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Gene Therapy in Oral Cancer: A Review

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ABSTRACT

Background: Squamous cell carcinoma of the head and neck includes cancer of the oral cavity, oropharynx, larynx, hypopharynx, nasal cavity and para-nasal sinuses. In India head and neck cancer contributes to about 45% of all malignancies of which one third are oral cancer. The two main etiological factors for head and neck cancer are alcohol and tobacco. There is a synergetic interaction between the two agents which may be super multiplicative, additive, or between additive and multiplicative. The other factors contributing to oral cancer are diet, viruses, occupational agents, environmental pollutants and genetic influences. The molecular events leading to cancer development involves activation of proto oncogenes or inactivation of tumor suppressor genes. The unique presence of somatic molecular alterations in cancer cells has provided scope for improved diagnostic and gene based therapy of HNSCC (Head and Neck Squamous Cell Carcinoma). Gene therapy involves both modification of existing genetic material or introduction of new genetic material. The main objective of gene therapy is introduction of genetic material into target cancer cell without any damage to the surrounding normal cells. The purpose of this article is to review the role of gene therapy in oral cancer.

Keywords: Oral cancer, Gene therapy, Adenoviruses, Oncolytic viruses, immunotherapy.

INTRODUCTION

Squamous cell carcinoma of the head and neck includes cancer of the oral cavity, oropharynx, larynx, hypopharynx, nasal cavity and para nasal sinuses. In India head and neck cancer contributes to about 45% of all malignancies of which one third are oral cancer. [1] Men are two to three times more commonly affected than women. The two main etiological factors for head and neck cancer are alcohol and tobacco. There is a synergetic interaction between the two agents which may be super multiplicative, additive, or between additive and multiplicative. The other factors contributing to oral cancer are diet, viruses, occupational agents, environmental pollutants and genetic influences.

The pathogenesis of cancer requires an environment that promotes genetic aberrations leading to either an increased genetic damage or decreased genetic repair and protective mechanism .The molecular events leading to cancer development involves activation of proto oncogenes or inactivation of tumor suppressor genes.

These genetic variations results in cells with growth self sufficiency, insensitivity to antigrowth and cell death signals, limitless replicative potential and ability to detach from and invade surrounding structures and spread to distant anatomic sites. The molecular signaling pathways commonly affected in HNSCC includes P53 pathway, retinoblastoma pathway, epidermal growth factor receptor pathway, P13-kinase pathway and several DNA repair and genetic instability, angiogenesis pathway, cellular adhesion, dissociation, invasion, migration and metastasis pathway.

The unique presence of somatic molecular alterations in cancer cells has provided scope for improved diagnostic and gene based therapy of HNSCC.

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Gene therapy has been defined as the "genetic modification of cells of a patient in order to fight a disease".[2] Gene therapy involves both modification of existing genetic material or introduction of new genetic material. The main objective of gene therapy is introduction of genetic material into target cancer cell without any damage to the surrounding normal cells.

The steps in gene therapy are: identification, isolation and amplification of the gene to be used in the treatment, extraction and in vitro culture of tissue cell from the patient to be treated, transfer of therapeutic gene into the cells via vector using a gene that contains promoting sequence to enable its expression and marker to identify the cells into which it is incorporated and transfer into patient of selected gene containing cells.[3] Gene therapy can be viral or non viral. The non viral gene therapy includes injection of naked DNA, electroporation, the gene gun, use of oligo nucleotides, dendrimers and inorganic nano particles. The non viral vectors are inhibited by the serum components limiting the efficiency. The idea of gene transfer for treating human diseases was first put forward by Cusack and

Tarner, [4] in 1998. The first successfully treated disease was X-linked severe combined immune-deficiency by *ex vivo* gene replacement therapy. The viruses commonly used as vector are retro virus, adeno virus, herpes simplex, lenti, vaccinia, pox virus and adeno associated virus. The viral DNA is removed and they act as vehicles to deliver the therapeutic DNA into host cells. These particles are activated through intra peritoneal, intra hepatic, arterial, intra tumoral and intravenous routes.

The purpose of this article is to review the role of gene therapy in oral cancer.

Addition gene therapy:

The cells grow under the control of cyclin dependent kinases and eventually undergo programmed cell death known as apoptosis. Mutations and over expression of these cell cycle regulators, or inhibition of tumor suppressor genes leads to uncontrolled proliferation of cells. Gene addition therapy aims at introducing a normal, functional gene into the genome so as to restore the normal function of the cell.[5] Addition gene therapy involves introduction of tumor suppressor genes through viral vectors thereby inactivating the carcinogenic cells. Several genetic alterations have been described in oral cancer including mutations of P53, P16, P21.Since the protein P53 plays a role in cell cycle regulation and apoptosis,P53 gene transfer was initially tested in squamous cell carcinoma patients by injecting the primary or regional tumor with an adenoviral vector expressing wild-type P53.Adenoviral P53(Ad-P53)was demonstrated to be safe and well tolerated. [6] Studies on Ad-P53 as a surgical adjuvant showed good results.[7] Studies are being carried out on adenovirus vector Ad5CMV-P53, which is applied by intramucosal injection followed 2 hours later by a mouthwash. From next day, it is administered as a mouthwash twice daily for 2-5 days. This treatment is repeated every 28days and has shown a capacity to inhibit disease progression in pre-cancerous lesions with no toxic effect.[8]

Ta-Jen Liu *et al*, [9] reported growth suppression of human head and neck cancer cells by the introduction of mild type P53 gene via a recombinant adenovirus. Wong *et al*, [10] demonstrated that the delivery of an oncolytic herpes virus to a primary tumor site after surgical excision may have a significant impact on reducing both primary site recurrence and regional nodal metastases.

P27KIP1 acts as a cyclic dependent kinase inhibitor and it has been detected in cancer cells as well as normal cells in addition to its role as cyclin gene inhibitor. P27KIP1 is a tumor suppressor gene, regulator of drug resistance in solid tumor and promoter of apoptosis. [11]Over expression of P27KIP1 induced cell cycle arrest and apoptosis and that suppress the ability of invasion and metastasis in oral cancer cell line. Koh *et al*, [12] reported growth inhibitory effect of mild type P27 gene transfer on head and neck cancer. Kudo *et al*, [13] reported growth inhibitory effect of mild type P27 gene transfer on oral carcinoma cell lines.

In a study conducted by Li *et al* , [14] (2014) where in a combination of recombinant adenoviral P53 gene therapy and intra-arterial delivery of chemotherapeutic agents of oral squamous cell carcinoma patients was assessed, it showed a

greater treatment response as compared with either treatment alone.

Gene therapy using oncolytic viruses:

Oncolytic viruses have the ability to replicate only tumor cells. The discovery of adenovirus lacking E1B which did not grow in normal cells but grew in cells without P53 which is common in cancer cells lead to this modality of gene therapy. The virus must be genetically modified to attenuate its toxicity in normal tissue while maintaining its oncolytic activity against malignant tumor replication. Competent attenuated mutants of herpes simplex virus type 1 (HSV-1) have been shown to be efficacious for tumor therapy.

ONYX-015 is a type of 2/5 chimeric adeno virus that has been genetically modified by alteration of E1B-55KDa gene.E1B-55KDa gene in complex with E4ORF6 binds to and inactivates P53 tumor suppressor gene product. ONYX-015 has been found to have a synergistic effect when combined with 5FU (5-Fluorouracil)/Cisplatin chemo therapy in head and neck cancer.

A phase I-II trial by Galanis *et al*, [15] of ONYX – 015 in combination with MAP (Metomycin, Doxorubicin, asplatin) chemo therapy in patients with advanced sarcomas reported that intra tumoral administration of ONYX-015 + MAP is well tolerated with no significant toxicity. Detectors of viral DNA in post treatment tumor specimens by ISH (In situ hybridization) and detection of ONYX-015 genome in peripheral blood by quantitative PCR up to 7 days after the last viral dose provide evidence for adeno viral replicators. There was evidence of anti tumor activity in one out of six patients. An alternative approach to increase the oncolytic activity of selectively replicating adenoviruses involves engineering these vectors to express exogenous therapeutic gene.

John Nemunaitis *et al*, [16] carried out a Phase II clinical testing of intratumoral and peritumoral ONYX-015 injection in 37 patients with recurrent head and neck carcinoma. Patients received ONYX-015 at a daily dose of 1×10^{10} plaque-forming units (pfu) via intra tumoral injection for 5 days during week 1 of each 3-week cycle (n=30; cohort A), or 1×10^{10} pfu twice a day for 10 days during weeks 1 and 2 of each 3-week cycle. Post treatment biopsies documented selective ONYX-015 presence and/or replication in the tumor tissue of 7 of 11 patients biopsied on days 5–14,but not in immediately adjacent normal tissue (0 of 11 patients;P50.01).Tissue destruction was also highly selective; significant tumor regression(>50%) occurred in 21% of evaluable patients, whereas no toxicity to injected normal peritumoral tissues was demonstrated. p53 mutant tumor were significantly more likely to undergo ONYX-015-induced necrosis (7 of 12) than were p53 wild-type tumor (0 of 7; P =0.017). High neutralizing antibody titers did not prevent infection and/or replication within tumor. ONYX-015 is the first genetically engineered replication competent virus to demonstrate selective intratumoral replication and necrosis in patients.

Future approaches also include the addition of therapeutic gene with anti tumoral effects to ONYX-015 (i.e.,using ONYX-015 as a delivery vehicle), so called “armed therapeutic viruses”. ONYX-015 has several favorable characteristics as a vector for gene delivery, inherent antitumoral activity and selectivity, potential amplification of transgene leading to high level expression and enhanced intratumoral spread versus non-replicating vectors. If these approaches are successful, viral therapy with genetically engineered viruses may become a novel therapeutic platform for the treatment of cancer.

Rudin *et al*, [17] in their study on “an attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia” reported that topical administration of ONYX-015 for chemoprevention in the oral cavity has some theoretical advantages over systemic drug therapy. First, topical ONYX-015 administration limits exposure to the involved oral mucosa, minimizing the potential for adverse systemic effects. Second, this approach targets a signaling pathway implicated in malignant transformation. Unlike isotretinoin, which functions primarily as a differentiating agent, ONYX-015 has been shown to be selectively cytotoxic for cells with defects in p53-dependent response pathways. The lack of adverse effects with ONYX-015 combined with evidence of potential efficacy supports the rationale for phase II studies of this agent both in patients with oral dysplasia and as preventive therapy in patients at high risk of local relapse after

resection of oral cancer. Finally, ONYX-015 may have potential synergy with retinoid therapy in oral cancer chemoprevention. High-dose isotretinoin is primarily active against dysplastic lesions without upregulated p53 and does not suppress p53 levels in dysplasia. Through its differentiating activity, retinoid therapy may promote transient resolution of the hyperplastic and hyperkeratotic thickening of mucosa associated with early dysplastic lesions. Retinoid therapy might be predicted to increase the accessibility of the basal mucosal layers of dysplastic lesions to viral infection with ONYX-015. In addition to phase II studies of single-agent ONYX-015 in chemoprevention for oral cancer, analysis of the combined efficacy of the differentiating activity of retinoid therapies with the cytotoxic effect of ONYX-015 is warranted.

J Nemunaitis *et al*, [18] in another study on "pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients" demonstrated elevations in neutralizing antibody titers within 4 weeks of the infusion of ONYX-015. Serum levels of IL-6, IL-10, tumor necrosis factor-α, and interferon-ɣ increased within 6 hours of viral infusion, suggesting immune activation. This response was more pronounced in the cohort of patients who received 2×10^{12} particles per infusion. Two patients demonstrated uptake of viral particles in malignant tissue by quantitative PCR (Polymerase chain reaction). Electron microscopy confirmed selective cytoplasmic viral particles within malignant cells but not within adjacent normal tissue in a third patient. In conclusion ONYX-015 can be administered safely in combination with CPT11, 5FU or low-dose IL 2 (Interleukin-2) and is able to access malignant tissue following intravenous infusion.

Suicide gene therapy:

Suicide gene therapy involves introduction of viral or bacterial genes into tumor cells which in turn convert a non toxic pro drug to a toxic one. [19] It is the most commonly used gene therapy which uses thimidine kinase or other chemosensitizing genes. [20] The cytosine deaminase gene (CD) of E.coli converts the pro drug 5FC (5-Fluorocytosine) to 5FU or HSV-tk (Herpes simplex virus –thymidine kinase) converts GCV (Ganciclovir) to GCVMP (Ganciclovir monophosphate) converted by cancer cells enzymes to ganciclovir triphosphate.

The HSV-tk/ GCV system produce a delay in S phase and G2 phase and apoptosis that results in delayed proliferation process. In addition, mitochondrial damage was observed to be induced, [21] and caspase-8, Chk1 (Checkpoint kinase-1) activation was associated with extensive cell death. [22,23]

Several receptors on living cells such CEA (Carcinoembryonic antigen), VEGFR (Vascular endothelial growth factor receptor), EGFR (Epidermal growth factor receptor), CD44s (Cluster of differentiation-44) have been used as targets for suicide gene therapy.

Bystander effect:

Suicide gene therapy produces bystander effect where the therapeutic consequences spread beyond the transfected tumor cells, distant tumor lesions were found to regress.

The five mechanisms for the bystander effect are: release of soluble formulations, passage through gap junctions, passive transportation, stimulation of local microenvironment and endocytosis of apoptotic vesicles. [24]

The CD (Cluster of differentiation)/5-FU system demonstrates stronger local bystander effect than the HV-tk/GCV. The 5-FU diffuses efficiently within the tumor cells, and does not require cell to cell contact. The GCV trisphophate mediated bystander effect uses different mechanisms. An additional, mechanism which the HSV-tk/GCV exploits, is the local inflammation and devascularization, which probably enhance the vascular permeability and the formulation diffuses more efficiently. [25] The distant tumor regression effect was initially attributed to the immune system stimulation. [26] However, tumor metastasis regression was also observed in immunodeficient SCID (Severe combined immunodeficiency disease) mice, which was associated with the release of soluble factors from the primary site of transfection. [27] Regarding the CD/5-FC system the distant tumor control has been linked to the stimulation of natural killer cells, CD4+ and CD8+. In summary, the CD/5-FC system does not use the cell to cell contact to diffuse efficiently,

while the HSV-tk/GCV mainly uses the five previously mentioned mechanisms.

Ambade *et al*, [28] in their study on "effect of suicide gene therapy in combination with immunotherapy on antitumor immune response & tumor regression in a xenograft mouse model for head and neck" reported that- IL-2 could induce proliferation of both NK (Natural killer) cells (DX5+) and dendritic cells (CD11c+) *in vivo*. Apoptosis was higher in combination therapy group as compared to HSVtk/GCV alone or IL-2 alone and was mediated through caspase-3 dependent pathway. Significant reduction in tumor volume was seen in all 3 treatment arms as compared to controls and concluded that combination of suicide gene therapy and immunotherapy leads to successful tumor regression in a HNSCC xenograft mouse model. Immunotherapy could help in a systemic long lived anti-tumor immune response which would prove powerful for the treatment of metastatic cancers, and also for minimal residual disease. The results of this study may form the basis for Phase 1 clinical trials.

Gene therapy to inhibit tumor angiogenesis:

The role of angiogenesis in cancer is well established and angiostatin has been found to be an important factor to inhibit neovascularization. Microencapsulation is a form of somatic gene therapy in which the therapeutic protein is delivered by encapsulated recombinant cells. The microcapsules are permeable to the recombinant products and nutrients but not to the host immune mediators because of their large size. Angiostatins delivered becomes localized to the tumor .The rationale behind this localization is suggested to be that the receptors for angiostatin are ATP (Adenosine triphosphate) synthase present on the surface of human endothelial cells and αvβ3 intergrin and vitronectin. Kirsch *et al*, [29] reported that angiostatin could also inhibit VEGF bFGF (Fibroblast growth factor), mRNA (Messenger RNA) generation. Cirone et al, [30] on their studies on antiangiogenic cancer therapy with microencapsulated cells concludes that encapsulated myoblasts maintained a constant level of CMV (Cytomegalovirus)-driven angiostatin gene, and this low dose was sufficient for tumor to suppress progression, decrease in tumor volume, increase in apoptosis and necrosis. Antiangiogenic therapies are not fruitful if it is started at a later stage and tumor have been found to sustain their own vascular bed despite loss of endothelial cells. Tumor with "Vasculogenic mimicry" where there is micro vascular channel regulation by genetically deregulated aggressive tumour cells are not ameanable for this therapy.

The disadvantages of this therapy include prolonged therapy, repeated high dose, cost and risk of toxicity. Niethammer *et al*, [31] reported a study on use of DNA vaccine against VEGF receptors 2 in mice. VEGFR 2 has an important role in tumor growth invasion and metastasis.

Several approaches have been used to block FLK-1 (Fetal liver kinase 1), including dominant-negative receptor mutants, germ line disruption of VEGFR genes, monoclonal antibodies against VEGF and synthetic RTK (Receptor tyrosine kinase) inhibitors. FLK-1 based DNA vaccine causes collapse of tumor vessels by T-cell mediated immune response against proliferating endothelial cells.

Immunotherapy:

This form of therapy activates the host immune response to the tumor.

Ehrlich, [32] in 1980 first proposed the concept of control of malignant cells by immune system. The term immune surveillance refers to the destruction of premalignant cells by the immune system before tumor formation can occur. HNSCC has been found to be an immune-suppressive disease with lower absolute lymphocyte count, impaired natural killer (NK) cell activity and poor antigen presenting function.

Impairment of tumor infiltrating T lymphocytes has also been reported in HNSCC. Suppressor regulatory T cells (Tregs) have been linked to HNSCC tumour progression. Tregs by secreting suppressive cytokines such as TGF-β (Transforming growth factor beta) and IL-10, express cytotoxic T lymphocyte associated protein (CTLA-4) and correlate with tumor progression. Hence immunomodulatory therapies such as cancer vaccines using tumor peptide antigens, or viral, bacterial and DNA-based vectors as well as tumor

specific monoclonal antibodies (moAbs) have been tried for oral cancer.

The most successful HNSCC-targeted immune therapy is the HPV targeted immune-prevention vaccines. These vaccines by targeting L1 capsid protein evoke a humoral antibody response, thereby preventing viral entry and infection. [33]

However since an established HPV (Human papillomavirus) infection leads to viral DNA integration and expression of intracellular E6 and E7 oncogenes, and loss of L1 expression, they are not effective in previous infections and in established HPV associated cancers.

Adoptive T cell transfer is another form of immunotherapy where the T cells are removed from the patient, genetically modified or treated with antigens to enhance their activity and then reintroduced into the patient to improve the patient immune system's anti cancer response.

The most extensively used (moAbs) in HNSCC is cetuximab- a mouse –human chimeric immunoglobulin G1 (IgG1) anti EGFR moAb.

Immune checkpoint has been found to prevent excessive inflammatory responses as well as development of auto immunity. Tremelimumab a moAb against CTLA-4 has been found to have some clinical benefit.

Excision gene therapy:

Excision gene therapy is aimed at removal of defective oncogenes thereby inhibiting the growth of tumor cells. Primary response or immediate early genes are induced by mitogenic stimuli in absence of denovo protein synthesis, Early growth response (Egr-1) is a nuclear transcription factor and is implicated in the regulation of number of genes involved in immune response. Okadaic acid is toxic polyether fatty acid produced by dinoflagellates and is a potent inhibitor of protein phosphatases type 1 and 2A. Okadaic acid has been found to reduce Egr-1 leading to decrease cell proliferation and division. [34]

Antisense RNA:

The antisense RNA inhibits the RNA complimentary to DNA thereby leading to control of expression of genes associated with tumor growth.[35]

The oncogenes such as myc, fos, ras and viruses like HSV-1, HPV, HTLV-1 (Human T-lymphotropic virus-1) can be controlled by this therapy, preclinical studies using antisense sequences under the control of six small RNA promoters demonstrated anti tumor effect with minimum toxicity. [36, 37] Difficulty of introducing sufficient quantity of antisense molecules to inhibit the tumor growth limits the use of this technique. [38]

Studies are under way in patients with advanced oral cancer to evaluate the safety and biological effects of administering liposome-mediated intratumoral epidermal growth factor receptor by means of antisense gene therapy. Results have been positive, showing low toxicity and high efficacy. [39]

RNA interference (RNAi):

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. [40]

Disadvantages:

The disadvantage of gene therapy is that the effects are short term as some cells prevent gene therapy, the effectiveness of gene therapy can be reduced by hosts immune system, the viral vectors can lead to toxicity, immune and inflammatory responses, induction of tumor if DNA is placed into a wrong place in genome.

Advantages:

The functional gene can replace a defective gene, aids in prevention of toxic effects of other therapies, also has a synergistic effect with other therapies and improves the patient lifestyle for longer period.

CONCLUSION

A review of literature has shown that the research on gene therapy in oral cancer is still in its primitive stage in the clinical scenario, but days are not far away where it may play a major role as the definite treatment for oral cancer and pre cancerous lesion and even have an adjuvant additive effect when used with other current therapies. The use of gene therapy will definitely reduce the mortality and morbidity associated with this deadliest disease of mankind. It is an emerging field of biomedicine. The added advantage of gene therapy is that it targets only the cancer cells sparing the normal cells in contrast to the other available treatment modalities for oral cancer. However more clinical trials, larger randomized and control studies are needed to establish the role of gene therapy in the management of oral cancer.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

REFERENCES

1. Philip B Sugerman, Neil W Savage. Current concepts in oral cancer. Aust Dent J. 1999; 44: 147-156.

2. Xi S, Grandis JR. Gene therapy for the treatment of oral squamous cell carcinoma. J Dent Res. 2003; 82: 11-16.

3. Singh R, Singh S, Kumar T, Kumar A, Kumar A, Nazeer J. A novel approach in the treatment of oral cancer-gene therapy an update. World J Pharm Pharm Sci.2017;6:283-289.

4. Kevin J Scanlon. Cancer Gene Therapy: Challenges and Opportunities Kevin J Scanlon. Cancer Gene Therapy: Challenges and Opportunities. Anticancer Res. 2004; 24: 3-7.

5. Saraswathi TR, Kavitha B, Priyadharsini JV. Gene therapy for oral squamous cell carcinoma: an overview. Indian J Dent Res. 2007; 18: 120.

6. Clayman GL, El-Naggar AK, Lippman SM, Henderson YC, Frederick M,Merritt JA, *et al.* Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma. J Clin Oncol 1998;16:2221-2232.

7. Ayllón Barbellido S, Campo Trapero J, Cano Sánchez J, Perea García MA, Escudero Castaño N, Bascones Martínez A. Gene therapy in the management of oral cancer: Review of the literature. Med Oral Patol Oral Cir Bucal 2008;13:E15-21.

8. Indu S, Ramesh V, Oza N, Prashad KV. Gene therapy: An overview. J Orofac Sci. 2013;5:83.

9. Liu TJ, Zhang WW, Taylor DL, Roth JA, Goepfert H, Clayman GL. Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus. Cancer Res. 1994;54:3662-3667.

10. Wong RJ, Joe JK, Kim SH, Shah JP, Horsburgh B, Fong Y. Oncolytic herpesvirus effectively treats murine squamous cell carcinoma and spreads by natural lymphatics to treat sites of lymphatic metastases. Hum Gene Ther. 2002;13:1213-1223.

11. Katayose Y, Kim M, Rakkar AN, Li Z, Cowan KH, Seth P. Promoting apoptosis: a novel activity associated with the cyclin-dependent kinase inhibitor p27. Cancer Res. 1997;57:5441-5445.

12. Koh TY, Park SW, Park KH, Lee SG, Seol JG, Lee DW, et al.Inhibitory effect of p27Kip1 gene transfer on head and neck squamous cell carcinoma cell lines. Head Neck-J Sci Spec 2003;25:44–49.

13. Kudo Y, Kitajima S, Sato S, Ogawa I, Miyauchi M, Takata T.Transfection of p27Kip1 threonine residue 187 mutant type gene, which is not influenced by ubiquitin-mediated degradation,induces cell cycle arrest in oral squamous cell carcinoma cells. Oncol 2002;63:398–404.

14. Li Y, Li LJ, Wang LJ, Zhang Z, Gao N, Liang CY, Huang YD *et al*. Selective intra- arterial infusion of rAd-p53 with chemotherapy for advanced oral cancer: a randomized clinical trial. BMC Med 2014; 30: 12-16

15. Galanis E, Okuno SH, Nascimento AG, Lewis BD, Lee RA, Oliveira AM, et al. Phase I-II trial of ONYX-015 in combination with MAP chemotherapy in patients with advanced sarcomas. Gene Ther. 2005; 12 :437-445.

16. Nemunaitis J, Ganly I, Khuri F, Arseneau J, Kuhn J, McCarty T, Landers S, Maples P, Romel L, Randlev

B, Reid T. Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. Cancer Res. 2000; 60: 6359-6366.

17. Rudin CM, Cohen EE, Papadimitrakopoulou VA, Silverman S Jr, Recant W, El-Naggar AK, et al. An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia. J Clin Oncol. 2003; 21:4546-4552.

18. Nemunaitis J, Cunningham C, Tong AW, Post L, Netto G, Paulson AS, Rich D, Blackburn A, Sands B, Gibson B, Randlev B. Pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients. Cancer Gene Ther. 2003;10 :341.

19. Sunil PM, Joseph I, Varghese SS. Gene Therapy In Oral Squamous Cell Carcinoma-A Short Review. J Oral Maxillofac Pathol. 2011 1;2.

20. Gardlik R, Celec P, Bernadic M. Targeting angiogenesis for cancer (gene) therapy. Bratisl Lek Listy. 2011;112:428-434.

21. Tomicic MT, Thust R, Kaina B. Ganciclovir-induced apoptosis in HSV-1 thymidine kinase expressing cells: critical role of DNA breaks, Bcl-2 decline and caspase-9 activation. Oncogene. 2002;21:2141-2153.

22. Beltinger C, Fulda S, Kammertoens T, Meyer E, Uckert W, Debatin KM. Herpes simplex virus thymidine kinase/ganciclovir-induced apoptosis involves ligand-independent death receptor aggregation and activation of caspases. Proc Natl Acad Sci U S A. 1999;96:8699-8704.

23. Abate-Daga D, Garcia-Rodríguez L, Sumoy L, Fillat C. Cell cycle control pathways act as conditioning factors for TK/GCV sensitivity in pancreatic cancer cells. Biochim.Biophys Acta , Mol. Cell. Res. 2010;1803:1175-1185.

24. Zarogoulidis P, Darwiche K, Sakkas A, Yarmus L, Huang H, Li Q, Freitag L, Zarogoulidis K, Malecki M. Suicide gene therapy for cancer–current strategies. J Genet Syndr Gene Ther. 2013; 4.

25. Floeth FW, Shand N, Bojar H, Prisack HB, Felsberg J, Neuen-Jacob E, Aulich A, Burger KJ, Bock WJ, Weber F. Local inflammation and devascularization—in vivo mechanisms of the "bystander effect" in VPC-mediated HSV-Tk/GCV gene therapy for human malignant glioma. Cancer Gene Ther. 2001; 8: 843.

26. Bi W, Kim YG, Feliciano ES, Pavelic L, Wilson KM, Pavelic ZP, Stambrook PJ. An HSVtk-mediated local and distant antitumor bystander effect in tumors of head and neck origin in athymic mice. Cancer Gene Ther. 1997; 4: 246-252.

27. Dilber MS, Abedi MR, Bjorkstrand B, Christensson B, Gahrton G, Xanthopoulos KG, Smith CI. Suicide gene therapy for plasma cell tumors. Blood. 1996;88:2192-2200.

28. Ambade AV, Joshi GV, Mulherkar R. Effect of suicide gene therapy in combination with immunotherapy on antitumour immune response & tumour regression in a xenograft mouse model for head & neck squamous cell carcinoma. Indian J Med Res. 2010; 132:415.

29. Kirsch M, Strasser J, Allende R, Bello L, Zhang J, Black PM. Angiostatin suppresses malignant glioma growth in vivo. Cancer Res. 1998;5 8: 4654-4659.

30. Cirone P, Bourgeois JM, Chang PL. Antiangiogenic cancer therapy with microencapsulated cells. Hum Gene Ther. 2003; 14: 1065-1077.

31. Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, KarstenG, et al. A dna vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. Nat Med. 2002; 8: 1369-1375.

32. Herberman RB, Holden HT: Natural cell-mediated immunity. Adv Cancer Res . 1978;27:305-377.

33. Ferris RL. Immunology and immunotherapy of head and neck cancer. J Clin Oncol. 2015; 33: 3293.

34. Kumar MS, Masthan KM, Babu NA, Dash KC. Gene therapy in oral cancer: A review. J Clin Diag Res. 2013 ;7:1261-1263.

35. Xi S, Grandis JR. Gene therapy for the treatment of oral squamous cell carcinoma. J Dent Res. 2003; 82: 11-16.

36. Bertrand JR, Pottier M, Vekris A, Opolon P, Maksimenko A, Malvy C. Comparison of antisense oligonucleotides and siRNAs in cell culture and in vivo. Biochem Biophys Res Commun. 2002; 296:1000-1004.

37. Bali A, Bali D, Sharma A. An overview of gene therapy in head and neck cancer. Indian J Hum Genet. 2013; 19: 282-290.

38. Clayman GL, Trapnell BC, Mittereder N, Liu TJ, Eicher S, Zhang S, *et al.* Transduction of normal and malignant oral epithelium by an adenovirus vector: The effect of dose and treatment time on transduction efficiency and tissue penetration. Cancer Gene Ther 1995;2:105-111.

39. Okamura H, Morimoto H, Fujita M, Nasu F, Sasakia E, Haneji T. Suppression of Egr-1 expression in human oral squamous carcinoma cells by okadaic acid. Oral Oncol. 2002 Dec;38:779-784.

40. Xu D, McCarty D, Fernandes A, Fisher M, Samulski RJ, Juliano RL. Delivery of MDR1 small interfering RNA by self-complementary recombinant adeno-associated virus vector. Mol Ther. 2005; 11:523-530.