# Designing a Vaccine for Cancer

## A Look Into Dendritic Cell Cancer Vaccine Research

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*Teach the body to fight cancer? Find out how current research may make this a reality in the coming years...*

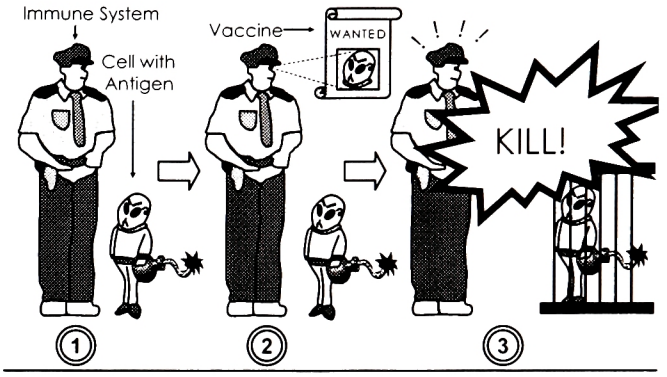
Turning the body's immune system into a weapon against cancer cells is an area that is currently brimming with breakthrough potential. Although the idea has been around since the 1890s, cumulative research findings have finally paved the way for making this idea a reality (Waldman, 2003). Scientists, many from the McMaster academic community, are now exploring the use of modified white blood cell injections as a potential therapeutic vaccine against this lethal disease. If successful, this vaccine will revolutionize the future of cancer treatment. Alone, the vaccine may cause tumour regression. Used in combination with current therapies, the fight against cancer may be won.

To help you best understand this promising new technology, this article has been divided into two parts and supplemented with several hand-made diagrams. In the first section, we will explore the basic mechanisms of how this vaccine of the future will work. The second section brings you to the edge of current immunological research and delves into a few of the fascinating techniques and concepts investigators are employing to overcome the remaining barriers to the vaccine's success.

### Section 1: The Basics of Dendritic Cell-Based Cancer Vaccines

The Basic Idea

Vaccines work by training the body's immune system to recognize and neutralize antigens, compounds that have the potential to generate immune responses. Usually, these are foreign proteins or peptide sequences from viruses, bacteria or other unwanted interlopers (Fig. 1). When antigens are associated with cells, the entire cells can be destroyed by the immune reaction as well. In the case of the common prophylactic vaccine, infectious organisms or viruses containing this marker are destroyed by an immune system that has been trained to recognize their antigens *specifically*. However, if the antigen is specific to cancer cells, vaccines can mount a therapeutic immune response against these cells, too (Abou-Jawde et al., 2003).




1. The naïve immune system (cop) cannot recognize the antigen (face)
2. The vaccine (wanted poster) trains the immune system to recognize it
3. The immune system can now recognize and kill cells that express the antigen

**Figure 1**
*A cop-alien analogy to how a vaccine mounts an immune response against antigens.*

How the Dendritic Cell Cancer Vaccine Works

*What is a dendritic cell and why use it for a vaccine?*

Dendritic cells (DCs) are potent antigen-presenting cells (APCs). One of the main roles of APCs in the body is to ingest, digest and present antigens to other cells of the immune system (Fig. 2). Presentation of antigens to other white blood cells is a crucial step in the development of an adaptive immune response; it activates 'naïve' or 'inert' T cells whose T cell receptor is specific for the particular antigen being presented by the APC. The cytotoxic T lymphocyte (Killer T cell) adaptive immune response is the principle way in which tumours can be destroyed by the body. The dendritic cell cancer vaccine exploits the powerful antigen-presenting capacity of the dendritic cell and uses it to develop therapeutic immunity against cancer-associated antigens (Janeway, 2001).

*How do dendritic cells 'present' antigens to the immune system? Why is it important?*

After a dendritic cell has ingested and processed an antigen, it must communicate its finding to the rest of the immune system. This may be achieved by

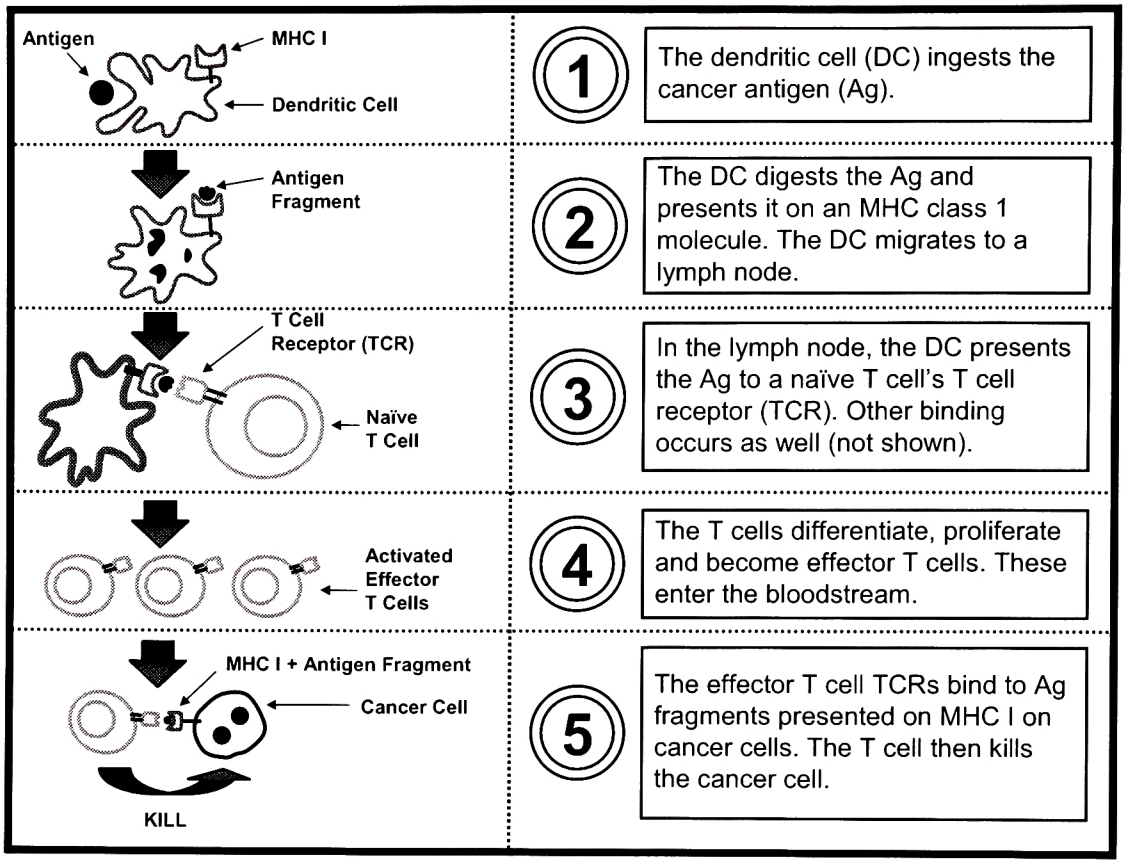



**Figure 2**

*Steps in developing a cytotoxic T lymphocyte (Killer T cell) response from a dendritic cell to an activated cytotoxic T lymphocyte.*

physically bringing the pieces of the antigen to other immune cells. However, since other cells do not have ready access to the engulfed particle inside the cell, the antigen fragment must be presented on the cell surface. One of the ways this is achieved is through antigen binding to a special 'presenting' molecule, MHC I (**M**ajor **H**istocompatibility **C**omplex - Class **I**). This allows the small morsel of antigen to be held in place on the cell surface and gives context to other immune cells, allowing them to respond properly (Janeway, 2001).

Usually, proteins that APCs ingest (exogenous proteins) are presented on MHC II, not MHC I; that is, MHC I is reserved for fragments of proteins that cells produce themselves (endogenous proteins). However, APCs have a special ability to cross-present exogenous antigens on MHC I, which allows APCs to activate cells that can recognize tumour cells expressing tumour-specific antigens in the context of MHC I (Janeway, 2001).

*How do dendritic cells cause an immune response against cancer-associated antigens?*

After a dendritic cell has successfully presented an antigen bound to an MHC I molecule on its cell surface, it migrates to a lymph node where many other white blood cells are waiting. Here, dendritic cells interact with CD8+ T lymphocytes. Dendritic cell antigen/MHC I complexes bind with T cell receptors on CD8+ T lymphocytes. This contact, in conjunction with other co-stimulatory and adhesive processes, causes CD8+ T lymphocytes to multiply and mature into selective cellular assassins, commonly known as killer T cells (or cytotoxic T lymphocytes). These cells then migrate from the lymph node back into the blood and throughout the body in search of the antigen by which they were stimulated. When they find cells that express the antigen, presented with an MHC I molecule, they destroy them. Since cells normally present bits of their internal proteins on MHC I molecules, cancer cells produced antigens can be recognized and destroyed in this way (Janeway, 2001).

*What are the steps to making and administering a dendritic cell-based cancer vaccine?*

Most dendritic cell-based vaccines are usually composed of the following four basic steps (Fig. 3):

1) Collect dendritic cells
2) Culture dendritic cells *in vitro*
3) Expose dendritic cells to the cancer antigen(s) of your choice
4) Administer the dendritic cells into a patient as a vaccine

(Chen et al., 2000)

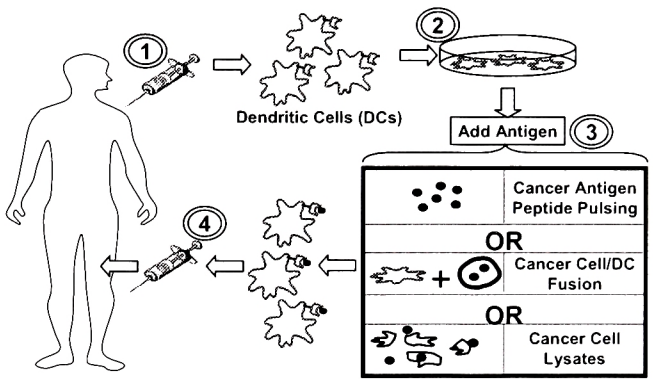



**Figure 3**
*The 4 basic steps in a dendritic cell cancer vaccine.*

## Section 2: Current Techniques and Concepts to Improve the Vaccine

Currently, mild therapeutic effects of DC vaccines based on the theory in section 1 have been documented (Engleman, 1997; Hsu et al., 1996). However, with recent advances in this area, scientists are now looking for new ways to increase this therapeutic effect. This section discusses some of the concepts and techniques that are being used to bring a curative vaccine for cancer closer to fruition.

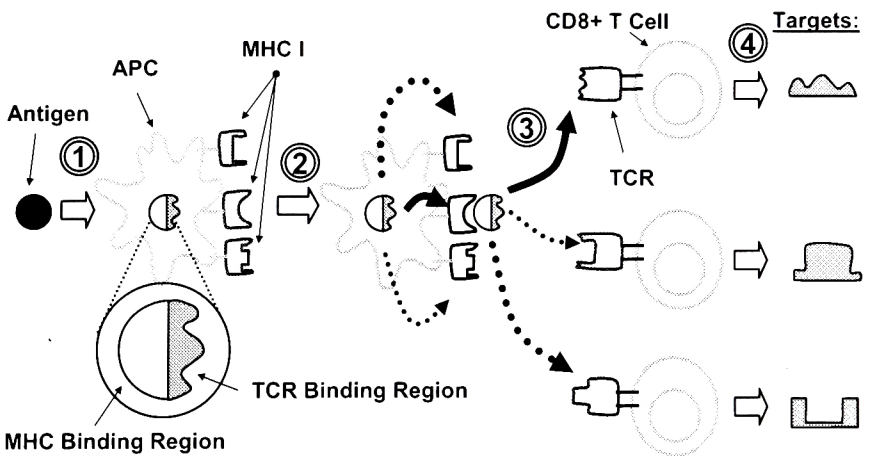
### Heteroclitic Peptides

Heteroclitic peptides are those with greater ability to generate an adaptive immune response than the original peptides. By changing the amino acid sequences of existing peptides, these molecules can be generated (Dyall et al., 1998).

For the purpose of understanding this concept, it is helpful to think of the antigenic fragments that result after dendritic cell processing as having two important regions (Fig. 4):

1. The TCR binding region (TCR: T cell receptor)
2. The MHC binding region (MHC: Major Histocompatibility Complex)

Heteroclitic peptides may have substituted amino acids in either or both of these regions; however, only heteroclitic peptides involving alteration to the MHC




1. The antigen is engulfed, digested and processed by an antigen presenting cell (eg. Dendritic cell).
2. The antigen fragment binds to an MHC I molecule. The affinity depends on the complementarity between MHCI and the MHC binding region.
3. The TCR binding region binds a corresponding TCR molecule. Under the right conditions, the CD8+ T cell is activated to become a killer T cell.
4. The killer T cell then seeks to destroy cells expressing molecules similar in shape to the TCR binding region.

**Figure 4**
*After digestion, an antigen fragment has an MHC binding region and a TCR binding region that serve different functions.*

binding region will be discussed here (Dyall et al., 1998; Parkhurst et al., 1996).

The TCR-binding region determines the specificity of the resulting immune response. It is specific for a particular T cell receptor. Since each T cell contains a T cell receptor specific for one antigen, the TCR-binding region determines the T cell variant that is activated, and thus, the antigen to which an immune reaction will be developed (Janeway, 2001).

The MHC-binding region determines the binding affinity of the antigenic fragment to its corresponding MHC molecule. By altering the MHC-binding region, the binding affinity between antigen fragment and MHC molecule can be increased (Parkhurst et al., 1996). Stronger binding affinity for MHC I has been correlated with a greater capacity to develop of a killer T cell response. It is thought that this relationship is caused by the unstable nature of the peptide:MHC 1 complexes. Increasing binding affinity would increase the available time for presentation of the peptide to T cell receptors (Sette et al., 1994).

Therefore, altering the cancer antigen MHC-binding region to increase its MHC-binding affinity will increase the effectiveness of the vaccine by increasing the likelihood of evoking an immune response. As long as the TCR-binding region remains complementary to the same T cell receptor, the specificity of the resultant immune response should be the same.

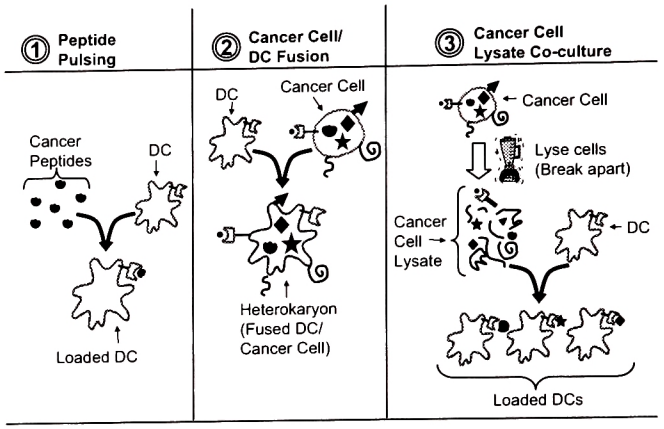
Patient-Specific Vaccines

Cancer antigens between individuals and between cancer types vary substantially. Therefore, it is believed that the effectiveness of the vaccine can be increased if the chosen cancer vaccine antigens were those taken for the individual patient. Tailoring the vaccine to patient-specific cancer antigens ensures that the immune response, if any, will be properly targeted to that person's cancer cells (Nadler & Shultze, 2002).

There are currently three commons methods used to expose dendritic cells to antigens *ex vivo* (Fig. 5). Of these, all can be used to develop patient-specific vaccines (Fecci et al., 2003; Fay, 2002; Rea et al., 2001).

Peptide pulsing with isolated antigens previously determined to be patient-specific is the most straightforward method of the three. It produces immunity to the cancer antigens that the particular patient's tumour is expressing (Fecci et al., 2003).

Peptide pulsing with tumour cell lysates involves exposing the dendritic cells *ex vivo* to the dispersed cell contents of the individual's tumour cells. This provides




Once the dendritic cells (DCs) have been harvested (Step 1: Figure 3), three common methods used for loading them with cancer antigens are peptide pulsing (1), cancer cell/DC fusion (2) and cell lysate co-culture (3).

**Figure 5**

*The 3 common methods of loading dendritic cells with cancer antigens.*

an opportunity for the dendritic cells to ingest all the possible antigens found in the cancer cell (Fecci et al., 2003).

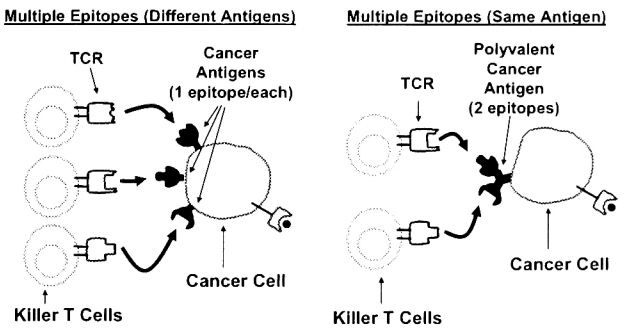
Another way to create patient-specific vaccines is to meld dendritic cells with the patient's tumour cells *ex vivo*. This brings the antigen-presenting ability of dendritic cells together with all the antigens found in the cancer cell. As a result, dependence on APC ingestion of the antigen is eliminated and the process of cross-presentation can be bypassed. All the important cancer antigen factors, including 'MHC I/cancer antigen complexes', are combined with the immune system stimulating properties of the dendritic cell (Fecci et al., 2003).

Polyvalent Vaccines

Usually lacking proper genetic repair mechanisms, cancer cells accumulate mutations in their own proteins at an elevated rate. This high mutation rate allows cancer cells within a patient to express different antigens, gaining or losing the expression of different proteins. This phenomenon of ever-changing tumour antigen expression has been verified by Brasseur and Lehmann's studies (Renner et al., 2001). Therefore, a vaccine targeting only one antigen is more likely to allow some altered cancer cells to avoid immune surveillance. To circumvent this problem, it is beneficial to generate polyvalent vaccines that target multiple antigens. By broadening the diversity of the immune response, one can minimize the probability of tumour escape due to a lost antigen (Zhou et al., 2002; Smith et al., 2001; Scanlan & Jager, 2001).

A polyvalent vaccine may also refer to the multiple epitopes of any particular antigen (Fig. 6). An epitope refers to the region of an antigen that the T cell receptor binds. I hypothesize that targeting several epitopes on one antigen may increase the likelihood that cells expressing that antigen will be recognized and destroyed. Also, it may prevent cancer cells from escaping through random mutations as previously discussed. If the gene encoding a cancer antigen undergoes a point mutation and change in one amino acid in the peptide chain, it may maintain another epitope that can be recognized by the immune system. Despite this speculation, currently I am unaware of any research that supports this notion.

Polyvalent vaccines can be made using the three techniques earlier described to introduce antigens to dendritic cells (Fig. 5): exposing dendritic cells to tumour lysates, inducing tumour cell-dendritic fusion or directly pulsing dendritic cells with multiple antigens.




Polyvalent cancer vaccines target several different cancer epitopes (instead of only one: monovalent). There are two ways polyvalency of a vaccine can be achieved.

Mount an immune response against:

1. Multiple epitopes on different antigens (Left panel)
2. Multiple epitopes on the same antigen (Right panel)

**Figure 6**

*There can be 2 types of polyvalency.*

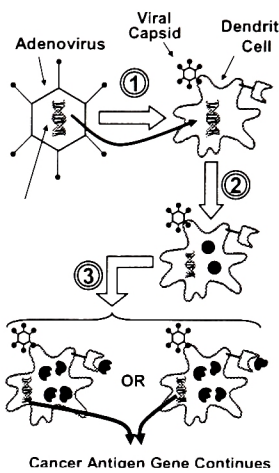
Gene Transduction

In addition to methods that apply the antigen to the DC directly, it is also possible to transfer the gene encoding the tumour-specific antigen into the DC (Fig. 7). Such an approach can be beneficial because it provides:

1) A continuous production of antigenic fragments
2) An intracellular source of antigen, easily accessible to the MHC I pathway

(Okada et al., 2001; Rea et al., 2001)

Continuous production of antigen allows for prolonged availability for loading into the MHC I pathway. Compared with peptide-pulsing techniques that provide short-term exposure, antigen gene transduction provides long-term exposure. Given that MHC I/antigen complexes




Adenoviruses can be used to insert cancer antigen genes into dendritic cells (DCs).

1. The adenovirus containing the desired gene is used to infect DCs. This process inserts the gene into the DC.
2. The foreign gene instructs the production of cancer antigens inside the DC.
3. The antigens are digested by the DC and the various fragments are presented on MHC I molecules.

Two benefits of this process are:

A. Continuous production of cancer antigen for loading onto MHC I (Instead of one-time exposure as in peptide pulsing).

B. An intracellular source of antigen, easily accessible to the MHC I pathway

**Figure 7**

*Using viral gene transduction to introduce antigen genes into a dendritic cell.*

are unstable and degrade relatively rapidly with time, it is believed helpful to have constant antigen present for continuous loading onto MHC I (Rea et al., 2001).

By providing an intracellular antigenic source, gene transduction improves the access of antigen fragments to the MHC I pathway. Exogenous antigen sources, as in peptide pulsing, are normally presented on the MHC II pathway and require cross-presentation by the dendritic cell. However, if the antigen is produced within the cell, it will be naturally loaded onto MHC I without the need for the less-efficient cross-presentation process (Rea et al., 2001).

To effect gene transduction, viruses can be used. Currently, one of the most effective techniques for dendritic cell gene transduction makes use of genetically-modified adenoviruses, a technology developed at McMaster by Dr. Frank Graham and colleagues. The adenoviral vector boasts high transfection rates and allows for several vectors to be introduced into the same DC population (Rea et al., 2001; Chen et al., 2001). In addition, this technique can also be used to transduce genes encoding immunostimulatory cytokines that stimulate the killer T lymphocyte response (cytotoxic T lymphocyte response) (Chen et al., 2001).

At McMaster University's Centre for Gene Therapeutics, gene technology has been united with dendritic cell cancer vaccine research. The results have been promising. In 2001, McMaster researchers Dr. Ronan Foley, Dr. Jack Gauldie and Dr. Yonghong Wan published results in *Gene Therapy* showing that DC vaccine effectiveness could be increased by a combination of both antigen and immunostimulatory cytokine gene transduction. The cytokine IL-12 was

chosen for this experiment because of its ability to activate immune cells and strengthen the killer T cell response. Using adenoviral vectors, the team simultaneously introduced a breast cancer antigen (ErbB-2/neu) and an IL-12 gene into dendritic cells *ex vivo* before administering the vaccine (Fig. 8). The result was a significant strengthening of the protective and therapeutic immunity of mice against injected breast cancer cells (Chen et al., 2001).

## Remaining Challenges for Cancer Vaccine Research

Four of the major remaining challenges for cancer vaccines involve identifying more cancer antigens so that more patients may be treated, developing more effective immunization strategies, suppressing tumour escape mechanisms, and preventing autoimmunity (Scanlan & Jager, 2001).

The hypothesis of tumour escape is one that attempts to explain the current lack of robustness of cancer vaccines. It is based on the concept of natural selection for cancer cells that can evade the immune system. Since tumour cells are highly mutagenic, they have been known to lose or down-regulate MHC I, cancer antigens, and other crucial molecules to the immune response. Furthermore, they may mutate to have defective death receptors that prevent destruction by killer T cells, secrete immunosuppressive cytokines or express death signals to cause T cell self-destruction. Such processes, if present and selected for, would greatly interfere with vaccine effectiveness (Khong & Restifo, 2002; Vicari et al., 2002; Garcia-Lora et al., 2003).

Since cancer cells ultimately derive from self-cells, there is the risk that a killer T cell response against normal, healthy cells may develop after a cancer vaccine is administered. This phenomenon of self-reactivity is known as autoimmunity and results in damage to the body's tissue. With this lingering possibility, future research must be cautious to avoid vaccines that produce immune responses that do not discriminate between self and cancer.

## The Future of DC Cancer Vaccine Research

Research in the area of dendritic cell cancer vaccines is moving forward at breakneck speeds. Each month, new discoveries are made that push our knowledge to greater heights, always moving closer to a cure. With an increased understanding of antitumour immunity and an enormous toolbox of immune enhancing techniques at our disposal, a therapeutic revolution is waiting to be had. When will the cure for cancer come? Feedback from current and future clinical trials will begin to set the stage for the routine use of DC cancer vaccines in the clinic. However, development of a highly effective or curative vaccine will require the integration of carefully selected immunological concepts, only a few of which have been discussed here. We have only graced the tip of the knowledge iceberg - a vast sea of discovery still awaits you. Bon voyage! M

*A special thank-you goes to Terry Ng and Deborah Leung for their research contributions and mind-expanding discussions. I am also indebted to Ryan Wiley for helping guide me into my immunological adventures – through forests of facts I would have been otherwise lost in.*

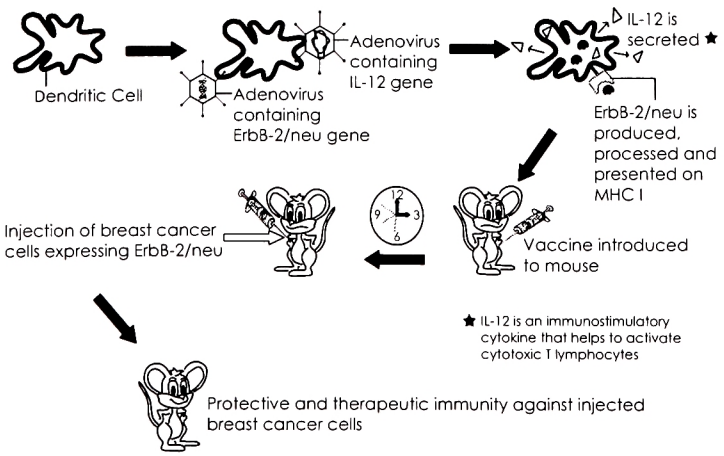



**Figure 8**
*McMaster researchers successfully transduced breast cancer antigen, ErbB-2/neu, and IL-12 genes into dendritic cells before administering the DCs as a vaccine to induce protective and therapeutic immunity against injected breast cancer cells (Chen et al., 2001).*