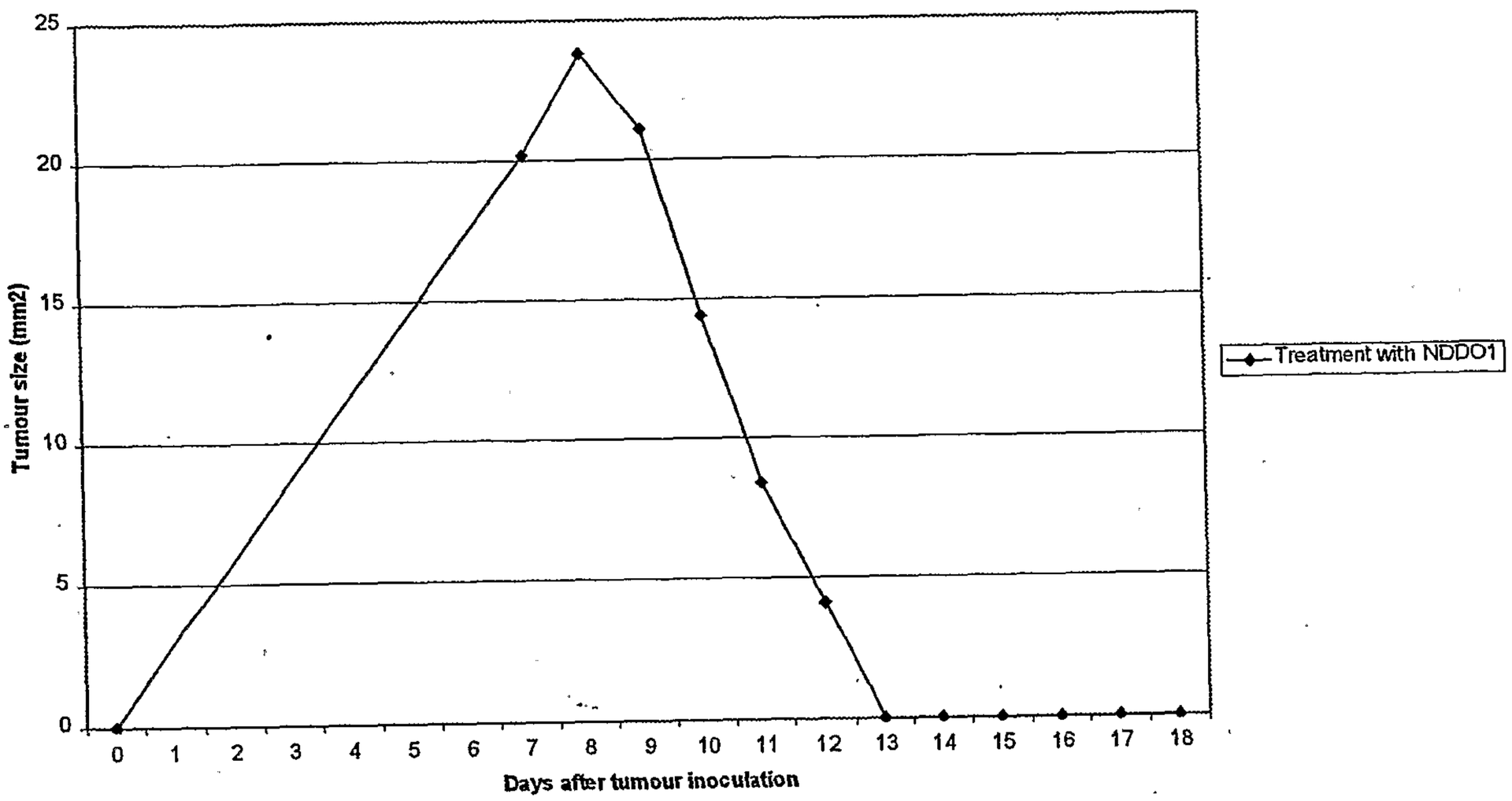
5
10
15
20
25
Number of days after inoculation
Control
Mod 28
Mod 39

Figure 5


25
20
15
10
5
0
Tumour size (mm2)
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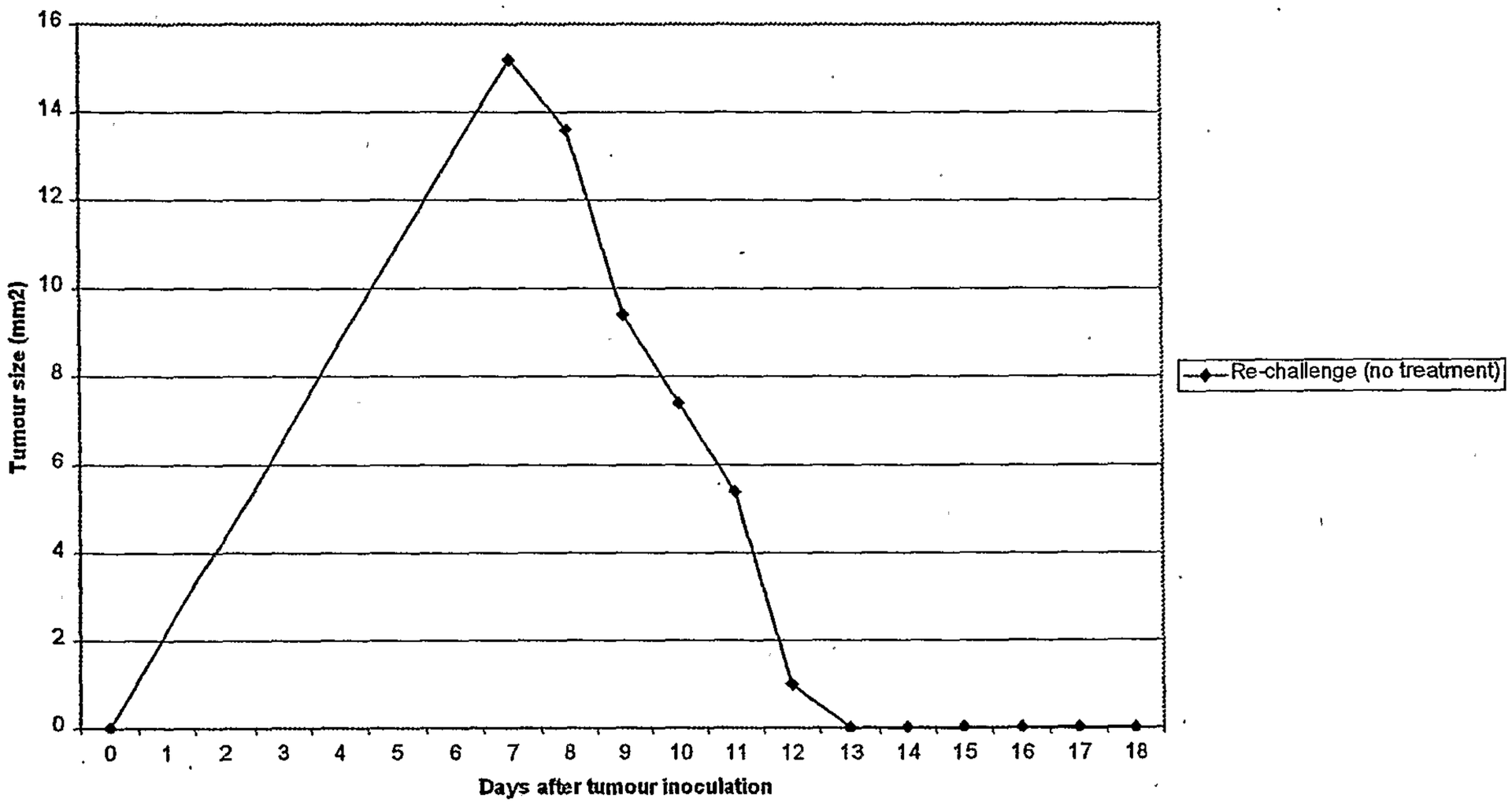
Days after tumour inoculation
Treatment with NDDO1

Figure 6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14
12
10
8
6
4
2
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Tumour size (mm2)
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Days after tumour inoculation
Re-challenge (no treatment)

Figure 7

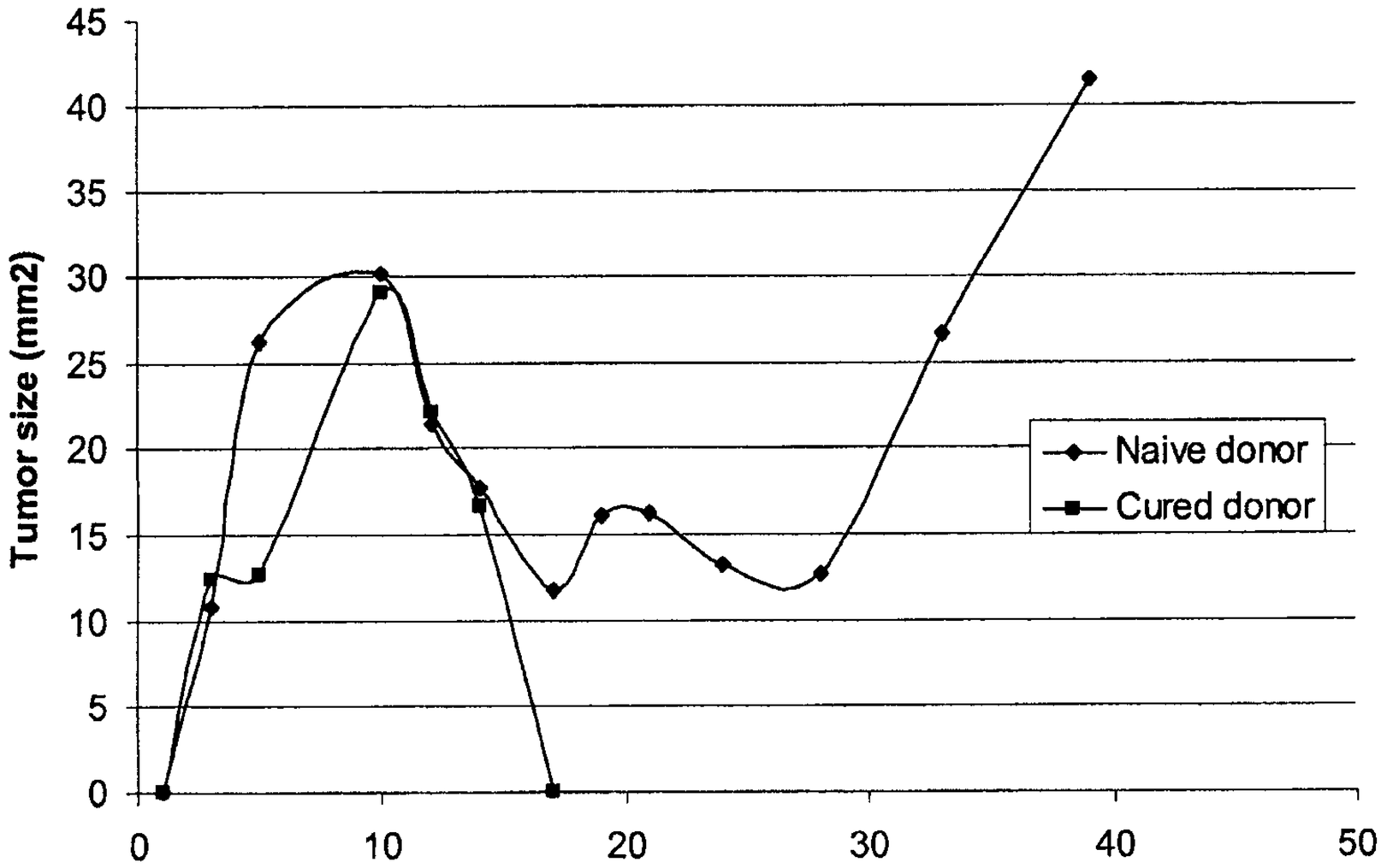
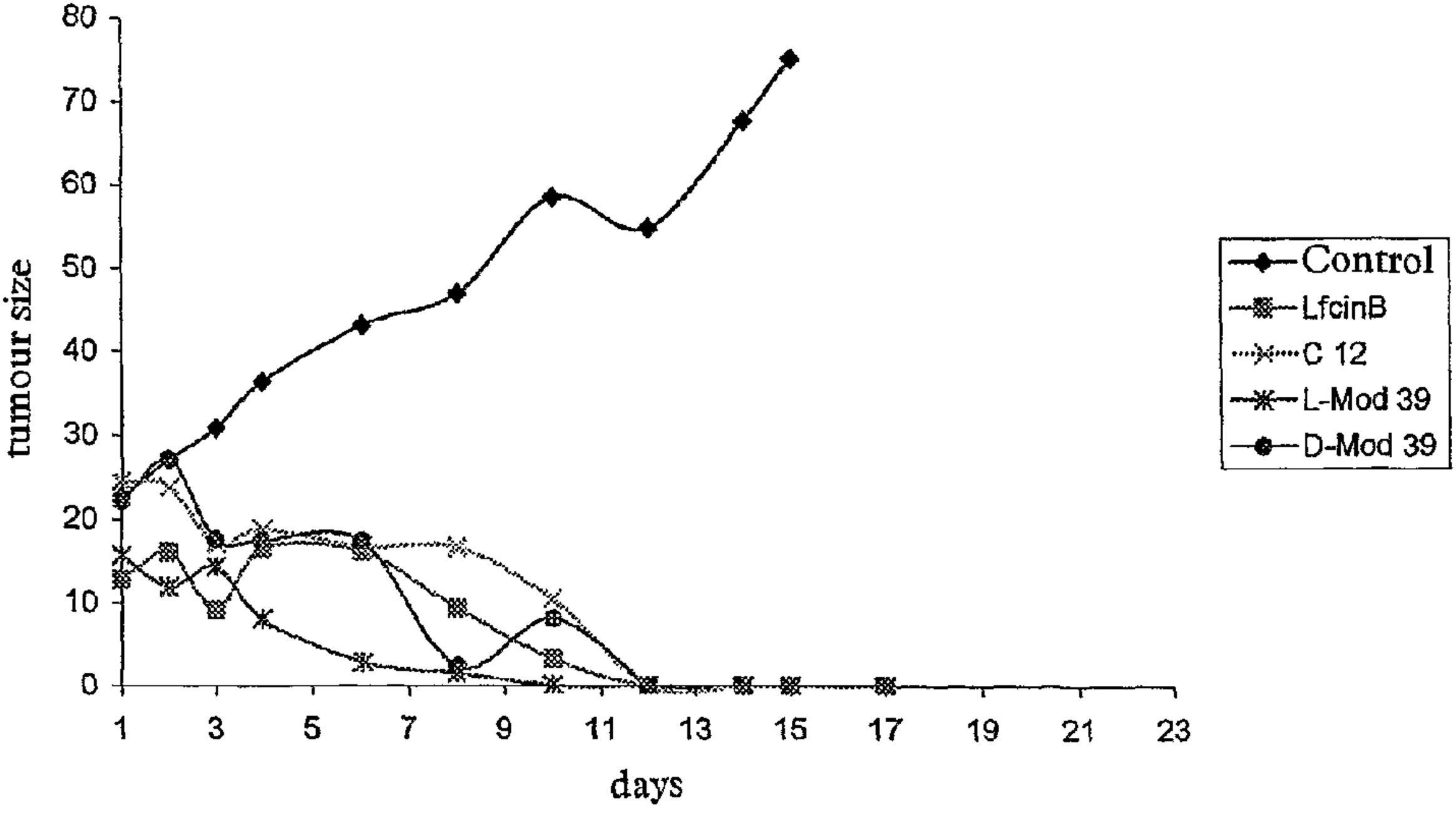

45
40
35
30
25
20
15
10
5
0
Tumor size (mm2)
0
10
20
30
40
50
Days
Naive donor
Cured donor

Figure 8


tumour size
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70
60
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15
17
19
21
23
days
Control
LfcinB
C 12
L-Mod 39
D-Mod 39