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# Targeted Molecular drugs for Cancer: Molecular Pathophysiology of Oral Cancer

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**Running Title: Molecular therapy**

**Clinical Significance: It is very important for a physician treating a cancer patient to have knowledge about the recently introduced therapeutic drugs.**


## ABSTRACT

Head and Neck cancers are extremely difficult to treat using standard radiotherapy and surgery, due to its toxicity and its effect on the neighbouring normal cells which mayresult in grave, long-term side effects. Therefore, 'targeted drugs' and predictive 'biomarkers' are necessary to improve treatment and minimise the treatment toxicity, and also allow the selection of patients who are more likely to gain from both non-selective and targeted therapies**.**

**Objectives:** The aim of this article was to scan reliable published data that discussed some of the new and commonly used targeted treatments in cancer; which lays stress on their mechanism of action and their clinical applications.

**Materials and methods:** The terms such as cancer, drugs for cancer, monoclonal antibodies and targeted treatments were used to search MEDLINE for English-language studies in humans; published between 2010 and 2016. Publications addressing the objectives of the article were identified and selected for review.

**Results:** Drugs such as rituximab, alemtuzumab, imatinib, gemtuzumabetc are recently developed cancer therapies that target specific types of cells and receptors. Cetuximab has been approved by the FDA to be used for targeted therapy of cancer.

**Conclusion**: Targeted treatment is the recently used approachin the treatment of cancer. This field has been expanding rapidly, with usage of new technology and continued medical research. The clinical use of such agents, delivered either in isolation or in combination with other established chemotherapeutic agents and radiation, may finally lead to better schedules and enhanced clinical responses.


## INTRODUCTION

Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by obstructing specific molecules ("molecular targets") that are involved in itsprogression. Targeted cancer therapies are known by various names:"molecularly targeted drugs" being one of them. [1]

Targeted therapies are different from standard chemotherapy in that[1]

| Standard Chemotherapeutic drugs | Targeted chemotherapy |
|---|---|
| 1. Act on the rapidly dividing normal and cancerous cells | 1. Act on specific molecular targets associated with cancer |
| 2. Standard chemotherapies identified because they kill cells. | 2. Deliberately chosen or designed to interact with their target |
| 3. Chemotherapeutic agents are cytotoxic (that is, they kill tumor cells) [1] | 3. Cytostatic (that is, they block tumor cell proliferation)<br>4. Targeted therapies are usually better endured than traditional chemotherapy[1] |
| 4. Patients experience toxicities of gastrointestinal symptoms, alopecia, and myelosuppression | 5. Associated with several adverse effects, such as cardiac dysfunction, acneiform rash, hypertension, thrombosis, and proteinuria. |
| 5. The treatment is general[6] | 6. The treatment is patient centric[6] |
| 7. Although the conventional cytotoxic chemotherapy is the preferred treatment for many malignancies, targeted therapies are considered as a mode of treatment for breast, pancreatic, colorectal and lung cancers, as well as multiple myeloma, lymphoma and leukemia.[6] | |

At present, treatment of an individual cancer is typically dependent,within a multidisciplinary setting; on the histological subtype, subsite, staging information; patient fitness, baseline swallow and airway function, guiding management decisions. [2]

**Steps leading to carcinogenesis**

Hahn and colleagues have demonstrated 6 steps believed to be necessary for the development of cancer:

- ✓ Autonomous proliferative signalling
- ✓ Inhibiting the growth inhibitory signals
- ✓ To escape programmed cell death
- ✓ To be immortalised
- ✓ Angiogenesis and
- ✓ Tissue invasion and metastasis

All the above mentioned steps necessary for tumorigenesisoffer a potential target for molecular therapy.

Scientists, while studying methods that would eliminate the tumor cells, discovered that the receptors located on the normal and cancer cells were different. They also found out that all molecules on cancer cells were not necessarily good targets for cancer therapy at the molecular level.

It was discovered that in order for the cell to be an ideal target they should have certain properties such as:

- Receptors must commonly be found on cancer cells and only on cancer cells
- They must be differentially expressed or be differentially functional in tumorcells as opposed to nontumor host tissues
- They must be the cause for tumorprogression, cell viability, or both

- They must also be involved in the carcinogenesis pathway
- They should be quantifiable in diagnostic tumor material
- They should also be able to exhibit and be able to be quantified, for either the positive or the null effect on the tumor tissue. [3]
  This may be done by performing tumor biopsies together with the evaluation of EGFR, phosphorylated EGFR, its downstream signalling molecules and their phosphorylated forms (Erk, Akt, etc). Some studies have utilized measures of proliferation, such as immunohistochemical staining, Ki67 and imaging of tumor metabolic activity with positron-emission tomography.[4]
  Some of the targets in use for head and cancers include
    - epidermal growth factor receptors (EGFRs),
    - interleukin-13 receptor (IL-13R),
    - protein kinase activator, and
    - others that are in various phase I, II, and III of studies.

**The development of targeted therapy**

**Autonomous proliferative signalling**

Cells transform from proto-oncogenes to oncogenes. Proto-oncogenes transform into oncogenes without the help of growth factors in case of cancer. In normal circumstances they facilitate intracellular signalling and enable cell growth and differentiation.

The goal of gene therapy is to introduce new genetic material into cancer cells that selectively kills them without causing toxicity to the surrounding cells. This task can be accomplished by replacing tumor suppressor genes that have been lost or mutated, selectively inserting genes that produce cytotoxic substances, or modifying the immune system to destroy the tumor cells. The major barrier in successful gene therapy is producing a vector that selectively infects all tumor cells within a tumor. Various techniques have been developed for targeting cancer cells:

- gene therapy,
- monoclonal antibodies (MAbs),
- antibody toxin conjugates,
- small-molecule inhibitors,
- antisense molecules, and
- tumor vaccines.

Small molecules or monoclonal antibodies form the basis of target drugs. Small molecules are able to enter the cells easily whereas the monoclonal antibodies are somewhat large and cannot enter cells hence they are used only for targets on the cell surface or outside the cells.[1]

MAbs and antibody toxin conjugates can be targeted to specific receptors or proteins found on cancer cells. MAbs can block receptors and prevent potential growth signals. Antibodies can also be conjugated to toxins and specifically kill the tumor cells they bind.

Antisense molecules are a small, complementary, single-stranded type of DNA that binds targeted messenger RNA (mRNA) within the cell and prevents specific protein translation. Antisense molecules can be targeted toward specific proteins that are crucial in tumorigenesis.

Small-molecule inhibitors can bind and inhibit specific receptors or enzymes in cancer cells. These small-molecule inhibitors can be targeted towards any marker, involved in the crucial steps oftumorigenesis.

Tumor vaccines act to stimulate the patient's immune system to attack cancer cells. These tumorvaccines can be developed from the patient's tumor cells. In this process, mRNA is isolated from a tumor biopsy sample, amplified,

and incorporated into human antigen-presenting cells (APCs). The APCs are then intravenously given to the patient to stimulate the patient's immune system to activate antitumor T cells.[3]

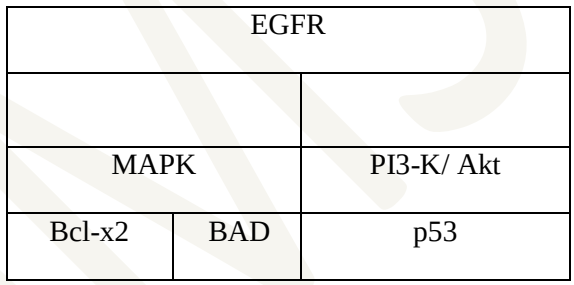
**EGFR signalling pathway**

The molecular pathways most often targeted in the treatment of solid tumors are those of the epidermal growth factor receptor (EGFR, also known as HER1), HER2/neu and the vascular endothelial growth factor (VEGF). [2]

EFGR is a receptor tyrosine kinase playing a major role in cancers. Its multiple downstream signalling pathways indirectly influence the cell growth, angiogenesis, and invasion of cancer cells. It activates the mitogen-activated protein kinase (MAPK) pathway as well as the phosphatidylinositol 3-kinase (PI3-K)/protein kinase B (Akt) pathway. Activation of the MAPK pathway leads to increased expression of antiapoptotic proteins like Bcl-x2 and inhibition of proapoptotic proteins like BAD. Signaling through the PI3-K/Akt pathway ultimately leads to inhibition of the tumor suppressor gene p53. All of theseresult in a proliferative state, and inhibition of tumor suppressorfunction.[5] EGFR is composed of an extracellular ligand-binding domain, acytoplasmic domain

**Figure 1**

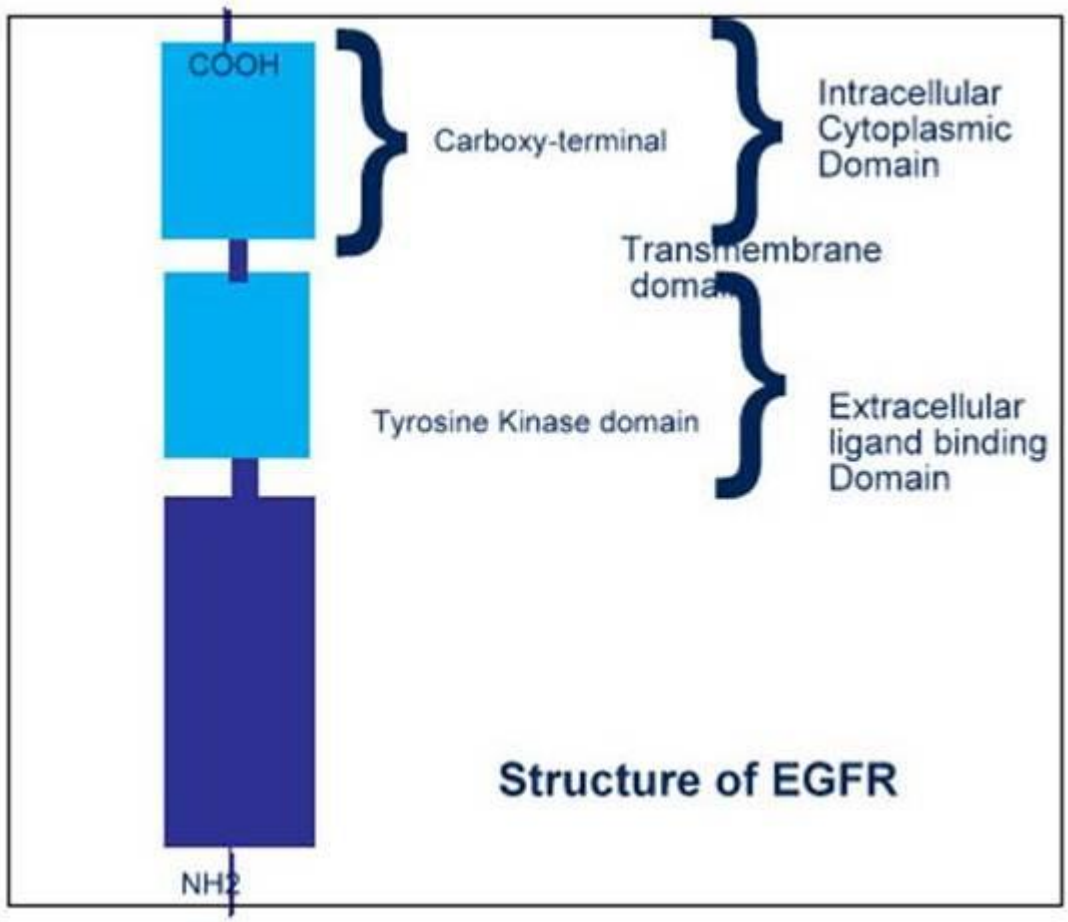
**Figure 1: Flowchart showing the downstream signalling pathway of the EGFR for targeted drug therapy.**




transmembrane segment and with tyrosine kinase activity.[2]The EGFR is one of a family of receptor tyrosine kinases that, when bound to one of several possible ligands, dimerizes and initiates phosphorylation-driven signalling cascades that result in cell proliferation, decreased apoptosis, angiogenesis, and other mechanisms associated with tumor progression and metastasis

The HER (erbB) family is made up of transmembrane receptor tyrosine kinases is one of the cytostatic targets in the growth of tumor cells and its survival. The binding domain of the HER receptor communicates through the EGFR and reaches into the cell to enable the cell growth and proliferation. An overexpression of EGFR has been recognized in more than 80% of squamous cell cancers, and this overexpression has been linked to poor prognosis. Drugs (EGFR inhibitors) interrupting the signalling has been shown to inhibit tumor cell propagation and/or feasibility both in vitro and in vivo. Selective compounds have been developed that target either the extracellular ligand-binding region of the EGFR (including a number of monoclonal antibodies [MAbs], immunotoxins, and ligand-binding cytotoxic agents) or the intracellular tyrosine kinase region (including various small-molecule inhibitors). The most rigorously studied monoclonal antibody, cetuximab, has shown an enhanced ability to kill tumor cells in synergy with radiation and chemotherapy.

Cetuximab C225 (Erbitux, Imclone Systems Inc, New York) 1 is the only European Medicines Agency-approved and FDA approved drug. [3] It has been the most religiously studied for targeted therapy of head and neck, lung, and colorectal cancers.1 In combination with radiotherapy and not with radiotherapy alone it advances life of the patient.[5] But the drawback of this drug is the reduced angiogenesis and the inability of DNA repair after exposure to radiation.[3]




**Dysregulation of HER-1/EGFR activity may be caused by mechanisms such as ligand overproduction, receptor overexpression, the presence of active mutant receptors, and cross-connection with varied amplified receptors and signalling systems, among others.** [3]

Panitumumab is a fully human anti-EGFR monoclonal antibody commonly used for the treatment of metastatic colorectal cancer. [5]

Nimotuzumab is a humanized anti-EGFR monoclonal antibody.

Zalutumumab (HuMax-EGFr) is a fully human IgG1κ monoclonal antibody targeting EGFr.[4]

Alemtuzumab (Campath) which targets CD52 is available as Humanized, unconjugated and is used for treatment of Chronic lymphocytic leukemia

Bevacizumab (Avastin) acts on VEGF. Available as Humanized, unconjugated for treatment of Colorectal cancer, non-small cell lung cancer.

GemtuzumabOzogamicin (Mylotarg) against CD33 and availalble as Humanized, toxin conjugate (calicheamicin) for treatment of Acute myeloid leukemia

90Y-Ibritumomab Tiuxetan (Zevalin) acts on CD20 available as Murine, radioisotope conjugate (yttrium-90) used in treatment of Non-Hodgkin's lymphoma

Rituximab (Rituxan) on CD20 available as Chimeric, unconjugated; acts on Non-Hodgkin's lymphoma, rheumatoid arthritis

131I-Tositumomab (Bexxar) on CD20 available as Murine, radioisotope conjugate (iodine-131) acts in Non-Hodgkin's lymphoma

Trastuzumab (Herceptin) on HER2/neu, available as Humanized, unconjugated acts in case of Breast cancer with HER2/neu overexpression.[6]

US Food and Drug Administration (FDA) has approved the clinical use of tyrosine kinase inhibitor targeting drugs such as oral quinazolineserlotinib (OSI-774, Tarceva; Genentech, South San Francisco, CA) and gefitinib (ZD 1839, Iressa; AstraZeneca, Wilmington, DE) [3]being developed by the NCI.[4]Gefitinib and erlotinib, currently being used in lung cancer, inhibit only EGFR and have not been shown to be efficacious in HNSCC till date. [4] These agents will be used in combination with chemotherapy (taxanes and platinums) and radiation therapy. [4]

Lapatinib is a reversible tyrosine kinase inhibitor that acts against both EGFR and HER2 [3]and is used in Breast cancer with HER2/neu overexpression[6]

Other tyrosine kinase inhibitors are afatinib, sunitinib, dacomitinib, and vandetanib for the treatment of advanced HNSCC.[3]

Bortezomib(Velcade) acts on 26S proteasome and is used in Multiple myeloma, mantle cell lymphoma (a subtype of non-Hodgkin's lymphoma)

Dasatinib (Sprycel) acts on BCR-ABL, SRC family, c-KIT, PDGFR and used in Chronic myeloid leukemia, acute lymphocytic leukemia

Imatinib (Gleevec) acts on BCR-ABL, c-KIT, PDGFR and is used in Acute lymphocytic leukemia, chronic myeloid leukemia, gastrointestinal stromal tumor, hypereosinophilic syndrome, systemic mastocytosis. This is the most effective among tyrosine kinase acting, set of drugs.

Rituximab has revolutionized the treatment of non-Hodgkin's lymphoma.[6]

NOTCH1's role in the complex signalling pathway of HNSCC needs to be examined, and therefore could potentially represent another therapeutic target. Both the NOTCH1 pathway inhibitors, that inhibit γ-secretase and NOTCH1 pathway activators, through inhibition of histone deacetylase, are presently undergoing clinical investigation.[2]

Nuclear factor kappaB (NF-kB), a transcription factor enables cell proliferation during inflammation.Inhibition of NF-kB can act as a therapeutic mode for HNSCC. NF-kB can enhance TNF mediated apoptosis.

Ras oncogene enables cell proliferation.[3]Ras is a guanosine nucleotide binding protein localised on the plasma membrane. Ras is bound to guanosinediphosphate (GDP) and activation converts Ras to the guanosine triphosphate (GTP)-bound form; Ras-GTP binds to and activates Raf-1. Raf-1 is phosphorylated by kinases MEK1 and MEK2 that in turn activate the MAP kinases ERK1 and ERK2. These then translocate to the nucleus and target genes involved in cell growth, proliferation and survival.[2]Ras can also activate the PI3K signalling cascade. Farnesyltransferase inhibitors (FTIs) can specifically inhibit the ras oncogene and have been shown to inhibit growth of tumor cell lines. Phase II clinical trials have been unable to show any significant response and further investigation is warranted.[3]

Sorafenib is a tyrosine kinase inhibitor that targets multiple ligands including Raf, PDGFR (platelet derived growth factor receptor) and VEGF (vascular endothelial growth factor receptor).

Trametinib, an MEK inhibitor has recently been approved for use in metastatic melanoma and investigation is underway to identify its usage in combination with AKT inhibition in solid tumours, such as the HNSCC. [2]

Mutations in STATs (Signal transducers and activators of transcription (STATs),that are a family of proteins transmit growth signals from the cell surface to the nucleus), specifically signal transducer and activator of transcription 3 (STAT3), have been shown to play a role in the development of HNSCC.

Small-molecule inhibitors have also been developed to inhibit the tyrosine kinase activity of EGFR. These molecules are typically adenosine triphosphate (ATP) acting.

Nucleic acid–based molecules that have been developed to interfere with translation of EGFR protein include the antisense oligodeoxynucleotides and small interfering mRNA. However, these are still in the early stages of investigation.

Gene therapy techniques are being used to restore TP53, INK4, Cyclin D1 and RB function lost in HNSCC.[3] Up to 90% of squamous cell cancers of the head and neck may have abnormalities in the cyclin D1/Rb/p16 pathway. Clinical trials are underway for Ad5CMV-p53 (virally mediated transduction of the p53 gene), flavopiridol (a cyclin-dependent kinase inhibitor), and the proteasome inhibitor PS-341 (inhibits degradation of signalling or modulatory molecules, such as p53, p27, and I kappaB). [4]

Telomerase can be targeted for treatment of HNSCC.[3]

It is known that angiogenesis is induced by hypoxia. [4]Phase III study of bevacizumab (Avastin) which is a humanized monoclonal antibody (MAb) was found to inhibit VEGF; a protein for angiogenesis, delays tumor growth in patients with metastatic colon cancer.[3]Interferon-alpha, [4] basic fibroblast growth factor (bFGF), interleukin-8 (IL-8) and platelet-derived endothelial cell growth factor (PD-ECGF), are also potent angiogenic factors.[3]

Tyrosine kinase receptor of VEGF are targeted by drugs that include Sunitinib, sorafenib, vandetanib, semaxanib, and foretinib, which are undergoing phase II clinical trials. [5]

Tumour oxygenation strategies have included the usage of hyperbaric oxygen, nicotinamide, carbogen, hypoxic cytotoxintirapazamine and radiosensitisation using nitroimidazoles foreg Nimorazole.[2]

Basement membrane proteins may also facilitate molecular target therapy in HNSCC.

For action on EpCAM, VB4-845 (Proxinium; Viventia Biotech, Inc) drug is currently being investigated in [2]phase II clinical trials. This drug is a recombinant fusion protein produced by E coli that expresses a humanized single-chain antibody fragment exclusive for EpCAM and linked to a truncated Pseudomonas exotoxin A.

IL-13R, COX-2, CEA, and tgDCC-E1A have also been used in targeted drug therapy for HNSCC.[3]

Metformin a Type 2 diabetes drug in combination with paclitaxel is being investigated in a phase II trial in metastatic/recurrent HNSCC. [2]Hence diabetics on metformin are at a reduced risk for cancer.[2]

MicroRNAs (miRNAs) are intracytoplasmic, tiny, non-coding RNAs consisting of 18–25 nucleotides that control and enhance genetic expression at both translational and transcriptional levels. The miR-221: miR-375 ratio can differentiate between normal and malignant tissue, and an elevated expression of miR-181 and miR-211 in oral SCC is correlated with metastasis to lymph nodes, vascular invasion and poor prognosis. The miRNAs have the potential in predicting the response to cancer treatment and can also be used as a therapeutic target. [2]

EphB4 and EphrinB2 are potentially useful biomarker and can be used for drug targeting. [5]

| o **Adverse effect** | o **Drug (affects >1% of patients)** |
|---|---|
| o Diarrhoea | o dabrafenib, dasatinib, erlotinib, gefitinib, lapatinib,<br>o nilotinib, pazopanib, sorafenib, sunitinib |

| | |
|---|---|
| ○ acneiform rash, | ○ |
| ○ cardiac dysfunction, | ○ |
| ○ thrombosis | ○ |
| ○ Hypertension | ○ pazopanib, sorafenib, sunitinib |
| ○ Prolongation of QT interval | ○ dabrafenib, dasatinib, lapatinib, nilotinib, pazopanib,<br>○ sorafenib, sunitinib |
| ○ Proteinuria | |
| ○ Constipation | ○ lenalidomide, thalidomide |
| ○ Fever | ○ dabrafenib |
| ○ Hypothyroidism | ○ imatinib, pazopanib, sunitinib |
| ○ Pulmonary complications | ○ dasatinib, imatinib, erlotinib, gefitinib, lapatinib |
| ○ Venous thromboembolic events | ○ lenalidomide, thalidomide, pazopanib, sorafenib,<br>○ sunitinib, |
| ○ Bleeding | ○ sorafenib, dasatinib, erlotinib, pazopanib,<br>○ sunitinib, gefitinib, |
| ○ Reduction in left ventricular ejection fraction | ○ dasatinib, sunitinib, lapatinib, , sorafenib,<br>○ trametinib, pazopanib |
| ○ Oedema | ○ dasatinib, nilotinib, everolimus, imatinib |

**TABLE 1**

**Table 1: Adverse effects of targeted drugs:**[7]

**Disadvantages of targeted therapy include:**

Cancer cells can become resistant to the drugs.

Resistance can occur in two ways:

- ✓ there is the target itself undergoing mutation so that the targeted therapy no longer interacts well with it, and/or
- ✓ the tumortakes its own new pathway to achieve tumor growth.[1]

**CONCLUSION**

Identification of specific epigenetic, genetic and metabolic aberrations, and using it collaboration with the traditional techniques will help an oncologist in tailoring therapy based on both patient and tumour characteristics. An increased understanding of the molecular biology through usage of high-throughput technology points to a future of personalised medicine.

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