Targeted Approach in anti cancer therapy
"Targeted therapy" refers to a new generation of cancer drugs designed to interfere with a specific target protein that is believed to have a critical role in tumor growth or progression \[^{[1]}\]. Targeted approach in anti cancer therapy means a more specific way in the approach of treating the cancer, where in specific tumor therapies are generated either by directly targeting the proteins involved in the neoplastic process or by targeting drugs to the tumor.

Most cancer chemotherapeutics that are in common use at present have little selectivity for cancer cells and can lead to increased toxicities against normal tissues such as the bone marrow, gastrointestinal tract and hair follicles. Non selectivity also results in failure of therapy often accompanied by the development of drug resistance and metastatic disease. The selectivity of an anticancer drug can be increased by either increasing the dose of the drug that reaches the diseased tissue or by decreasing the dose that reaches normal tissues.

Various approaches for improving the selectivity of anticancer therapeutics include:

1. **Tyrosine kinase receptor inhibitor**: includes Epidermal Growth Factor Receptor (EGFR) inhibitors include small molecule inhibitors: gefitinib for non-small cell lung cancer (NSCLC) and erlotinib for metastatic renal cell carcinoma.
   
   Imatinib, for Philadelphia chromosome positive chronic myeloid leukemia (CML). It also inhibits c-kit and Platelet Derived Growth Factor Receptors (PDGF-R) which are mutated in numerous solid tumors such as gastrointestinal stromal tumors, lung cancer, prostate cancer, breast cancer, gliomas and seminoma.

2. **Angiogenesis inhibitor** includes bevacizumab, a vascular endothelial growth factor (VEGF) receptor inhibitor used in treatment of metastatic colorectal cancer, metastatic renal cell carcinoma, multiple myeloma and prostate cancer.

3. **Monoclonal antibodies (MAbs)**: MAbs for hematological malignancies: Rituximab for B-cell lymphoma and B-cell lymphoproliferative disorders. Rituximab (anti CD20) and Alemtuzumab (anti CD52) have proven efficacy in different clinical trials for the treatment of NHL and CLL, respectively.
   
   MAbs for non hematological malignancies: Trastuzumab for advanced breast cancer, Cetuximab for EGFR-expressing, metastatic colorectal cancer.

4. **Other targeted approaches**: **Proteasome inhibitor**: Proteasome is a structure inside the cell which breaks down proteins that have been labeled to undergo degradation and recycling. By binding part of the proteasome, a drug can inhibit the breakdown of some of these proteins that have been marked for destruction. Bortezomib, a proteasome inhibitor, is used in multiple myeloma patients on whom other therapies failed.
Various targeted approaches in Anti cancer therapy

I. Targeted approach using small molecules: Protein phosphorylation regulates most aspects of cell life, whereas abnormal phosphorylation is a cause or consequence of disease especially in cancer biology, such as abnormal proliferation, anti-apoptosis and angiogenesis\(^2,3,4\).

Many studies have indicated that activation of protein phosphorylation-related pathways in tumors can occur through mutation or over expression if compared to normal cells \(^5,6\).

Small molecule inhibitors of protein kinases have emerged as indispensable for studying target therapy. The protein kinases that have been targeted most intensively for drug development are plasma membrane-associated protein tyrosine kinases.

The following are the protein tyrosine kinase inhibitors (also called signal transduction inhibitors):

- **Imatinib mesylate** is approved to treat Philadelphia chromosome positive chronic myeloid leukemia (CML) leukemia, gastrointestinal stromal tumor (a rare cancer of the gastrointestinal tract), dermatofibrosarcoma protuberans, myelodysplastic/myeloproliferative disorders, and systemic mastocytosis. The drug targets several members of a class of proteins called tyrosine kinase enzymes that participate in signal transduction. These enzymes are overactive in some cancers, leading to uncontrolled growth. It is a small-molecule drug, which means that it can pass through cell membranes and reach targets inside the cell.

- **Dasatinib** is approved to treat some patients with CML or acute lymphoblastic leukemia. The drug is a small-molecule inhibitor of several tyrosine kinase enzymes.
Targeted Approach in anti cancer therapy

- **Nilotinib** is approved to treat some patients with CML. The drug is another small-molecule tyrosine kinase inhibitor.

- **Lapatinib** is approved for the treatment of certain types of advanced or metastatic breast cancer. This small-molecule drug inhibits several tyrosine kinases, including the tyrosine kinase activity of HER-2. Lapatinib treatment prevents HER-2 signals from activating cell growth.

- **Gefitinib** is approved to treat patients with advanced non-small cell lung cancer (NSCLC). Gefitinib inhibits the tyrosine kinase activity of EGFR, which is overproduced by many types of cancer cells. This small-molecule drug is restricted to use in patients who, in the opinion of their treating physician, are currently benefiting, or have previously benefited, from gefitinib treatment.

- **Erlotinib** is approved to treat metastatic non-small cell lung cancer (NSCLC) and pancreatic cancer that cannot be removed by surgery or has metastasized. This small-molecule drug inhibits the tyrosine kinase activity of EGFR.

- **Vandetanib** is approved to treat patients with metastatic medullary thyroid cancer who are ineligible for surgery. This small-molecule drug binds to and blocks the growth-promoting activity of several tyrosine kinase enzymes, including EGFR, several receptors for vascular endothelial growth factor receptor (VEGF), and RET.

- **Crizotinib** is approved to treat certain patients with locally advanced or metastatic non-small cell lung cancer. This small-molecule drug inhibits the tyrosine kinase activity of a fusion protein called EML4-ALK, resulting in decreased tumor cell growth, migration, and invasiveness.

- **Sorafenib** is a small-molecule inhibitor of tyrosine kinases that is approved for the treatment of advanced renal cell carcinoma and some cases of hepatocellular carcinoma. One of the kinases that sorafenib inhibits is involved in the signaling pathway that is initiated when VEGF binds to its receptors. As a result, new blood vessel development is halted. Sorafenib also blocks an enzyme that is involved in cell growth and division.

- **Sunitinib** is another small-molecule tyrosine kinase inhibitor that is approved for the treatment of patients with metastatic renal cell carcinoma, gastrointestinal stromal tumor that is not responding to imatinib, or pancreatic neuroendocrine tumors that cannot be removed by surgery, are locally advanced, or have metastasized. Sunitinib blocks kinases involved in VEGF signaling, thereby inhibiting angiogenesis and cell proliferation.

- **Pazopanib** is approved to treat patients with advanced renal cell carcinoma and advanced soft tissue sarcoma. Pazopanib is a small-molecule inhibitor of several tyrosine kinases, including VEGF receptors, c-kit, and platelet-derived growth factor receptor.
II. Antibody targeted therapy: Paul Ehrlich first came with the concept of “magic bullet” in the treatment of disease as early as 1900s. Kohler and Milstein have developed methods for the production of specific monoclonal antibodies against a particular antigen [7].

Antibodies developed against a particular antigen are known as monoclonal antibodies. They are produced by clones of hybrid cells in the laboratory by fusion of B-lymphocytes with tumor cell.

Structure of an immunoglobulin (IgG)

There are two types of monoclonal antibodies used in the treatment: Unconjugated, where in the monoclonal antibodies are without any drugs or radioactive materials attached to them. Another is conjugated monoclonal antibodies, where monoclonal antibodies are attached to drugs, toxins or radioactive atoms.

From the evolution point, monoclonal antibodies are classified as:

- **First generation**: These include murine antibodies, developed following injection of antigens to the animal. The proteins are isolated and purified. Disadvantage is development of human anti mouse antibodies which block the effectiveness of therapy by prematurely clearing the treatment antibodies and limiting possibilities for future immunotherapy [8].

- **Second generation**: These include chimeric, humanized, primatized, or pure human MAbs. Here DNA technology or genetic engineering is used to construct hybrids composed of human antibody regions linked with a murine or primate backbone [9,10].

- **Chimeric Abs**: These are composite of antibodies from two different species. The antibody combines the antigen binding parts (variable region) of the mouse antibody with the effector parts (constant region) of a human antibody e.g., Rituximab.

- **Humanized Abs**: Human antibody containing the complementarity-determining region from a non-human source. The antibody combines only the amino acids responsible for making the Ag binding site (the hypervariable region) of a mouse antibody with the rest of human antibody molecule thus replacing its own hypervariable regions, e.g., Daclizumab, Trastuzumab. **Primatized Abs**: It is a composite of primate variable regions and human constant regions. **Human MAbs**: Can be produced by Recombinant DNA technology, Transgenic technology and Phage display technique.
Strategies for the employment of antibodies for anti-cancer immunotherapy include:

(1) Immune reaction directed destruction of cancer cell: where in antibody gets attached to a particular antigen and activates the complement system and causes the lysis of the tumor cell.

(2) Interference with the growth and differentiation of malignant cells, where in antibody interferes with the signaling of growth factors. Eg: Trastuzumab interferes with the signaling of HER-2/neu protein.

(3) Antigen epitope directed transport of anti-cancer agents to malignant cells, where in the antibody recognizes the specific antigen on the tumor cell and delivers anti cancer agents. In addition, a variety of different agents (e.g., toxins, radionuclides, chemotherapeutic drugs, etc.) have been conjugated to mouse and human MAbs for selective delivery to cancer cells [11].

Applications of Monoclonal antibodies in cancer therapy:

I. Unconjugated antibodies:

Trastuzumab is approved to treat certain types of breast cancer as well as some types of gastric or gastroesophageal adenocarcinoma. This monoclonal antibody that binds to the human epidermal growth factor receptor 2 (HER-2), a receptor with tyrosine kinase activity and prevents HER-2 from sending growth-promoting signals. Pertuzumab is approved to be used in combination with trastuzumab and docetaxel to treat metastatic breast cancer that expresses HER-2 and has not been treated with chemotherapy or a HER-2-directed therapy. Pertuzumab is a monoclonal antibody that binds to HER-2 at a region distinct from trastuzumab. This region allows HER-2 to interact with other receptors, such as the epidermal growth factor receptor (EGFR), to send growth-promoting signals. The drug likely prevents HER-2 from sending growth signals and induces the immune system to attack HER-2-expressing cells. Cetuximab is a monoclonal antibody that is approved to treat some patients with squamous cell carcinoma of the head and neck or colorectal cancer. The drug binds to the external portion of EGFR, thereby preventing the receptor from being activated by growth signals, which may inhibit signal transduction and lead to antiproliferative effects. Panitumumab is approved to treat some patients with metastatic colon cancer. This monoclonal antibody attaches to EGFR and prevents it from sending growth signals. Rituximab is a monoclonal antibody that is approved to treat certain types of B-cell non-Hodgkin lymphoma and, when combined with other drugs, to treat chronic lymphocytic leukemia (CLL). The therapy recognizes a molecule called CD20 that is found on B cells. When Rituximab binds to these cells, it triggers an immune response that results in their destruction. Rituximab may also induce apoptosis. Alemtuzumab is approved to treat patients with B-cell CLL. The therapy is a monoclonal antibody directed against CD52, a protein found on the surface of normal and malignant B and T cells and many other cells of the immune system. Binding of alemtuzumab to CD52 triggers an immune response that destroys the cells. Ofatumumab is approved for the treatment of some patients with CLL that does not respond to treatment with fludarabine and alemtuzumab. This monoclonal antibody is directed against the B-cell CD20 cell surface antigen. Ipilimumab is approved to treat patients with unresectable or metastatic melanoma. This monoclonal antibody is directed against cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), which is expressed on the surface of activated T cells as part of a “checkpoint” to prevent a runaway immune response. By inhibiting CTLA-4, ipilimumab stimulates the immune system to attack melanoma cells.
II. Conjugated antibodies:

**Tositumomab** and **311-I-tositumomab** is approved to treat certain types of B-cell non-Hodgkin lymphoma. The therapy is a mixture of monoclonal antibodies that recognize the CD20 molecule. Some of the antibodies in the mixture are linked to a radioactive substance called iodine-131. The 311-I-tositumomab component delivers radioactive energy to CD20-expressing B cells specifically, reducing collateral damage to normal cells. In addition, the binding of tositumomab to the CD20-expressing B cells triggers the immune system to destroy these cells.

**Ibritumomab tiuxetan** is approved to treat some patients with B-cell non-Hodgkin lymphoma. The therapy is a monoclonal antibody directed against CD20 that is linked to a molecule that can bind radioisotopes such as indium-111 or yttrium-90. These radiolabeled forms deliver a high dose of radioactivity to cells that express CD20. **Denileukin diftitox** is approved to treat some patients with Cutaneous T-Cell Lymphoma (CTCL). Denileukin diftitox consists of interleukin-2 (IL-2) protein sequences fused to diphtheria toxin. The drug binds to cell surface IL-2 receptors, which are found on certain immune cells and some cancer cells, directing the cytotoxic action of the diphtheria toxin to these cells.

**II. Angiogenesis inhibitor / vascular endothelial growth factor (VEGF) receptor inhibitor:** Bevacizumab is a recombinant human MAb that inhibits the biological activities of vascular endothelial growth factor (VEGF) receptor, a protein involved in the neovascularization of malignant tumors. Studies have shown that bevacizumab has both cytostatic and cytotoxic effects, resulting in a reduction in tumor growth and increase in median survival time and time to tumor progression. In addition to these effects it decreases the interstitial pressure in the tumor and increases the delivery of other anti cancer drugs in the tumor. Bevacizumab is available as an intravenous agent and carries FDA-approved labeling for use in the first-line treatment of metastatic colorectal cancer (CRC) in combination with fluorouracil-based chemotherapy and irinotecan-based chemotherapy. Bevacizumab has also received FDA approval for **metastatic HER2 Negative Breast Cancer**, non-small-cell lung, pancreatic, prostate and hepatic cancers, as well as for melanoma and acute myelogenous leukemia[13].

The efficacy and safety of bevacizumab in the front-line treatment of patients with epithelial ovarian, fallopian tube or primary peritoneal cancer as add-on therapy to carboplatin and paclitaxel was demonstrated in a phase III trial when compared to the chemotherapy regimen alone[13].

**IV. Other targeted approaches:**

**Bortezomib** is approved to treat some patients with multiple myeloma and some patients with mantle cell lymphoma. Bortezomib causes cancer cells to die by interfering with the action of a large cellular structure called the proteasome, which controls the degradation of many proteins that regulate cell proliferation. Drugs that block this process are called proteasome inhibitors. Proteasome inhibitors affect normal cells to a lesser extent. **Carfilzomib**, is approved to treat some patients with multiple myeloma whose disease has progressed after treatment with bortezomib. Carfilzomib is another proteasome inhibitor. **Pralatrexate** is approved for the treatment of some patients with peripheral T-cell lymphoma. Pralatrexate is an antifolate, which is a type of molecule that interferes with DNA synthesis. Other antifolates, such as methotrexate, are not considered targeted therapies because they interfere with DNA synthesis in all
Targeted Approach in anti cancer therapy

dividing cells. However, pralatrexate appears to selectively accumulate in cells that express RFC-1, a protein that may be overexpressed by some cancer cells.

**Conclusion:** The use of targeted therapy has markedly changed outcomes for some diseases especially in malignancies. Imatinib has had a dramatic effect on chronic myeloid leukemia, and rituximab, sunitinib, and trastuzumab have revolutionized the treatment of non-Hodgkin’s lymphoma, renal cell carcinoma, and breast cancer, respectively. They also increased the over-all survival rates in certain malignancies like advanced pancreatic cancer (addition of erlotinib to standard chemotherapy). In addition to prolonging survival in patients with certain cancers, targeted therapies provide treatment options for some patients who may not otherwise be candidates for anticancer therapy. For instance, nonsmall cell lung cancer and non-Hodgkin’s lymphoma primarily affect elderly patients, many of whom have medical comorbidities that limit the use of standard chemotherapy. Targeted therapies such as erlotinib and rituximab are often less toxic and better tolerated than traditional chemotherapy, offering these patients additional treatment options. Thus Paul Ehrlich’s conception of “magic bullet” has also become fruitful in the treatment of cancer and has individualized the treatment in cancer patients.

**References:**


