

## TUMOR IMMUNOLOGY

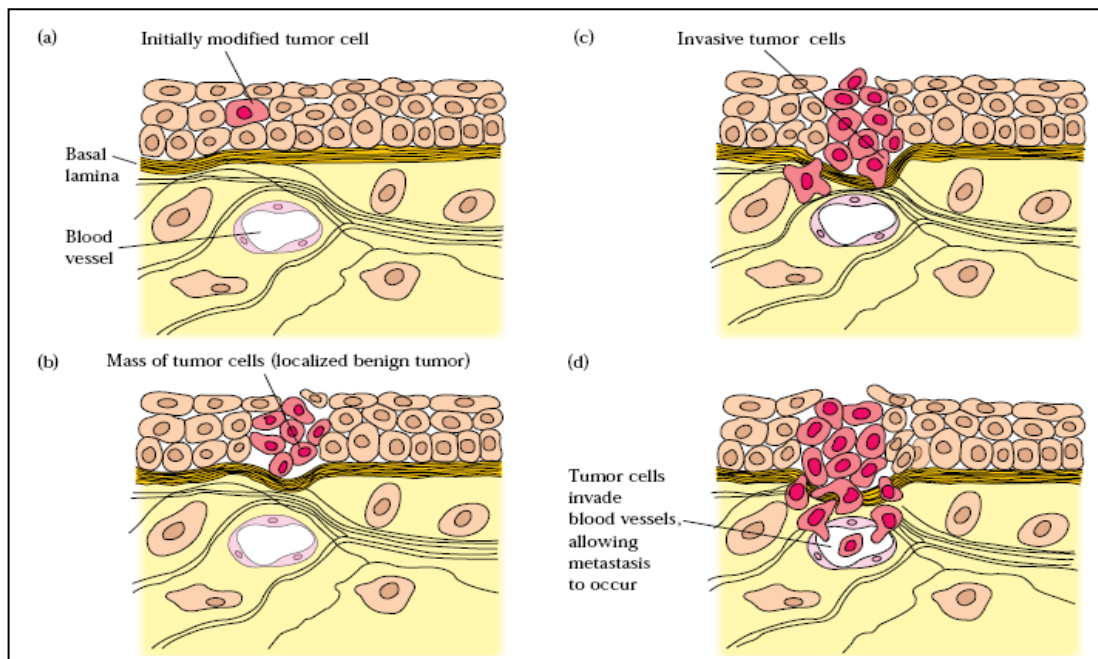
In most organs and tissues of a mature animal, a balance is usually maintained between cell renewal and cell death. The various types of mature cells in the body have a given life span; as these cells die, new cells are generated by the proliferation and differentiation of various types of stem cells. Under normal circumstances, the production of new cells is regulated so that the number of any particular type of cell remains constant. Occasionally, though, cells arise that no longer respond to normal growth-control mechanisms. These cells give rise to clones of cells that can expand to a considerable size, producing a tumor, or **neoplasm**.

**Benign:** A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is **benign**.

**Malignant:** A tumor that continues to grow and becomes progressively invasive is **malignant**.

The term **cancer** refers specifically to a malignant tumor.

In addition to uncontrolled growth, malignant tumors exhibit **metastasis**. It is a process in which, small clusters of cancerous cells dislodge from a tumor, invade the blood or lymphatic vessels, and are carried to other tissues, where they continue to proliferate. In this way a primary tumor at one site can give rise to a secondary tumor at another site (figure).



**Classification of cancers:** This is based on the embryonic origin of the tissue from which the tumor is derived.

- 1) **Carcinomas:** These are tumors derived from endodermal and ectodermal tissues such as skin or the epithelial lining of internal organs and glands. Eg: Cancer of colon, breast, lung and prostate carcinomas.
- 2) **Leukemias and lymphomas:** These are malignant tumors of hematopoietic cells of the bone marrow and account for about 9% of cancer incidence in the United States. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses.
- 3) **Sarcomas:** These arise less frequently (around 1% of the incidence in the United States), are derived from mesodermal connective tissues such as bone, fat, and cartilage.

**Malignant Transformation of cells:** Treatment of normal cultured cells with chemical carcinogens, irradiation, and certain viruses can alter their morphology and growth properties. In some cases this process, referred to as **transformation**.

This makes the cells able to produce tumors when they are injected into animals. Such cells are said to have undergone malignant transformation, and they often exhibit properties in vitro similar to those of cancer cells. For example, they have decreased requirements for growth factors and serum, are no longer anchorage-dependent, and grow in a density-independent fashion. Moreover, both cancer cells and transformed cells can be subcultured indefinitely; that is, for all practical purposes, they are immortal.

Various chemical agents like **DNA-alkylating reagents** and physical agents like **ultraviolet light** and **ionizing radiation** that cause mutations have been shown to induce transformation. Induction of malignant transformation with chemical or physical carcinogens appears to involve multiple steps and at least two distinct phases: **initiation and promotion**.

Initiation involves changes in the genome but does not, in itself, lead to malignant transformation. After initiation, promoters stimulate cell division and lead to malignant transformation. The importance of mutagenesis in the induction of cancer is illustrated by diseases such as **xeroderma pigmentosum**. This rare disorder is caused by a defect in the gene that encodes a DNA-repair enzyme called **UV-specific endonuclease**. Individuals with this disease are unable to repair UV-induced mutations and consequently develop **skin cancers**.

A number of DNA and RNA viruses have been shown to induce malignant transformation. Two of the best-studied are **SV40** and **polyoma**. In both cases the viral genomes, which integrate randomly into the host chromosomal DNA, include several genes that are expressed early in the course of viral replication. The proteins encoded by

these genes plays a role in the malignant transformation of virus-infected cells. **Retrovirus** the RNA viruses induced transformation due to the presence of **oncogenes**, or “cancer genes,” carried by the retrovirus. One of the best-studied transforming retroviruses is the **Rous sarcoma virus**. This virus carries an oncogene called *v-src*, which encodes a 60-kDa protein kinase (**v-Src**) that catalyzes the addition of phosphate to tyrosine residues on proteins.

**Cancer associated genes:** Homeostasis in normal tissue is maintained by a highly regulated process of cellular proliferation balanced by cell death. If there is an imbalance, either at the stage of cellular proliferation or at the stage of cell death, then a cancerous state will develop. Oncogenes and tumor suppressor genes have been shown to play an important role in this process, by regulating either cellular proliferation or cell death. Cancer-associated genes can be divided into three categories that reflect these different activities.

**a) Genes that induce cellular proliferation:** In normal cells, the expression of growth factors and their receptors is carefully regulated. Usually, one population of cells secretes a growth factor that acts on another population of cells that carries the receptor for the factor, thus stimulating proliferation of the second population. Inappropriate expression of either a growth factor or its receptor can result in uncontrolled proliferation.

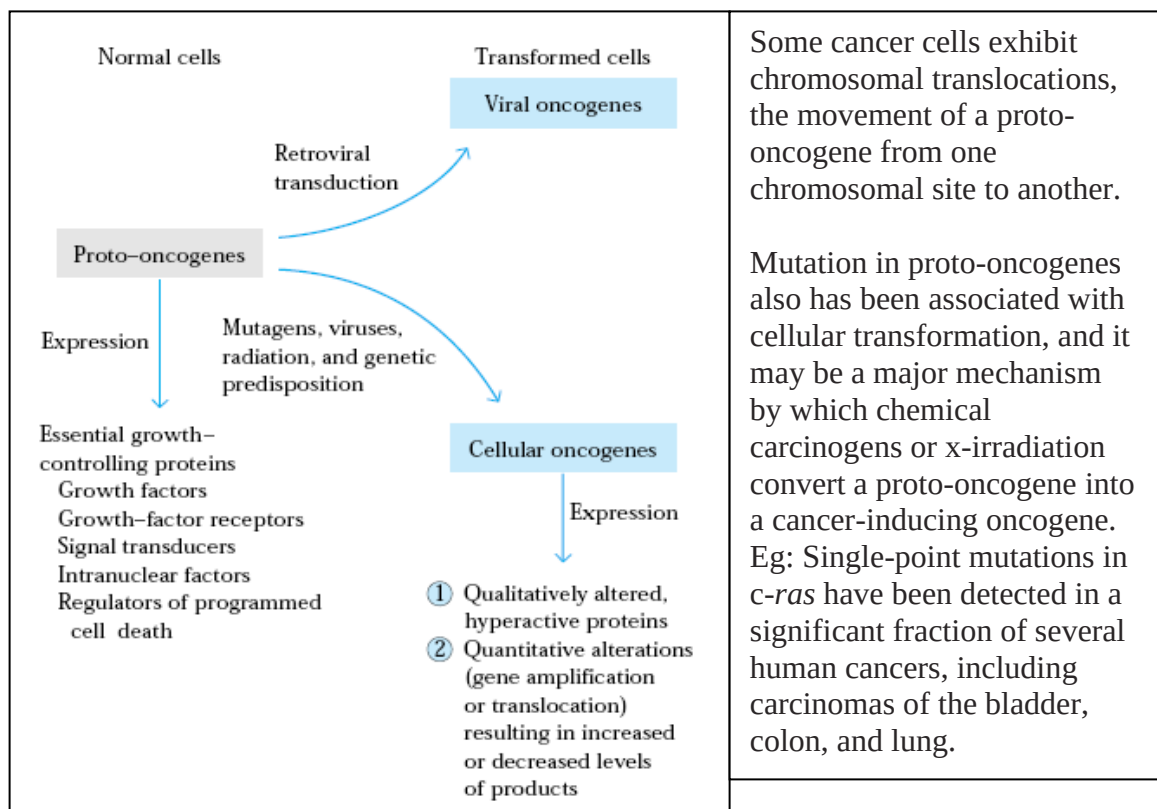
Eg: Growth factor (*sis*) which is a platelet-derived growth factor, Growth factor receptors (*fms*, *erbB*, *neu*, *erbA*), which code for receptors of CSF-1, Epidermal growth factor and Thyroid hormone. They also include signal transducers (*src*, *abl*, *Ha-ras*, *N-ras*, *K-ras*), which code for tyrosine kinases and GTP binding proteins with GTPase activities etc.

**b) Tumor-suppressor genes and Inhibitors of cellular proliferation:** A second category of cancer-associated genes called **tumor suppressor genes**, or **anti-oncogenes**—encodes proteins that inhibit excessive cell proliferation. Inactivation of these results, in unregulated proliferation. The prototype of this category of oncogenes is *Rb*, the retinoblastoma gene. Hereditary retinoblastoma is a rare childhood cancer, in which tumors develop from neural precursor cells in the immature retina. Probably the single most frequent genetic abnormality in human cancer is mutation in *p53*, which encodes a nuclear phosphoprotein. Over 90% of small-cell lung cancers and over 50% of breast and colon cancers have been shown to be associated with mutations in *p53*. Other examples include, DCC, APC, NF1, WT1 which are suppressors of colon carcinoma, adenomatous polyposis, neurofibromatosis and Wilm’s tumor.

**c) Genes that regulate programmed cell death:** A third category of cancer-associated genes regulates programmed cell death. These genes encode proteins that either block or induce apoptosis. Included in this category of oncogenes is *bcl-2*, an anti-apoptosis gene. This oncogene was originally discovered because of its association with B-cell follicular lymphoma. It has been shown to play an important role in regulating cell survival during hematopoiesis and in the survival of selected B cells and T cells during maturation.

### Induction of Cancers is due to conversion of Protooncogenes to Oncogenes:

Mutations or genetic rearrangements of proto-oncogenes by carcinogens or viruses might alter the normally regulated function of these genes, converting them into potent cancer-causing oncogenes.

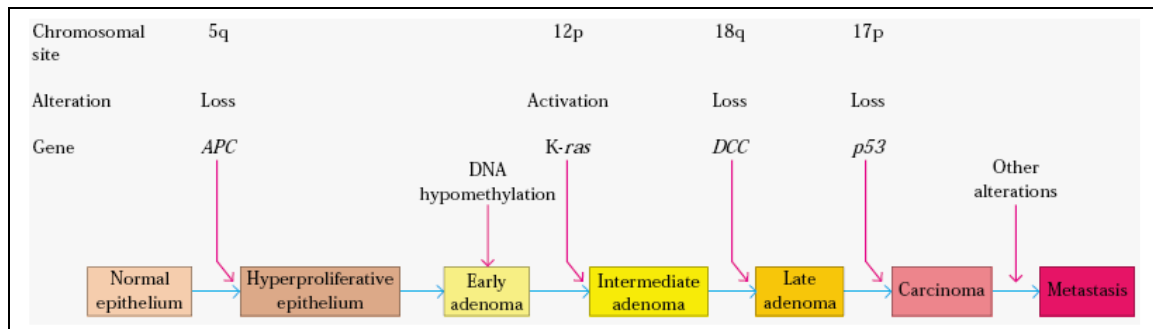


Viral integration into the host-cell genome may in itself serve to convert a proto-oncogene into transforming onco-receptors.

**Induction of cancer is a multistep process:** The development from a normal cell to a cancerous cell is usually a multistep process of clonal evolution driven by a series of somatic mutations that progressively convert the cell from normal growth to a precancerous state and finally a cancerous state.

The steps involved in conversion of a normal epithelial cell into a metastatic cancerous cell are given in figure. These well-defined morphologic stages of colon cancer have been

correlated with a sequence of gene changes involving inactivation or loss of three tumor-suppressor genes (*APC*, *DCC*, and *p53*) and activation of one cellular proliferation oncogene (*K-ras*).



**Tumors of Immune system:** Tumors of the immune system are classified as lymphomas or leukemias. **Lymphomas** proliferate as solid tumors within a lymphoid tissue such as the bone marrow, lymph nodes, or thymus. **Leukemias** tend to proliferate as single cells and are detected by increased cell numbers in the blood or lymph. Leukemia can develop in lymphoid or myeloid lineages.

Leukemias are classified as **acute or chronic** according to the clinical progression of the disease. The acute leukemias appeared suddenly and progressed rapidly, whereas the chronic leukemias were much less aggressive and developed slowly as mild, barely symptomatic diseases.

The major distinction between acute and chronic leukemias is the maturity of the cell involved. Acute leukemias tend to arise in less mature cells, whereas chronic leukemias arise in mature cells. The acute leukemias include, **Acute lymphocytic leukemia (ALL)** and **acute myelogenous leukemia (AML)**; these diseases can develop at any age and have a rapid onset. **The chronic leukemias include chronic lymphocytic leukemia (CLL)** and **chronic myelogenous leukemia (CML)**. These diseases develop slowly and are seen in adults.

A number of B- and T-cell leukemias and lymphomas involve a proto-oncogene that has been translocated into the immunoglobulin genes or T-cell receptor genes. One of the best characterized is the translocation of *c-myc*. The *c-myc* is translocated from chromosome 8 to the Ig heavy-chain gene cluster on chromosome 14. In some patients, *c-myc* remains on chromosome 8 and the  $\kappa$  or  $\lambda$  light-chain genes are translocated to a region 3' of *c-myc*. Kappa-gene translocations from chromosome 2 to chromosome 8 occur 9% of the time, and  $\gamma$ -gene translocations from chromosome 22 to chromosome 8 occur 16% of the time.

**Tumor Antigens:** Antigens on tumor cells are called tumor antigens. Two types of tumor antigens have been identified: **tumor-specific transplantation antigens(TSTAs)** and **tumor-associated transplantation antigens(TATAs)**.

These are unique for a tumor cell and are a result of mutation in the tumor cell. The novel peptides are presented with the MHC classI molecules inducing a cell-mediated response by tumor specific CTL's. The tumor antigens are classified into four major categories: These may be,

- Antigens encoded by genes exclusively expressed by tumors. These are induced by chemical or physical carcinogens while some are viral induced. It is difficult to induce immune response to such tumor antigens.

- Antigens encoded by variant forms of normal genes that have been altered by mutation. Methylcholanthrene and ultraviolet light are two carcinogens that have been used extensively to generate lines of tumor cells. In contrast to chemically induced tumors, virally induced tumors express tumor antigens shared by all tumors induced by the same virus.

- Antigens normally expressed only at certain stages of differentiation or only by certain differentiation lineages. **Oncofetal tumor antigens** are found not only on cancerous cells but also on normal fetal cells. These antigens appear early in embryonic development, before the immune system acquires immunocompetence; if these antigens appear later on cancer cells, they are recognized as nonself and induce an immunologic response. Two well-studied oncofetal antigens are **alpha-fetoprotein (AFP)** and **carcinoembryonic antigen (CEA)**.

- Antigens that are overexpressed in particular tumors. Antigens are also present in normal cells encoded by the corresponding proto-oncogene. In many cases, there is no qualitative difference between the oncogene and proto-oncogene products; instead, the increased levels of the oncogene product can be recognized by the immune system. For example, human breast-cancer cells exhibit elevated expression of the oncogene-encoded Neu protein, a growthfactor receptor, whereas normal adult cells express only trace amounts.

These antigens can induce both humoral as well as cell mediated immunity. Even NK cells can bind to Ab-coated tumor cells and kill the cells.

**Tumor cells evade the Immune system:**

-Even if antibodies are produced and administered by passive immunization these were reported to enhance growth of tumor. In some cases the tumor cells develop blocking factors against antibodies. In some cases, antitumor antibodies themselves were found to act as blocking factors and thereby mask Tc cells activity.

- Certain tumor-specific antigens have been observed to disappear from the surface of tumor cells in the presence of serum antibody and then to reappear after the antibody is no longer present. This phenomenon, called antigenic modulation, is readily observed when leukemic T cells are injected into mice previously immunized with a leukemic T-cell antigen (TL antigen).

- Malignant transformation of cells is often associated with a reduction (or even a complete loss) of class I MHC molecules, and a number of tumors have been shown to express decreased levels of class I MHC molecules. The decrease in class I MHC expression can be accompanied by progressive tumor growth as they are not recognized by the CD8 cells.

-T-cell activation requires an activating signal, triggered by recognition of a peptide-MHC molecule complex by the T-cell receptor, and a co-stimulatory signal, triggered by the interaction of B7 on antigen-presenting cells with CD28 on the T cells. Both signals are needed to induce IL-2 production and proliferation of T cells. The poor immunogenicity of many tumor cells may be due in large part to lack of the co-stimulatory molecules.

**Cancer therapy:**

- Tumor immunity can be enhanced by providing the co-stimulatory signal necessary for activation of CTL precursors (CTL-Ps).

Enhancement of APC Activity Can Modulate Tumor Immunity. Mouse dendritic cells cultured in GM-CSF and incubated with tumor fragments, then reinfused into the mice, have been shown to activate both TH cells and CTLs specific for the tumor antigens.

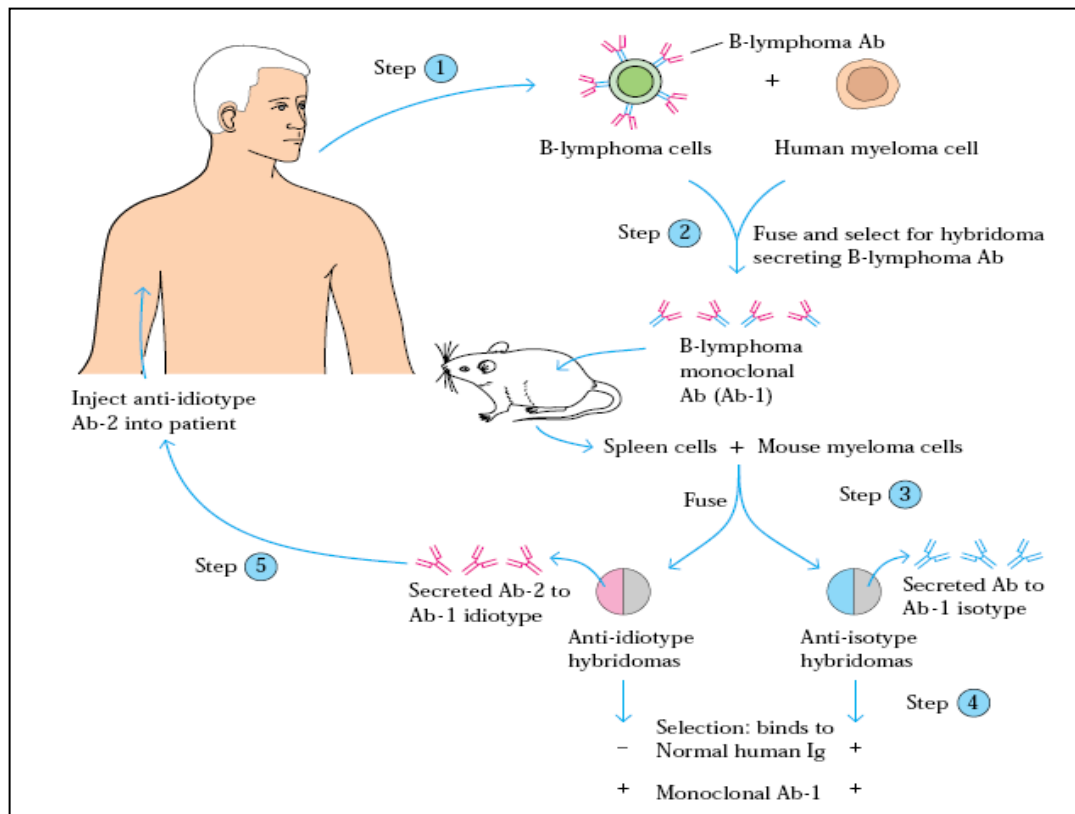
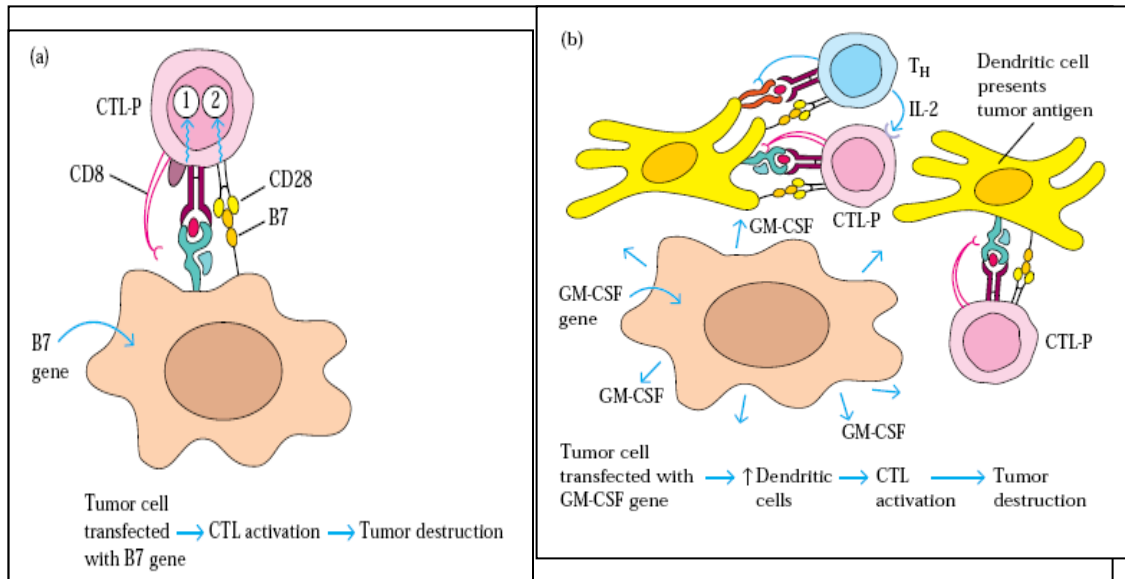
- Cytokine therapy can augment immune responses to tumors. cytokines that have been evaluated in cancer immunotherapy are IFN- $\alpha$ , $\beta$  and  $\gamma$ ; IL-1, IL-2, IL-4, IL-5, and IL-12; GM-CSF; and TNF. All these enhance the expression of MHC class I and class II molecules. Interferon gamma increases Tc cells, macrophages and NK cell activity. TNF cause hemorrhagic necrosis and regression of tumor.

- Lymphokine-activated killer cells along with IL-2 mediate tumor cell destruction.

- Monoclonal antibodies have been used in various ways as experimental immunotherapeutic agents for cancer. For example, anti-idiotypic monoclonal antibodies have been used with some success in treating human B-cell lymphomas and T-cell leukemias. In B-cell cancer, the membrane-bound antibody on all the cancerous cells had



the same idiotype. When Mouse monoclonal anti-idiotype antibody was injected into the patient, it bound specifically to the B-lymphoma cells, because these cells expressed that particular idiotype. Since B-lymphoma cells are susceptible to complement-mediated lysis, the monoclonal antibody activated the complement system and lysed the lymphoma cells without harming other cells. After four injections with this anti-idiotype monoclonal antibody, the tumors began to shrink, and this patient entered an unusually long period of complete remission.





A variety of tumors express significantly increased levels of growth-factor receptors, which are promising targets for anti-tumor monoclonal antibodies.

| TABLE 22-4 Some tumor-associated antigens under examination as potential targets for monoclonal-antibody therapy |                                          |                                                        |
|------------------------------------------------------------------------------------------------------------------|------------------------------------------|--------------------------------------------------------|
| Tumor antigen                                                                                                    | Tumor type                               | Target antigen                                         |
| LYMPHOID CELL-SURFACE MARKERS                                                                                    |                                          |                                                        |
| T-cell marker                                                                                                    | T-cell leukemia/lymphoma                 | CD5                                                    |
| B-cell marker                                                                                                    | B-cell lymphoma                          | CD20                                                   |
| Hematopoietic-cell marker                                                                                        | Acute myeloblastic leukemia              | CD45                                                   |
| Anti-idiotype                                                                                                    | B-cell lymphoma                          | Immunoglobulin                                         |
| NONLYMPHOID TISSUE MARKERS                                                                                       |                                          |                                                        |
| Cell-surface antigens                                                                                            |                                          |                                                        |
| Carcinoembryonic antigen (CEA)                                                                                   | Colon cancer (some others)               | Glycoprotein                                           |
| MUC1                                                                                                             | Breast cancer                            | Glycoprotein                                           |
| Gangliosides such as GD2 and GD3                                                                                 | Neuroectodermal tumors                   | Glycolipids associated with neural tissue              |
| Growth-factor receptors                                                                                          |                                          |                                                        |
| Epidermal growth-factor receptor (EGFR)                                                                          | Some lung, head, neck, and breast tumors | EGF-binding cell surface protein                       |
| HER2 (and EFG-like receptor)                                                                                     | Breast and ovarian tumors                | Cell-surface EGF-binding protein with homology to EGFR |

-Monoclonal antibodies also have been used to prepare tumor-specific anti-tumor agents. In this approach, antibodies to tumor-specific or tumor-associated antigens are coupled with radioactive isotopes, chemotherapy drugs, or potent toxins of biological origin. In such “guided missile” therapies, the toxic agents are delivered specifically to tumor cells. This focuses the toxic effects on the tumor and spares normal tissues. Reagents known as **immunotoxins** have been constructed by coupling the inhibitor chain of a toxin (e.g., diphtheria toxin) to an antibody against a tumor-specific or tumor-associated antigen. In vitro studies have demonstrated that these “magic bullets” can kill tumor cells without harming normal cells.

