

# Biologic therapy and cervical cancer

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Biologic therapy is considered as the fourth modality of cancer treatment. It is therapy geared toward the utilization of the patient's own biologic system or the use of natural biologic reagents to generate a rejection response against the malignant phenotype. This is a new method of cancer treatment and is gaining a large momentum because of the accelerated discoveries in both molecular biology and immunology of cancer.



There is no standard classification of biologic therapy. This term may be applied to many types of therapies that, in principle, affect the relationship between the biologic system of the patient and the malignant cell. These include, among others: bone marrow enhancing reagents, hormonal therapy, agents that affect tumor vascularization and immunotherapy. The last immunotherapy is usually used interchangeably with biotherapy and in this chapter the term biotherapy and biologic therapy will be used to describe immunotherapy for cancer.

This review focuses on the basic principals of biologic therapy. We have chosen cervical cancer as an illustrative example for how such discipline can be incorporated in modern practice of oncology.

## The immune system

The main function of the immune system is to distinguish "self" from "foreign" molecules, thereby providing the body with a defense mechanism against foreign antigens and harmful pathogens. The ability to distinguish between self and foreign molecules is a learning process and partly leads to the development of tolerance to self-molecules and the generation of a specific immune response against foreign antigens.

Lymphocytes employ two types of immunologic responses: humoral and cell-mediated. The former is antibody dependent. Antibodies are released by B-lymphocytes and are able to recognize extracellular domains of proteins that are expressed on the surface of intact cell, and antigens that reside in the extracellular space. These antibodies either "neutralize" pathogens, preventing their entry in the cell, or "opsonize" them and thus accelerate their clearance by the reticuloendothelial system

Cell-mediated immune response is a function of T lymphocytes, which can recognize intracellular antigens. These antigens can either be foreign proteins that originate from intracellular organisms such as viruses, parasites and some bacteria; or altered native proteins. Lymphocyte is able to recognize antigens with specific receptors on their surface, the T cell receptor (TCR). TCR recognizes antigens that are presented on the surface of the target cells by the major histocompatibility complex (MHC)<sup>1</sup>. The antigens are small peptides that are the resultant of endogenous protein processing. There are two types of T lymphocytes: T helper lymphocytes (CD4+), and cytotoxic T lymphocytes (CD8+). The CD4+ cells are able to recognize peptide antigens that

*1) Historically the term "MHC" has referred to the nomenclature of the mouse complexes, while the human counterpart has been referred to as human leukocyte antigens, or HLA, but the term "human MHC" is now acceptable as a general label.*

are presented in association with MHC class II (MHC II), while CD8+ cells are programmed to recognize antigens displayed on MHC class I (MHC I). MHC I molecules are expressed on all nucleated cells, while MHC II molecules are expressed on professional antigen presenting cells (APC): dendritic cells, B cells, and macrophages. When stimulated, T-helper cells secrete cytokines, such as IL2, which can stimulate the growth and differentiation of B-lymphocytes, activate macrophages, and induce the production of cytotoxic T lymphocytes CTL. Cytotoxic T lymphocytes then release mediators that can lyse or kill the target cells.

Antigens in nucleated cells are expressed on the cell surface to be recognized by T cells using a mechanism called "antigen processing and presentation" 1. Proteins are converted in this process into small peptides, and then transferred to the cell surface in association with the (MHC) molecules 2, 3. This is pivotal for the antigen to interact with the T cell receptor and to subsequently generate the immunologic response 4.

### The immunology of cancer

The immune system is well known to play a role in determining the natural history of cancer. This is obvious from many anecdotal and systematic observations including: 1) Immune suppressed post-transplant patients have an increased incidence of malignancy 5-9; 2) some tumors are known to spontaneously regress 10-12; and 3) biologic agents that have been shown to modify the immune response have also been demonstrated to be effective in treating tumors 13-16. The above indicate that tumors are potentially amenable for immune recognition, and thus are able to present antigens that are recognizable by the immune cells. These antigens are termed tumor-associated antigens "TAA".

TAA usually reflect new characteristics acquired by the malignant cells, as a

result of the accumulation of series in genetic changes that are either spontaneous or acquired. These genetics lead the transformed cells to express a novel antigenic profile which has the potential of being perceived by the immune system as novel 17, 18.

It is possible to categorize TAA into two separate classes reflecting their origin: Self, and Non-self. Self Antigens are produced by alterations that occur in the transformed tumor cells without changing the original protein sequence. Such alterations include the activation of a silent gene, such as MAGE1 in melanoma 19, or an oncofetal antigen like CEA 20. Another alteration gene is overexpression: of an oncogenic (e.g. HER-2/neu), or non-oncogenic antigen (e.g. the melanoma antigens MART1, gp100, and Tyrosinase 21).

The second class of TAA is Non-self Antigens. These can be the products of organism such as tumor viruses 22-24. The human papillomavirus (HPV), Epstein-Barr virus (EBV), Hepatitis B virus (HBV), and Hepatitis C virus (HCV), are some known examples of the oncogenic viruses. Alternatively, they can be the product of genetic mutations, such as the oncogene ras and the tumor suppressor gene p53, both of which can acquire point mutation in human cancer (20% and 50% respectively for all solid tumors).

### Types of biological therapies

The modalities of biologic cancer therapy can be either humoral, by the active administration of specific antibodies; or cellular, by generating a cellular immune response against tumor targets. Each can be either a defined-antigen directed or a non- defined antigen directed therapy:

Monoclonal Antibodies (MoAb) are biologic agents that are designed to target the extracellular domain of specific molecules expressed on the cell membrane or molecules that reside in the extracel-

lular space. They exert their anti-tumor effect in several ways, including blocking the targeted receptor thus preventing the transmission of proliferative signals to the nucleus; activating antibody-dependent cellular cytotoxicity (ADCC); or internalizing the receptor and hence delivering toxic agents into the cells.

Cytokines are soluble proteins that have a hormone-like action, and exhibit their effect in the immune system through regulating other cells. They can be produced by either lymphocytes, in which case they are called "Lymphokines"; or "monokines" by the monocytes. They include interleukins, interferons, tumor necrosis factor, and growth factors. 25

Cancer vaccines aim to actively stimulate the immune system to generate a specific response, either humoral or cellular, against antigens that are expressed by malignant or pre-malignant cells. They can be specifically directed against a known TAA, and administered either in a recombinant form that expresses the targeted antigen, or a synthetic form of the antigen itself. Alternatively, using whole cell vaccines or lysates as a source of TAA, vaccines may utilize the collection of potential cancer cell derived antigens that mostly have not been identified.

### Biologic therapy for cervical cancer:

Cervical cancer is one of the few human malignancies which is linked to a specific etiologic agent 26 27 28 where more than 99% of the cases are caused by the Human papillomavirus. This serves as a great model for developing molecular targeted therapy. Therapeutic strategies against HPV-induced malignancies are designed for either the prevention or the treatment of the disease. HPV's are divided into low-risk and high-risk types, based on the characteristics of clinical lesion with which they are associated. Low risk HPV's (e.g. HPV 6 and 11) are generally associated with benign

lesions, such as condyloma acuminata, and their DNA is usually maintained, their DNA in cells as extrachromosomal circular episomes. High risk types (e.g. HPV 16 and 18) are found in CIN II and III lesions and invasive cervical cancers 29. The genomes of high-risk type are integrated into the cellular DNA in most high-grade lesions and cervical carcinomas. The protein products of the early genes E6 and E7 in high-risk HPV types have been implicated in the oncogenic capability of the viruses. Thus, the continued expression of these proteins in cervical cells appears to be a critical event in the progression and maintenance of cervical neoplasia, 30. Therefore, the products of these two genes can be seen as very strong candidates to become targets for biologic therapy 31.

It has been observed that HPV E6 and E7 form TAA in cervical cancer. The latter, in turn, can induce cytotoxic lymphocytes (CTL) in patients with HPV positive cervical cancer, that are capable of recognizing and lysing tumor cells harboring HPV genome 32, 33. Furthermore, HPV E6 and E7 epitope-specific CTL can be generated from cervical cancer patients with HPV positive tumors 33.

The role of cytokines in cervical cancer has not yet been shown to be very promising. Interferon was tried in advanced disease in a multi institutional phase II trial conducted by the Eastern Cooperative Oncology Group with no tangible effect on this group of patients 34. Interferon  $\alpha$  was also used in combination with other agents like retinoic acid. Cytokines are currently in the process of being explored, mainly in conjunction with vaccine therapy, as immune enhancers.

Cervical cancer vaccines can either be prophylactic or therapeutic. Formerly based on inducing humoral immune response to generate neutralizing antibodies against the infectious agent. The traditional prophylactic use of viral vaccines has been living, attenuated or for-

malin-inactivated viral strains. Because of the difficulty of propagating HPV in tissue cultures, prophylactic vaccines against HPV lagged behind in development.

Recently, it has been shown that the HPV viron, composed of two late proteins, L1 and L2, is highly immunogenic and can generate humoral immune responses with high neutralizing titers 35-37. When expressed in non-mammalian cells, self assembly into HPV virus-like particles (VLP) resembling the native viron is observed 38-40, while exhibiting the same immunogenicity 35, 41. HPV-16 VLP were reported to induce specific humoral immune response against the virus 42, and to reduce the incidence of HPV-16 infection and related CIN 43. These encouraging results, among others, are paving the way for the development of prophylactic vaccines for cervical cancer.

E6 and E7 have also been shown to be potential targets for therapeutic vaccination. E6 and E7-derived peptides that form human CTL epitopes have been identified 44, 45, and several clinical trials have been conducted testing such peptide-vaccine in patients with advanced cervical disease or high grade CIN, with conflicting results. Full length HPV E7 protein has also been used in clinical trials in cervical cancer and pre-malignant disease. One example currently under study using a full length HPV16 E7 construct that is fused to the heat shock protein (Hsp65) in order to enhance antigen processing and presentation 46.

Human papillomavirus antigens, whether proteins or peptides, can also be administered as naked DNA or recombinant viral vectors. Because of concerns about using oncogenes like E6 and E7 proteins, functionally defective mutated versions of the proteins are used. Mini-genes that consist of chains identified as CTL epitopes can thus be expressed from these vectors. Recombi-

nant viruses such as vaccinia, adenovirus, and adenoassociated virus have been used to express HPV proteins 47, 48. Viral infections, however, need to be used with caution in immunocompromised patients with advanced disease. Naked DNA vectors, on the other hand, cannot be delivered as efficiently as viral vectors, but they do not have the same risks and are stable and easy to produce.

## Conclusion

Biologic therapy for cancer is one of the most diverse treatments in medicine. This is because of the many different ways of manipulating the biological system; the many antigens that have been discovered or will be discovered; the different strategies of targeting those antigens; and the possible combinations of such strategies. This diversity generates a tremendous amount of controversy regarding the best therapeutic strategy, trial design, study population, and the time to take these strategies into phase III trials.

In conclusion, biotherapy is a very promising therapy in the treatment of cancer. However, there are many issues in the area of biotherapy that continue to be highly debated, and further pre-clinical and clinical research will be essential if we want to bring this field into the main stream of cancer therapy.

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