

# Gene Therapy in the Treatment and Prevention of Oral Cancer: An Overview

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## ABSTRACT

Cancer is a disease characterized by uncontrollable and unwanted growth of body cells due to the loss of their normal regulatory controls. Oral cancer has a remarkable incidence worldwide, and a fairly poor prognosis. Gene therapy can be used to treat cancer patients who do not respond to traditional therapies. Gene therapy is a new treatment procedure in which an existing gene is manipulated, or a new gene is introduced, in order to slow the growth of the tumor or cause the death of the cancer cells. Currently, clinical trials in oral cancer have revealed evidence of gene expression and transduction, mediation of apoptosis, and pathological complete responses by way of clinical responses. A web-based search for articles published was done by using Medline/PubMed to review gene therapy in treatment and prevention of oral cancer. Articles published in English with no restriction on publication date (last update 2014) were included in the search. The search revealed the different treatment approaches for cancer gene therapy: Gene addition therapy (particularly p53), immunotherapy, gene therapy using oncolytic viruses, antisense ribonucleic acid (RNA), and RNA interference-based gene therapy. Published scientific data show promising pathways for the future development of more effective prognosis. In this paper, current approaches to gene therapy in oral cancer are discussed with an emphasis on dynamics of oral cancer, risk factors, and biomarkers.

**Keywords:** Biomarkers, Clinical trials, Dynamics of oral cancer, Gene therapy, Immunity, Oral cancer

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## INTRODUCTION

Globally, the 6<sup>th</sup> most common malignancy affecting mankind is head and neck cancer (HNC).<sup>1</sup>

The vast majority (more than 90%) are squamous cell carcinomas and affect approximately 405,000 people each year ([www-dep.iarc.fr](http://www-dep.iarc.fr)).<sup>2</sup> This aggressive epithelial malignancy is associated with severe morbidity and <50% long-term survival despite advances in treatment with surgery, radiation, and chemotherapy. The HNC disease can affect overall health and mental health, appearance, employment, social life, and family living. Furthermore, serious changes in the functioning of the upper aerodigestive tract that affect the quality of life of patients may occur.<sup>3</sup> Males are more prone to develop oral squamous cell carcinomas (OSCC) since they are exposed to various oral risk factors such as tobacco smoking, betel quid chewing, or alcohol consumption.<sup>2</sup>

The influence of family history in head and neck squamous cell carcinoma (HNSCC) development may be because of familial aggregations that may indicate that inheritable genetic factors play a role in HNSCC risk.<sup>3</sup>

Oral cancer can be diagnosed definitively only after becoming locally advanced in the majority of cases.<sup>3</sup> The ability to intervene prior to this advanced stage may improve treatment results. Furthermore, the understanding of disease development and its appearance can help in the treatment choice, as well as the symptoms analysis, and/or rehabilitation as necessary. This along with better organization and quality of care help in identifying aspects of impact on patient survival which in turn help the decision on the effectiveness of treatment through the clarification of the side effects of treatment.<sup>3</sup> Surgery combined with or without neck dissection, or a combination of surgery, and radiotherapy are used to treat primary OSCC. However, despite the radical nature of the treatment, recurrences are common.<sup>4</sup>

There is a clear need for new prognostic (read genetic) indicators, which could be used in diagnostics, as well as in selecting the most effective method of treatment.<sup>5</sup> Gene therapy has potential to target cancer cells while sparing normal cells.

The clinical application of gene therapy for the treatment of HNC will require gene delivery to be optimized while simultaneously determining the efficiency of transfection.<sup>6</sup>

This paper aims to recapitulate the dynamics of oral cancer, risk factors and biomarkers, current status, and future aspects of gene therapy in treatment and prevention of oral cancer.

## MATERIALS AND METHODS

A web-based search for articles published was done using Medline, PubMed, Google scholar databases for duration of 3 months from August 2014 to October 2014 with key words such as oral cancer, OSCC, tobacco smoking, alcohol consumption, genetic polymorphisms, biomarkers, risk factors, immunity, and gene therapy. Furthermore, reference lists of these articles were examined for any further relevant articles that had not been identified earlier.

The search was confined to articles published in English without restriction on publication date (last update 2014). A total of 182 articles were found in the initial search and among them total 26 were found relevant to the review based on study objectives. These included animal trials and human trials under various phases of clinical trial.

### Dynamics of Oral Cancer

Cancer is a disorder involving dynamic changes in the structure, as well as the function of the cellular genome, in the cancer cells. The changes observed in the cancer cells are as follows:

- Neoplastic differentiation
- Insensitivity to anti - growth signals
- Tissue invasion and metastasis
- Limitless replicative potential
- Evading apoptosis
- Sustained angiogenesis
- Self-sufficiency in growth signals.<sup>7</sup>

### Production of Cancer by Carcinogens

In order to understand tier dynamics of cancer, we need to understand cell proliferation and its regulation. Normal proliferation, differentiation, and growth in cells are sequentially controlled by the following events:

1. Binding of growth factors to specific receptors on the cell membrane.

2. Activation of growth factor receptors that further activates signal transducing proteins on the inner surface of the plasma membrane.
3. Subsequently an appropriate signal is transmitted to the nucleus through certain messenger proteins -across the cytoplasm.
4. Activation of transcription factors that bind at specific regions on the genome initiate DNA transcription.
5. Cell enters into mitosis after passing through the checkpoints and eventually undergoes nuclear and cytoplasmic division.<sup>8</sup>

The events mentioned above operate under strict genetic control. The abnormal proliferation of cells may result from mutations that alter the functions of genes governing cellular proliferation. Defective signaling in growth regulating pathways can lead to abnormal growth. Abnormal expression of components regulating signaling pathways is caused by mutations in the responsible genes (proto-oncogenes). A non-activated proto-oncogene can be transformed into activated cancer producing oncogene by a point mutation, chromosomal translocation, or viral infection. E.g., Cyclin D, Cdk4, epidermal growth factor receptor (EGFR), fibroblast growth factor receptor, *ras*, Bcl2, and Mdm2.<sup>9</sup> On the other hand, a normal cell also contains genes, which are called tumor suppressor genes. The function of these genes is to apply "brakes" to cell growth. Thus, they possess tumor suppressor activity. E.g., BRCA 1, BRCA 2, MLH 1, p53, p21, Rb 1, Bax, and APC.<sup>8</sup>

### Risk Factors and Biomarkers

The genetic aberrations in cancer cells can be identified well before the resultant cancer phenotypes are manifested.<sup>10</sup> Changes arising exclusively or preferentially in cancerous tissue can be used as molecular biomarkers.<sup>11</sup> A variety of nucleic acid-based biomarkers has been demonstrated as novel and powerful tools for the detection of cancers.<sup>9,12,13</sup> To date, most of these markers have been identified in cancer cell lines or biopsy specimens from advanced invasive and metastatic cancers.<sup>10</sup>

Asakage *et al.* found that the significant independent risk factors for oral and pharyngeal cancer overall among moderate-to-heavy drinkers were inactive ALDH21/2, less-active ADH1B1/1 among other factors.<sup>14</sup> In a Japanese study, this risk was associated with significant genetic interactions between ADH2 and ALDH2 polymorphisms, as well as gene-environment interactions, between these polymorphisms and alcohol drinking.<sup>1</sup>

Li *et al.* discovered, seven cancer-related mRNA biomarkers that exhibited at least a 3.5-fold elevation in

OSCC patients saliva ( $P < 0.01$ ). The utility of salivary transcriptome diagnostics for oral cancer detection was demonstrated in this study.<sup>10</sup> In a study by Garnis *et al.*, genome alterations in low - grade dysplasias progressing to invasive disease had a closer resemblance to those observed for later stage disease compared to non-progressing low -grade dysplasias. The data demonstrates that high -resolution genomic analysis can be used to evaluate progression risk in low - grade oral premalignant lesions.<sup>15</sup>

### Immunity

A significant and profound immunosuppression is often found in HNSCC patients in comparison with other malignancies.<sup>16</sup> Earlier studies of oral cancer patients have shown a higher antibody response<sup>17</sup> and impaired T-cell functions<sup>18</sup> that were also related to the poor prognosis of the patient.<sup>19</sup> However, the exact mechanism of altered immunity in these patients is not known. Qin *et al.* evaluated the efficiency of recombinant vaccinia virus expressing interleukin-2 (rvv-IL-2) as a tumor vaccine. They demonstrated the higher expression of IL-10, GM-CSF, TGF- $\beta$ , and NO synthetase in tumors. It was suggested that these molecules might play a role in immunosuppression. Hence, rvv-IL-2 has potential as a therapeutic vaccine for HNC, and it could be more effective provided the immunosuppression was reversed.<sup>20</sup>

### Gene Therapy

Gene therapy is defined as “gene transfer for the purpose of treating human disease, this includes the transfer of new genetic material, as well as manipulation of the existing genetic material.”<sup>6</sup> The main purpose of gene therapy is introduction of new genetic material into the target cells while leaving the surrounding normal cells undamaged.<sup>21</sup>

#### Modes of Gene Delivery in Gene Therapy

Viruses/bacteria and other non-viral methods are used to deliver genetic material into the host cells. All viruses bind to their hosts by introducing their genetic material into the host cells. In gene therapy, the viruses act as vehicles to deliver the therapeutic DNA into the host cells after the viral DNA has been removed.

The vectors in gene therapy are outlined in Figure 1.<sup>21</sup>

However, the efficiency of gene delivery *in vivo* by non-viral vectors is limited due to inhibition by the serum components. Despite the use of several non-viral methods, viruses provide a more efficient mode in gene therapy.<sup>21</sup>

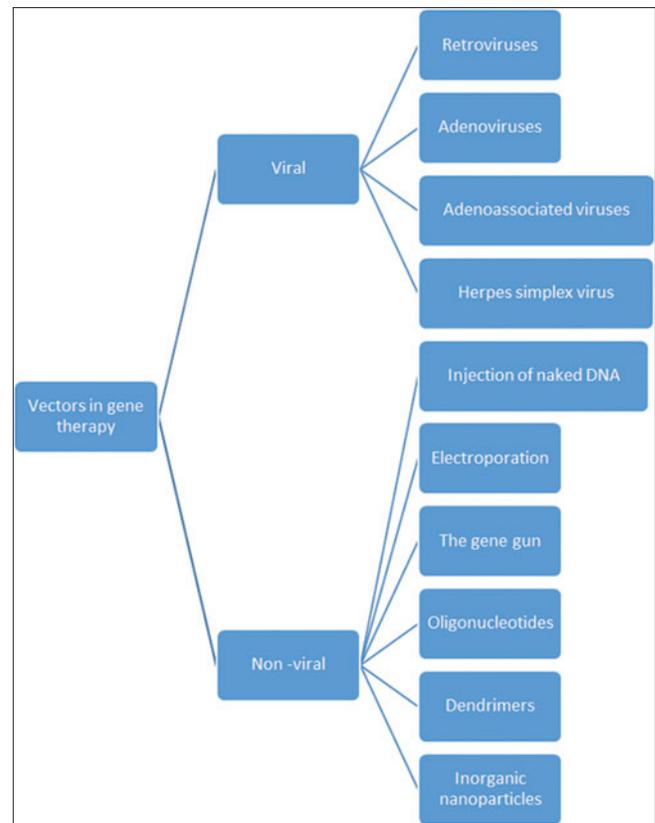


Figure 1: Vectors in gene therapy

### The Techniques of Gene Therapy

Gene therapies that result in cancer cell death include:

- Gene addition therapy
- Gene excision therapy<sup>21</sup>
- Antisense ribonucleic acid (RNA) therapy
- RNA interference (RNAi)-based gene therapy
- Immunotherapy
- Suicide gene therapy
- Gene therapy with the use of oncolytic viruses
- The delivery of drug resistance genes into normal tissues for protection against chemotherapy.<sup>6</sup>

**Gene addition therapy:** In this technique, the tumor growth is controlled by the introduction of tumor suppressor genes (p53, p21, p16), which inactivate the carcinogenic cells.<sup>6</sup> The p27 gene was found to inhibit the cell cycle of tumor cells. It was found that intraepithelial injections of recombinant human adenovirus-p53 were safe, feasible, and biologically active for patients with dysplastic oral leukoplakia.<sup>22</sup> In another study, it was found that 11R-p53 enhanced the cisplatin-dependent induction of apoptosis of the cells.<sup>23</sup>

**Gene excision therapy:** Removal of the defective oncogenes leads to an inhibition in the growth of tumor cells.<sup>21</sup> Okadaic acid is a highly toxic polyether that inhibits phosphorylation of Types 1 and 2A proteins, reducing expression of Egr-1 (related to cell proliferation and division), which in turn

inhibits tumor activity. Genes that control cell growth and cell cycle progression, including those that encode for tissue factors TGF- $\beta$ 1, PDGF-A, and PTEN are also regulated by the expression of Egr-1, making this a good therapeutic approach. It was demonstrated that inhibition of protein kinase C reduced the expression of this gene, triggering higher sensitivity of the tumor to radiotherapy.<sup>24</sup>

### Antisense RNA Therapy

The Antisense RNA inhibits the RNA, which is complementary to the strands of the DNA which expresses that particular gene thereby checking the tumor growth.<sup>21</sup> Inhibition of expression of these oncogenes (MYC, FOS, and RAS) may alter the phenotype, thus abrogating tumor growth.<sup>25,26</sup> Preclinical studies using antisense sequences under the control of six small RNA promoters demonstrated a powerful anti-tumor effect with minimum toxicity.<sup>26</sup> The difficulty of introducing a sufficient quantity of antisense molecules to inhibit tumor growth limits the use of this technique. Powerful promoters are currently being developed to overcome this drawback.<sup>6</sup>

**RNA interference (RNAi) based gene therapy:** Small interfering RNA (siRNA) is primarily involved in guiding the degradation of messenger RNA. This form of gene therapy consists of two approaches:

1. Plasmid or viral vector -mediated delivery of short hairpin RNA precursors and
2. Direct delivery of siRNAs or siRNA precursors to target cells.<sup>27</sup>

Self-complementary recombinant adeno-associated virus (sc AAV) efficiently delivered siRNA into multidrug-resistant human breast and oral cancer cells and suppressed multidrug resistance-1 (MDR-1) gene expression. This resulted in rapid, profound, and durable reduction in the expression of the P-glycoprotein multidrug transporter, and a substantial reversion of the drug resistant phenotype.<sup>28</sup>

**Immunotherapy:** Cancer immunotherapy is the use of the immune system to treat cancer. In this form of gene therapy, the immune system is provoked into attacking the tumor cells by targeting cancer antigens.<sup>10,11</sup> There are three main groups of immunotherapy: Antibody therapies, cell-based therapies, and cytokine therapies. All these therapies work on the basis that cancer cells often have subtly different molecules on their surface that can be detected by the immune system. These molecules, known as cancer antigens, are most commonly proteins but may also include carbohydrates.<sup>29</sup>

Cell - free vaccines that can be directly administered from easily stored and transported vials are usually

less immunologically active compared to the most immunologically active vaccines that require costly and laborious *ex vivo* cellular cultures.<sup>6</sup>

In a clinical trial, both overall survival and progression-free survival of patients who received RT + UFT + OK- 432 were significantly longer than those of patients who received RT + UFT ( $P = 0.0075$  and  $P = 0.0175$ , respectively).<sup>30</sup> In another trial, to find out the technical feasibility of intraoperative INGN 201 gene therapy with postoperative chemoradiotherapy, the 1-year progression free survival was estimated at 90%.<sup>31</sup>

**Suicide gene therapy:** This therapy involves enzymes, the expression of which transforms the non-toxic producing drug into an active cytotoxic substance. It is the most commonly used gene therapy and uses thymidine kinase or other chemosensitizing genes.<sup>21</sup> Viral Herpes simplex thymidine kinase (HSVtk) converts a nontoxic prodrug ganciclovir (GCV) into a toxic form thereby killing the cells expressing the enzyme.<sup>32</sup>

A phenomenon known as "Bystander Effect" is observed where cells neighboring those expressing HSVtk are also killed,<sup>33</sup> thereby, enhancing tumor cell kill.<sup>34</sup> Besides this, the HSVtk strategy is reported to enhance the NK cell killer activity *in vivo* by inducing the systemic immune response.<sup>35</sup> The lack of transfection efficiency is one of the major drawbacks of suicide gene therapy. However, due to the ability of transfected tumor cells to induce cell death in neighboring transfected cells (bystander effect), a high percentage of transfected cells do not appear to be required *in vivo*.<sup>6</sup>

Induction of a remarkable cytotoxicity with a bystander effect in human OSCC cells by adenovirus-mediated suicide gene therapy was discovered in an *in vitro* study, thus suggesting an effective treatment strategy for that tumor.<sup>36</sup> An Indian study reported that IL-2 could induce proliferation of both dendritic cells (CD11c+) and NK cells (DX5+) *in vivo*. Apoptosis mediated through the caspase-3 dependent pathway was higher in the combination therapy group in comparison to HSVtk/GCV alone or IL-2 alone. A significant reduction in tumor volume was seen in all three treatment arms as compared to controls. Therefore, a combination of suicide gene therapy and immunotherapy leads to successful tumor regression in an HNSCC xenograft mouse model.<sup>32</sup>

### Gene Therapy with the use of Oncolytic Viruses

In this therapy, a viral vector is genetically modified, which replicates and lyses the tumor cells. Adenovirus mediated gene therapy is used for advanced cancers than traditional therapies.<sup>21</sup> Adenovirus is an oncolytic virus,

which can be designed to lyse cancer cells by replicating selectively within them.<sup>6</sup> The most noteworthy adenoviral therapy to date is the ONYX-015 viral therapy.<sup>37</sup> A phase two clinical trial on 11 patients revealed selective ONYX-015 presence and/or replication in the tumor tissue of 7 of 11 patients but not in immediately adjacent normal tissue (0 of 11 patients;  $P = 0.01$ ). Tissue destruction was also highly selective. Significant tumor regression (>50%) occurred in 21% of evaluable patients, with no toxicity to injected normal peritumoral tissues. The p53 mutant tumors were significantly more likely to undergo ONYX-015-induced necrosis (7 of 12) than were p53 wild-type tumors (0 of 7,  $P = 0.017$ ). This agent demonstrates the promise of replication - selective viruses as a novel therapeutic platform against cancer.<sup>38</sup>

### The Delivery of Drug Resistance Gene(s) to Normal Tissues for Protection from Chemotherapy

Normal tissues that are vulnerable to destruction are protected by the drug resistance genes. MDR-1 gene is the drug resistance gene in humans. Bacterial nitroreductase gene and the dihydrofolate reductase mutants that protect against methotrexate are also included in this category of genes.<sup>21</sup>

### Prognostic Markers

Patients with tumors of the same clinicopathological features have heterogeneous responses to treatment. Therefore, the traditional risk factors such as extracapsular spread, pathologically positive nodes, and tumor depth have limited value.<sup>2</sup>

Chang *et al.* showed that expression of the EGFRvIII correlated with the T classification and TNM stage. Individual status of phosphorylated AKT and EGFRvIII led to significant differences in survival outcome. EGFRvIII and phosphorylated AKT were predictors for the patient survival and clinical outcome.<sup>39</sup>

Compared to those with the PP genotype, individuals with the TP53 R allele had a higher frequency of pathogenic somatic mutation. TP53 R allele patients with pathogenic somatic mutations demonstrated a significant association with a poorer disease free survival than other individuals (HR = 1.71, 95% confidence interval, 1.15-2.57,  $P = 0.009$ ). This phenomenon was observed only in patients who received adjuvant radiotherapy/chemoradiotherapy after surgery. Pathogenic mtDNA mutations are a potential prognostic marker for OSCCs. Furthermore, functional mitochondria may play an active role in cancer development and the patient's response to radiotherapy/chemoradiotherapy.<sup>40</sup>

Peng *et al.* found that besides MYC itself, a novel dysregulated MYC module plays a key role in OSCC

carcinogenesis. They identified a candidate molecular signature associated with poor prognosis in OSCC patients, which might ultimately facilitate patient-tailored selection of therapeutic strategies.<sup>2</sup>

### Advantages of Gene Therapy

- A functional gene can replace a defective gene
- Aids in the prevention of potentially toxic effects in the body that are caused by other therapies
- It decreases the cost of various therapies and improves the patient's lifestyle for a longer period.<sup>10</sup>

### Disadvantages of Gene Therapy

- Some cells prevent the gene therapy for long-term effects. Due to this patients may have to undergo multiple rounds of gene therapy
- It is possible that the effectiveness of the gene therapy may be reduced by the host's immune system and its response
- A variety of potential problems to the patient such as toxicity and immune; and inflammatory responses can occur due to the viral vectors
- A tumor can be induced if the DNA is introduced into a wrong place in the genome, for example, into a tumor suppressor gene.<sup>21</sup>

## CURRENT STATUS AND FUTURE PERSPECTIVES

Today, the research on gene therapy in oral cancer is increasing day by day, both in the laboratory and the clinical settings. Gene therapy is now moving from phase 1 and 2 trials to the next level.<sup>6</sup>

Considerable time and a large number of patients are required to demonstrate the true efficacy of the therapies. Furthermore, sequencing of the human genome can now be performed in about 90 min for about 1000\$ each.<sup>41</sup>

Combination gene therapy which uses several genes showed significant tumor regression in animal studies.<sup>21</sup> A phase 1 trial in patients with advanced oral cancer evaluated the safety and biological effects of administering liposome-mediated intratumoral EGFR by means of antisense gene therapy. The results showed a low toxicity and high efficacy.<sup>42</sup>

In India, Advanced Centre for Treatment, Research and Education for Cancer (ACTREC, Mumbai) was the 1<sup>st</sup> Centre dedicated to gene therapy research (1998). Rita Mulherkar's group from ACTREC is conducting gene therapy studies related to the treatment of HNC using viral vectors. The group has conducted preclinical studies using xenograft mouse models to test the

combined efficacy of herpes simplex virus thymidine kinase gene and ganciclovir treatment.<sup>43</sup> The results of their study may form the basis for Phase 1 clinical trials. Earlier this group had carried out pre-clinical studies using retroviral and recombinant adenoviral vectors in combination with immunotherapy.<sup>31,44</sup>

There is limited data available on the genetic modifiers of clinical outcomes in OSCC.<sup>4</sup> The discovery and validation of candidate DNA markers and dysfunctional gene modules for predicting prognosis will be further enabled by the explosion of genomic data.<sup>2</sup>

New advances coupled with precise knowledge of the requirement for the generation of a cellular immune response to tumor antigen will probably provide powerful, non-individualized cell-free vaccines. Furthermore, it is probable that gene therapy is likely to be very effective when combined with existing clinical regimens. A multitude of studies are now showing great potential for combining gene therapy and chemotherapeutic, immunological, and radiotherapeutic approaches to cause more effective cell destruction and in larger numbers.<sup>6</sup>

## CONCLUSIONS

The field of cancer gene therapy is rapidly growing and will be part of future cancer therapeutics. Because it targets cancer cells only, gene therapy is an attractive tool in the treatment of OSCC and pre-cancer. While not all the current trials will lead to a viable therapeutic agent, there is hope that these advances will help to treat cancer patients without offering suffering and death. As shown in this review, among patients suffering from cancer, gene therapy has been shown to be effective in initial clinical trials.

Gene therapy may contribute a definitive treatment for HNCs that will offer great effectiveness in comparison to current therapies and will significantly reduce the high mortality associated with these lesions in the near future.

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