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TRABAJO DE FIN DE GRADO

Grado en Odontología

COULD GENE THERAPY BE A TREATMENT FOR ORAL SQUAMOUS CELL CARCINOMA?

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Summary

Introduction: Oral Squamous Cell Carcinoma (OSCC) is with 90% the most common type of malignant lesion that can be found within the oral cavity. Its aetiology is based on the combination of both environmental and genetic factors. The early diagnosis within the dental clinic is essential for the treatment outcome. Nowadays there are available a wide variety of treatment approaches including gene therapy. Gene therapy is a recent approach, based on the concept of correcting or replacing a malfunctioning gene without damage to surrounding tissue.

Objectives: The main objective is to analyse the effectiveness of gene therapy in the treatment of oral squamous cell carcinoma in comparison to other treatment options.

Methodology: For the literature search electronic databases, relevant publications, abstracts, and books were accessed. The databases considered, included Medline, PubMed, Academic Search Ultimate and Google Scholar.

Results and Discussion: All the treatment approaches including chemotherapy, radiotherapy, surgery, immunotherapy, photodynamic therapy, COX-2 inhibition and gene therapy have their advantages and disadvantages. Their use depends on the stage of the disease as well as the individual characteristics of the patient.

Conclusion: Gene therapy is a suitable treatment approach for OSCC, especially for more advanced stages of OSCC. It needs to be considered that the most effective treatment outcomes can be found in combination with other treatment approaches.

Resumen

Introducción: El carcinoma oral de células escamosas (COCE) es con 90%, el tipo de lesión maligna más común que se puede encontrar dentro de la cavidad oral. Su etiología se basa en la combinación de factores ambientales y genéticos. El diagnóstico precoz dentro de la clínica dental es fundamental para el resultado del tratamiento. Hoy en día existe una amplia variedad de enfoques de tratamiento, incluida la terapia genética . La terapia genética es un enfoque reciente, basado en el concepto de corregir o reemplazar un gen que funciona mal sin dañar el tejido circundante.

Objetivos: El objetivo principal es analizar la efectividad de la terapia genética en el tratamiento del COCE en comparación con otras opciones de tratamiento.

Metodología: Para la búsqueda de literatura se accedió a bases de datos electrónicas, publicaciones relevantes, resúmenes y libros. Las bases de datos consideradas incluyeron Medline, PubMed, Academic Search Ultimate y Google Scholar.

Resultados y discusión: Todos los enfoques de tratamiento, incluyendo quimioterapia, radioterapia, cirugía, inmunoterapia, inhibición fotodinámica, inhibición de COX-2 y terapia genética, tienen sus ventajas y desventajas. Su uso depende de la etapa de la enfermedad y de las características individuales del paciente.

Conclusión: La terapia genética es un tratamiento adecuado para COCE, especialmente para estadios más avanzados. Debe tenerse en cuenta que los resultados de tratamiento más eficaces se pueden encontrar en combinación con otros enfoques de tratamiento.

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1. Introduction

Oral squamous cell carcinoma (OSCC) is a type of malignant lesion located in the oral cavity. It can be stated that OSCC is with 90%, the most common malignancy found in the oral cavity. At the same time, it is the 6th most frequent cancer in the whole world and 500.000 new cases can be identified each year (1).

This disease can be frequently found in the dental practice, therefore dental practitioners and medical doctors should have a sufficient knowledge of this disease in order to make an early diagnosis, including a proper screening and proceed with the most suitable treatment. Considering that, the main notions about OSCC's aetiology, pathology, diagnosis, and treatment options are going to be explained in order to review the main characteristics of this disease (1).

1.1. The disease

1.1.1. Aetiology

The aetiology of the disease can be explained thanks to a combination of different factors, including **environmental and genetic factors**.

With respect to environmental factors related to the lifestyle, two factors have shown to have a huge impact on OSCC. Hereby **smoking and drinking of alcohol**, have been stated to be the most important risk factors (2).

According to recent studies, cigarettes consist of more than 60 chemical carcinogens, such as polycyclic aromatic hydrocarbons, aromatic amines, aldehydes and volatile organic hydrocarbons, and metals (3). These can lead to side effects as for example mucosal alteration,

discoloration and edema of the skin (2). Also, the chewing of betel nuts and gutka, commonly practiced in South Asia is explained to have an impact on this disease (4).

Alcohol has been suggested to affect the metabolism of cells and hereby easing the entrance of carcinogens into the cells. However, it also must be considered that often alcohol and tobacco are consumed in combination, making it complicated to determine their individual effect as a risk factor (4).

Moreover, recent experiments suggest the **effect of biological factors**, including the role of viruses as a risk factor. Hereby especially the **Human Papillomavirus (HPV) and Herpes Simplex virus** need to be considered. Infections of both viruses are mainly sexually transmitted. The serotype HPV-16 serotype is most commonly related to OSCC, while other serotypes including HPV-18, HPV-31 and HPV-33 can also be involved. The presence of oral sores caused by the Herpes simplex virus is a possible precancerous stage in the development of OSCC(4).

Another biological factor that could be involved in the origin of the disease are **candida infections** within the oral cavity**.** They have been recognized to produce an increased amount of dysplasia and malignant transformation in the tissue present. However, this association has mainly been investigated in animals and there exists not sufficient research in humans (4).

Furthermore, a link has been made between a **diet rich in vegetables and fruits** and a decreased risk of developing OSCC (4). Hereby, a diet based on the consumption of high amounts of carotenoids, vitamin A and C, folic acid and antioxidants present in fruits and vegetables, could be protective and result in a preventative effect against OSCC (5).

However, there are not only environmental factors increasing the risk of being affected by OSCC but also genetic factors.

It has been reported that 10% of all OSCCs have been linked to a genetic susceptibility, with a high occurrence within certain families and ethnic groups. An example hereby is the Ashkenazi group in Israel with a twice as high occurrence rate in comparison to the general Jewish population (4). A possible correlation can be made due to endogamy caused by interbreeding within the same racial community, and hereby giving on defective gene sequences to the following generations (6).

Apart from this, it seems that **gender plays a role as a risk factor**. It has been identified that OSCC is more often found in males in comparison to females with a ratio of 2:1. Moreover the mean age of diagnosis with OSCC lies at 62.3 years. Hereby females (67 years) are diagnosed in average at an older age than males (59.9 years) (7).

Finally, oral squamous cell carcinoma has also been linked to specific genetic disorders **including Fanconi anaemia (FA)** and **Dyskeratosis congenita (DC)** (8).

FA is a is a rare but serious blood disease resulting in an impaired response to repair DNA damage. It is considered a bone marrow failure syndrome (mainly autosomal recessive) and patients show clinical signs of skin, skeletal, genitourinary, gastrointestinal, cardiac as well as neurological anomalies. FA is commonly diagnosed in young patients and is associated with an increasing loss of all bone marrow production of different hematopoietic cells, including red blood cells, white blood cells, and platelets. These individuals have an increased risk of developing a cancer in the bone marrow called acute myeloid leukaemia (AML), or tumours of the head, neck, skin, gastrointestinal system, or genital tract. As it has been mentioned

before, people affected by this disease, have an 800 times higher risk of being affected by **head and neck oral squamous cell carcinoma** (HNSCC) (8, 9).

DC is also a rare disease that encounters a bone marrow failure syndrome (X-linked or autosomal). Clinical signs include abnormal skin pigmentation, nail dystrophy and leucoplakia of the mucosa. Moreover, abnormalities in other locations as in the oral cavity, gastrointestinal tract, neurological system and lungs can be identified. DC is also most often diagnosed at a young age, and although the main cause of mortality in these patients is related to bone marrow failure, other causes such as the formation of malignant tumours and the development of pulmonary diseases could be involved. People affected by this disease have a 1100 higher risk of being diagnosed with HNSCC (8,9).

HNSCC is a classification of cancer which can include several different locations, including the pharynx, larynx, nasal cavity, middle ear, salivary and thyroid gland, soft tissue and bone in that area, as well as the oral cavity. Herby it can be stated that the most frequent locations (90%) where HNSCC can be found, include the oral cavity and larynx. Therefore, patients affected by FA and DC have an increased risk for squamous cell carcinoma in the head and neck area, with specific increased occurrence in the oral cavity (OSCC) (10).

1.1.2. Pathology and symptoms

Definition and Classification of Neoplasm

A neoplasm can be defined as an accumulation of abnormal tissue which is produced by a rapid and uncontrolled cell division without the presence of regular programmed cell death (apoptosis). Hereby the neoplasms can be classified into two different groups, the benign and the malignant neoplasm (11).

The **benign neoplasm** is described as a tumoral growth, which has the ability to increase in size, without advancing to surrounding tissues or organs (11).

The **malignant neoplasm** is outlined as a tumoral growth that rapidly increases in size and that advances to the surrounding tissue, blood, lymph system and other organs. Theses secondary tumours are called metastasis. Malignant tumours are also called cancer (11).

Tumour cells have specific characteristics which can explain their behaviour in the human body. These include the concept of **clonality, autonomy, anaplasia and metastasis (12).**

The basis of the formation of a tumour is the abnormal formation of one individual cell due to mutations (genetic changes) which then will continue dividing. This is the concept of **clonality (12).**

The cell development and division are disturbed by outer environmental effects, not allowing the normal cell cycle division. This concept is called **autonomy (12).**

For a normal cell development, it is essential for the cell to differentiate and specialize in a specific action, however this is absent in cancerous cells, making them poorly differentiated. This concept is called **anaplasia (12).**

Malignant tumours have the ability to advance and invade surrounding tissue. This concept is called **metastasis (12).**

OSCC is a type of malignant neoplasm and therefore also presents all the previously mentioned characteristics.

It is highly important the study of both **the macroscopical and microscopical characteristics** of OSCC.

From the **microscopical** point of view OSCC can be classified into five histological stages depending on the development of the epithelial tissue. The stages include normal mucosa, hyperplasia, dysplasia, carcinoma in situ and invasive carcinoma. Each stage shows both different microscopical differences as well as genetic variations (13).

Microscopical

The **normal mucosa,** when investigated under the microscope, shows no abnormal cellular alteration. According to the WHO classification (2005) the first stage of abnormal alteration is **hyperplasia** where a higher number of cells in different layers can be identified. Hereby also genetic modifications can be detected, including the 9p21 LOH and the CDKn2A inactivation (13).

The second stage is the **dysplastic stage**, which can be classified into mild, moderate, and severely dysplastic. In the mild dysplasia only the lower third of the epithelium shows abnormal cellular changes. In the moderate dysplasia it affects both the lower and middle part of the epithelium. In the severe dysplasia, there will be an affection of more than the lower

and middle part of the epithelium. Hereby also genetic alterations can be detected, including the 3p21, 17p13LOH as well as TP53 inactivation (13).

In the third stage, the **carcinoma in situ**, it can be identified an almost entire abnormal epithelium, as well as abnormal cellular structures. Hereby again new genetic changes can be identified, including 11q13, 13q21 and 14q32 LOH as well as CCND1 amplification (13).

The last stage is the development into an invasive carcinoma, where the abnormal cellular tissue will spread to surrounding areas. Herby abnormal genetic changes affect 6p, 8, 4q27 and 10q210q23 LOH and PTEN inactivation (13).

A graphic explanation can be seen below in **Figure 2**, showing the different microscopical stages and genetic changes.

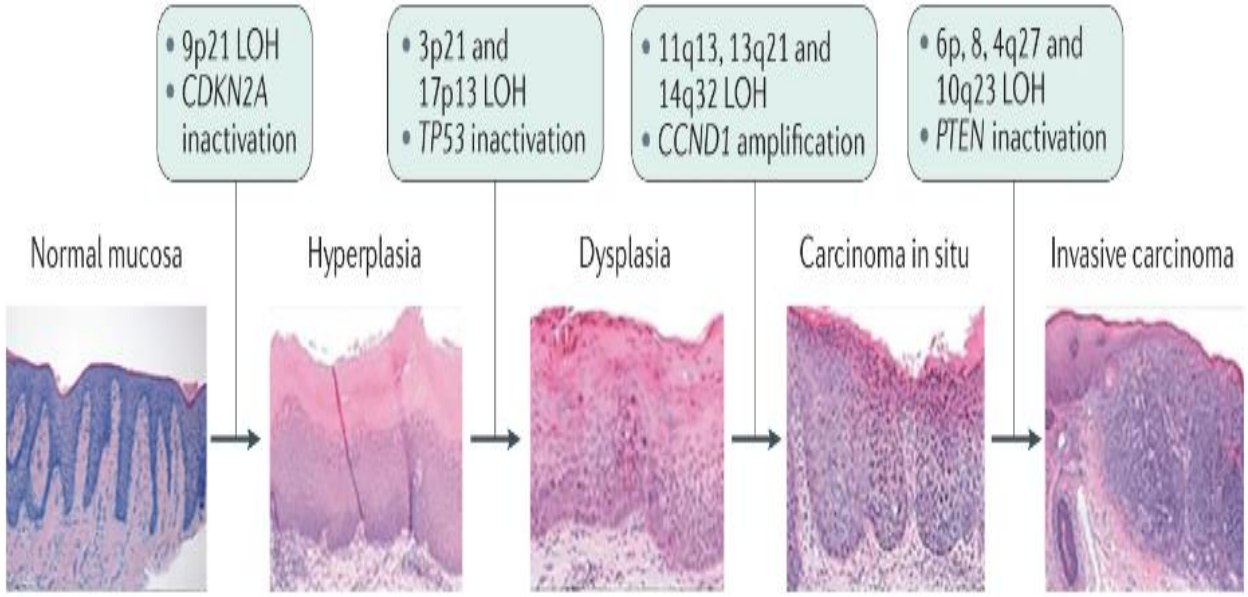

Figure 1. Progression of the different **Histological Stages** including Normal Mucosa, Hyperplasia (precancerous), Dysplasia (precancerous), Carcinoma in Situ (stage 0) to Invasive Carcinoma (stage I to IV, depending on extension and metastasis) and the corresponding **Genetic alterations** Of OSCC (14).

From a **macroscopical point** of view it is possible to detect precancerous as well as cancerous lesions. Lesions indicating for a possible affection with OSCC can include exophytic, endophytic as well as white or red patches or a combination of all (15).

- **Exophytic lesions** consist of a papillary or verrucous surface and the detailed characteristics can depend on the existence of ulceration and keratinization (15).
- **Endophytic lesions** consist of ulcerated lesions with fixated tissue below and raised margins. There is the possibility of white or red coloration around the margins (15).
- **Heterogenous red/white patches** which are called erythroplakia are considered more malignant than homogenous white lesions called leukoplakia (15).

However, there can also be a combination of several aspects, as for example ulcerated tissue with red or white lesions (15).

In the following figures, it can be seen different clinical appearances of precancerous lesions, which have the potential to develop into OSCC.

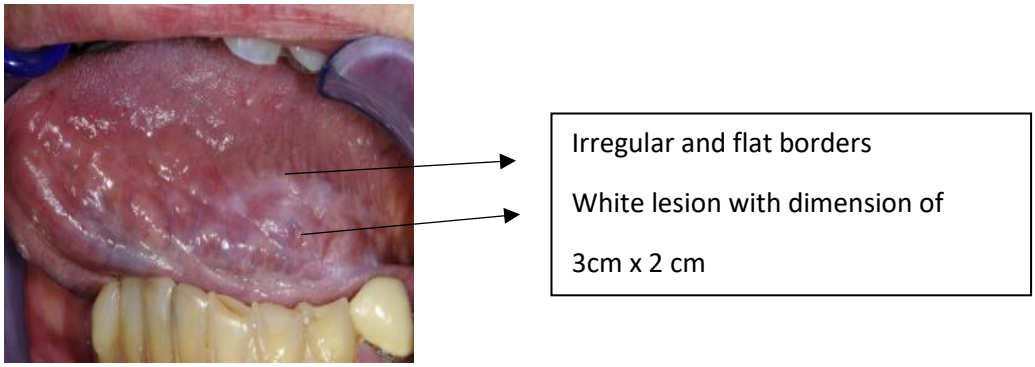

Figure 2. Precancerous lesion: Leukoplakia with a flat white appearance in the lateral side of the tongue (16).

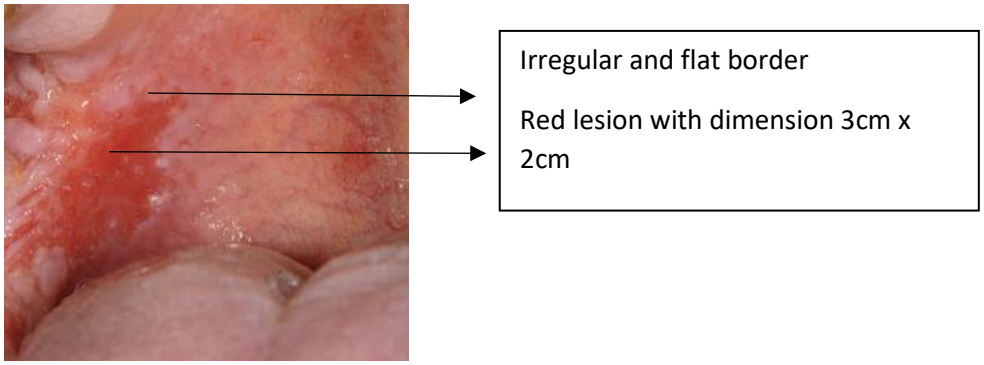

Figure 3. Precancerous lesion: Erythroplakia located on the soft palate with severe dysplasia (16).

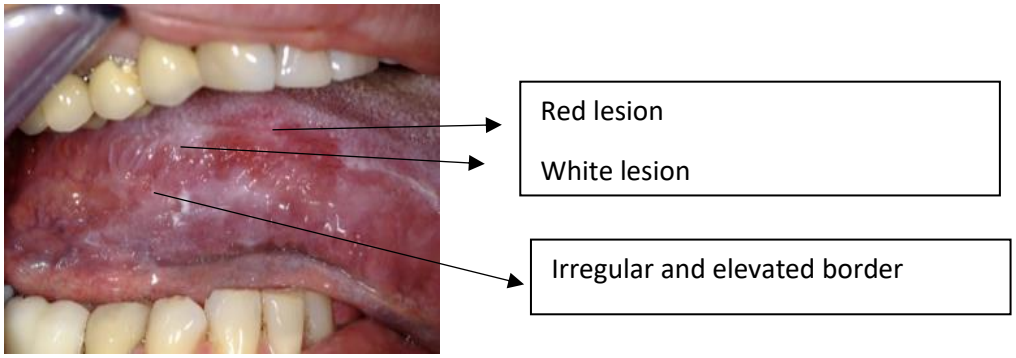

Figure 4. Precancerous lesion: Leukoerythroplakia in the lateral side of the tongue, elevated and irregular borders with white and red colour (16).

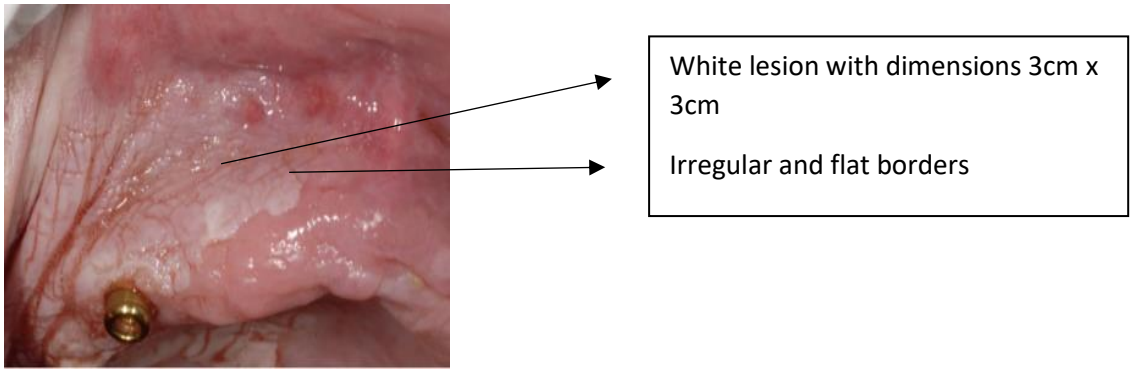

Figure 5. Precancerous lesion: Verrucous Leukoplakia present in the gingiva and alveolar mucosa in a patient without remaining teeth (16).

To determine the difference between a precancerous and cancerous lesion, it is essential to do a histological analysis of the tissue under the microscope. From a macroscopical point of view it can determined the abnormality of the cellular growth from a visual point of view, however the microscopical study is essential to determine the shape, size and structure of the cells giving an indication for the amount of cellular abnormalities within the epithelium. **Figure 6** hereby indicates the correlation between the microscopical and macroscopical appearance of both precancerous and cancerous lesions (17).

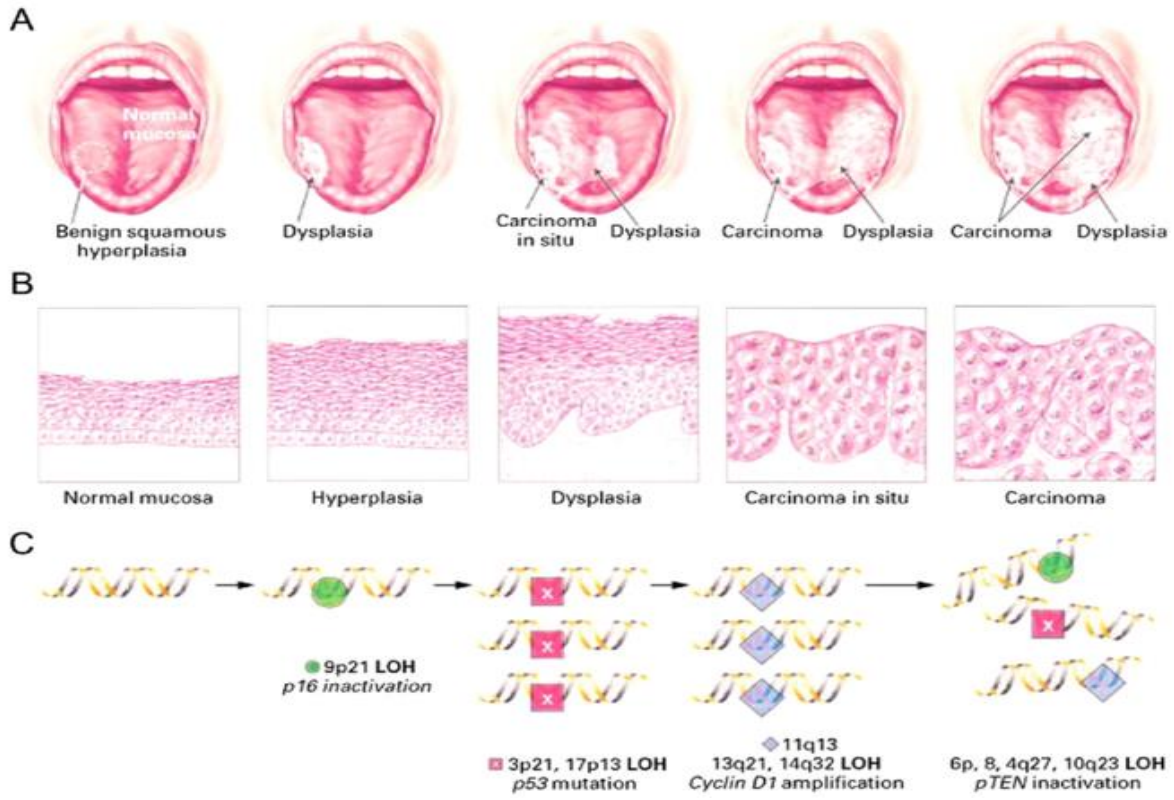

Figure 6. Correlation between macroscopical, microscopical aspects as well as genetic alterations of the OSCC (17). **A :** Indicates the progression from hyperplasia until carcinoma which can be identified within an intraoral examination **(macroscopical)** ; **B :** Indicates the progression from normal mucosa until carcinoma through **microscopical** study ; **C** : Indicates the **genetic alterations** during the development of OSCC

When diagnosing both precancerous and cancerous lesions, it is highly important to not only investigate the appearance of the lesion in terms of size, number, colour, surface and border but also take into account the **location** of the lesion. Hereby the location can give indications about the possible malignancy. The floor of the mouth, retromolar area and lateral border of the tongue have been identified as areas with increased risk of malignancy, and therefore development of an invasive carcinoma (7).

The most common site of location of OSCC is the border of the tongue, alveolar mucosa, gingiva, floor of the mouth and ventral surface of the tongue (from highest to lowest occurrence) (7).

The border of the tongue is the most frequent area of location in America and Europe, while the buccal mucosa is the most frequent location in South Asia due to the habit of tobacco chewing (7). At the time of diagnosis, the tumour generally already shows an increased size of up to 4 cm (mean size of 3.4 cm) (7).

Most patients realize about the existence of a lesion around 10 months (mean time) before being diagnosed with OSCC. This indicates that it takes 10 months from the appearance of the lesion until the correct diagnosis with OSCC is being made (7). This delay in diagnosis is caused by the lack of symptoms in the patient and the small size of the tumour in early stages of the disease. Generally, the delayed diagnosis of OSCC is a severe issue, and can be addressed by regular dental check-ups as well as a check-up by a specialist in oral pathology in case of uncertainness or insufficient knowledge by the dental professional (18). Hereby an early diagnosis of the precancerous lesions, histological study and follow up are essential basic aspects.

In order to stage the OSCC after its diagnosis, the TNM staging system (by the American Joint Committee) can be used. This is an essential part to determine the treatment approach later on. It is based on the **size of the tumour, lymph node affectation and metastasis** (18).

Hereby T1 is ≤2 cm; T2 is >2-4 cm; T3 is >4 cm and T4 indicates an invasive tumour (18). Furthermore N0 indicates that no lymph nodes are affected, N1 that one lymph node is affected with less than 3 cm, N2a that one lymph node of the same side is affected (3-6 cm), N2b that more than one lymph node on the same side is affected (3-6 cm) and N2c that both lymph nodes are affected or the lymph node on the opposite side of the cancer (3-6 cm) without invasion of surrounding tissue. N3a defines a lymph node affection of more than 6 cm and N3b an additional invasion of the surrounding tissue. M0 specifies the absence of metastasis, while M1 the presence of metastasis (19).

From the combination of size, lymph node affectation and metastasis we can then determine the stage of the disease. This can be seen in the table below (19).

	Stage O	**Stage I**	**Stage II**	**Stage III**	**Stage IVA**	**Stage IVB**	**Stage IVC**
Option 1	Carcinoma in situ (superficial layer)	T1 N0 M0	T2 N0 M0	T3 N0 M0	T4a N0 or N1 M0	Any T N3 M0	Any T Any N Any M
Option 2				T1/T2/T3 N1 M0	T1/2/3/4a N2 MO	T4b Any N M0	

Table 1. TMN Staging System indicating the different stages (0, I, II, III, IVA, IVB, IVC) and the corresponding options for each stage, determined by the size (T), lymph node affection (N) and metastasis (M) (19).

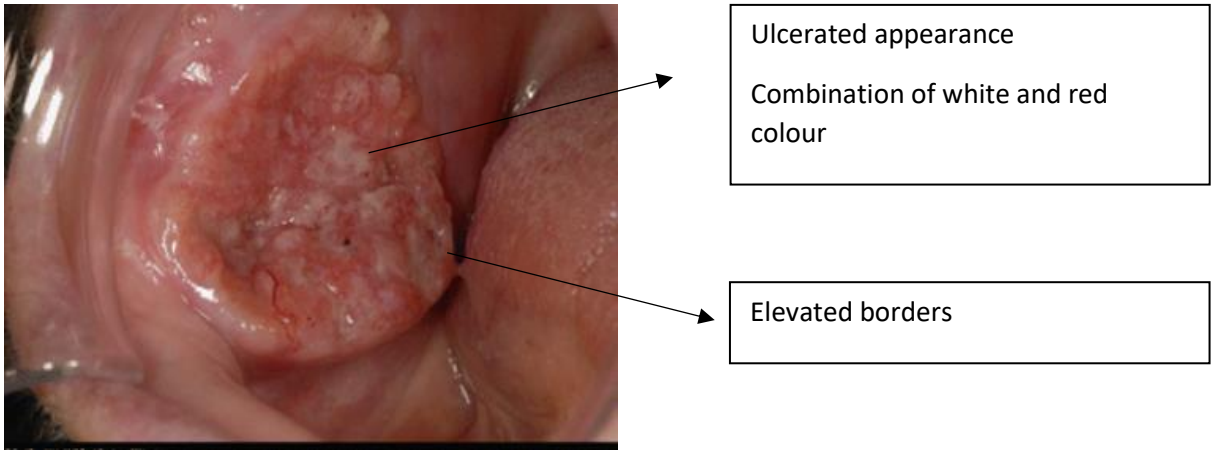

Figure 7. Example of TMN staging of Oral Squamous Cell Carcinoma in buccal mucosa (T2, N0, M0- Stage II) (15).

1.2. Diagnostic techniques of OSCC

There exists a variety of diagnosis techniques to ensure an early diagnosis in the dental office.

1) Vital Staining
2) Auto-Fluorescence
3) Histology
4) Cytology
5) Imaging (Positron Emission Tomography- PET)

To identify their main advantages and disadvantages a summary of each is explained in the following table (Table 1).

Type of Diagnosis Method	Description	Advantages	Disadvantages
Vital Staining (20)	Dyes (most commonly toluidine blue) are used to colour cells with different ranges of colours depending on their amount of reproduction.	Can detect areas of tissue with an increased amount of cell reproduction.	Not sufficient to make a final diagnosis. Biopsy is needed (invasive method).
Auto-Fluorescence (20)	Use of fluorescent light to detect differences between the cellular tissue.	Helps in the localization of lesions and can determine its limits.	Biopsy is needed to confirm malignancy (invasive method).
Histology (Biopsy) (20)	Excisional biopsy of the affected lesion with a safety margin to detect epithelial dysplasia.	Determines the degree of dysplasia (possible malignant transformation) Can identify OSCC at an early and yet invisible stage.	Invasive method (Biopsy).
Cytology (20)	Microscopic study of a sample of the tissue.	Not invasive as it takes samples from the mucosal surface through smears or fine needle aspiration.	Can only be used when the lesion is already visible.
Imaging (PET) (20)	Use of Positron emission tomography indicating the degree of metabolism of the tissue.	Not invasive technique takes cross sectional images of the tissue. Measures tissue metabolism and identifies dysplasia	Biopsy is needed to confirm the malignancy.

Table 2. Diagnosis techniques in OSCC and their corresponding Advantages and Disadvantages

Comparing the possibilities for an early diagnosis of OSCC, biopsy followed by a histopathological examination are the most reliable methods. These are the techniques which are most likely being used within the dental practice (20).

1.3. Treatments

There exist a variety of treatment options for OSCC. As an introduction, a description of the available treatments is going to be made. The advantages and disadvantages as well as their application in the different stages of OSCC will be explained in discussion and results.

A. Surgical Removal
B. Chemotherapy
C. Radiotherapy
D. Treatment with COX-2 Inhibitors
E. Photodynamic Therapy
F. Immunotherapy
G. Gene Therapy

A. Surgical removal is a highly relevant treatment approach and is often used in combination with Radiotherapy and Chemotherapy. Within the surgical excision of the OSCC it needs to be considered the value of the safety margin of at least 10mm, the size of the initial tumour, depth, ability of mouth opening, possible mandibular affection as well as the abilities of the oral surgeon (21).

B. Chemotherapy uses different drugs to achieve the treatment of different types of cancer. It is a treatment approach which works systemic as it is injected into the body. Chemotherapy drugs hinder the division and reproduction of cancer cells. They can be used individually or in combination and target either the cancer cells within the body

or specific sites or processes. Therefore, it has the ability to treat both primary and secondary tumours. There are available several types of drugs which can be used depending on the type of cancer, location and dimensions (22).

The most used include Docetaxel, Cisplatin, 5-fluorouracil and Carboplatin.

The function of **Docetaxel** as a chemotherapeutic drug is based on the tubulin polymer stabilization as well as the inhibition of the cell cycle of the cancerous cells. It stimulates the assembly of tubulin in stable microtubules by inhibiting their polymerization, which leads to a decrease in free tubulin. Therefore, it alters the tubular network of cells that is essential for the vital functions of mitosis (for the cell division of dividing cells) such as cancer cells (23).

Cisplatin and carboplatin are alkylating agents, impeding DNA synthesis by the construction of cross-links within or between DNA strands. However, the synthesis of proteins and RNA is not as much decreased. It also provides immunosuppressive, radio sensitizing and antibacterial effects (24).

In the case of **carboplatin**, it has a lower activity reported against cancer cells, but due to its low reactivity, it also has longer effects (25).

5- fluorouracil is part of the group of antimetabolites and is based on the process of modifying both the DNA and RNA of dividing cells, among them also the cancerous cells. Its effectiveness is based on the irreversible attachment to the enzyme thymidylate synthase, required for the production of thymine nucleotides (26).

C. **Radiation therapy** is a treatment approach where increased doses of radiation are used to damage the genetic material, leading to a decreased growth or death of the cells. There exist different types, including the external beam radiation therapy as well as the internal beam radiation therapy. The choice depends on the type of tumour, its location and dimensions. In external beam radiation the source of radiation will be applied from the outside of the body and will be directed to a specific area. In the internal radiation therapy, the source will be within the body. Brachytherapy which is a form of internal radiation consisting of a solid source, also aims a specific location within the body. Most used for the treatment of OSCC is the Brachytherapy, which can be divided into Low-dose radiation (LDR) or High dose radiation (HDR). LDR is an approach where less radiation is given over a longer time, while HDR is given over a short time with intense radiation. HDR is the more advanced approach which is more effective but at the same time also more aggressive (27).

D. **Treatment with COX-2 Inhibitors:** Cyclooxygenase (COX) enzymes are enzymes which are essential for the development of arachidonic acid into prostaglandins. COX-1 can be found within the body under normal conditions as for example in the gastric mucosa, while COX-2 can be found in elevated levels in case of an inflammation within the body (2). In the presence of cancerous cells, the COX-2 levels are elevated. Those elevated levels of COX2 could be found in correlation with a stop in apoptosis, decreased immune response, increased invasiveness of the tumour and higher probability of the formation of mutagens (28).

E. Photodynamic Therapy

Photodynamic Therapy is a non-surgical treatment approach which is based on photosensitive, using light and oxygen. The combination of these three parts together leads to a toxic process with causes the death of cells or necrosis of the cancerous tissue. It is applicable in dysplastic and premalignant lesions as well as cancerous lesions like OSCC (29).

F. Immunotherapy

Immunotherapy is a treatment approach based on the improvement of the immune response of the patient as well as increasing the immune potential of the cancerous cells within the tissue. Through research it has been seen that OSCC patients have fewer immune cells, like natural killer cells, T-lymphocytes and cytokines (30). There are different types of immunotherapy, including **Immune checkpoint inhibitors, T-cell transfer therapy as well as with Monoclonal antibodies.** The presence of immune checkpoints is essentially in inhibiting the occurrence of exaggerated immune responses, which could lead to the elimination of normal cells. However, these checkpoints can also inhibit the destruction of cancerous cells. This can occur when the proteins of the T cells (also called immune checkpoint proteins) bind to the cancerous cells, hereby causing a stop signal to the T cells and the cancerous cell is not eliminated. **Immune checkpoint inhibitors** hereby stop the binding of the two proteins and therefore no stop signal is sent to the T-cell. This results in the destruction of the cancer cell and is therefore useful in cancer therapy. **T-cell transfer therapy** is used to help the already present T-cells to act more strongly. Herby the most active immune cells within the cancerous lesion are extracted, modified and

amplified outside the body before they are being returned to the patient. **Monoclonal antibodies** have the ability to attach to selected locations on the cancerous cell, hereby marking the cell, which makes it easier to be detected by the immune system. They are produced within laboratory conditions before being implanted into the patient's body (31).

G. Gene therapy (GT)

Definition of gene therapy according to Spanish law that comes from the transposition of several European Directives about new advances in medicines.

According to the Spanish law *"RDL 1/2015 de 24 de julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios" (32)*

A "gene therapy drug" is considered a highly developed therapy drug which is fabricated through different procedures, so that the prophylactic, diagnostic or therapeutic gene (for example a part of the nucleic acid) can be carried through the in vivo or ex vivo technique to a cell. This system is based on the use of vectors. These vectors can be incorporated in a human or animal cell (32).

In general gene therapy can be described as a type of treatment where a therapeutic gene is inserted into cells, that replace or not the endogenous gene, or that modifies it in a way that a malfunctioning gene could be re-stablished. This should happen without leading to any damage to the neighbouring cells and tissues (33).

Gene addition therapy for OSCC is based on the approach of placing tumour suppressor genes into the cancerous cells, causing cell death. The required checkpoints within the cell cycle, which are responsible for stopping a mutated cell in its development, did not work probably, leading to the development of cancerous cells. The transcription factor p53 is hereby of high importance within the cell cycle, as it has the potential to cause the stop of the cell cycle or its death. Hereby it can arrest the cell cycle at G1 and G2/M phases in case of mutations as well as it can lead to transcription silencing, making the continuation of the cell cycle impossible and therefore DNA replication. As the cell is being checked already at the G1 phase, this makes it possible for the cell to be repaired before re-entering into the cell cycle. Therefore, p53 is both responsible for the checkpoints within the cell, as well as the possible repair in case of damages, giving it its name as the "guardian of the genome". It can be made a connection between the importance of the correct functioning of p53 and the prevention of the development of cancerous cells (34,35,36).

1.4. Gene therapy as a treatment in different diseases

In accordance with the previous definition, GT can be a treatment option for several pathologies, and therefore can be used for bone repair, salivary gland disorders, implant osseointegration, relief of chronic pain, periodontal problems as well as head and neck oral cancer (37).

As a background information, in this section, the main characteristics of gene therapy are going to be explained.

1.4.1. *Ex-vivo* versus *in-vivo* modifications.

In general terms, there are two main ways of practising GT options, either by the *ex-vivo* modification, or by *in-vivo* modification.

- In the *ex-vivo* technique, the transfer of the gene into the required cells is done outside the body. This involves the modification of the target cells from a particular tissue (such as the hematopoietic stem cells from blood or the mesenchymal stem cells from adipose tissues) in the laboratory, and then their re-infusion to the patient. After the successful modification, the modified cells are inserted back into the body of the patient (33).
- In the *in-vivo* technique the therapeutic gene is transferred directly into the target tissue of the patient, such as muscle cells in muscular dystrophy patients of Duchenne muscular dystrophy (DMD), or Becker muscular dystrophy (BMD) (38).

1.4.2. **Different types of GT**

According to different studies, nowadays there are three main types and techniques to apply gene therapy including:

a. **Gene addition therapy**
b. **Gene replacement either by means of gene correction or knock-in**
c. **Suppression strategy or gene excision therapy**

Gene addition therapy consists in the introduction of a therapeutic gene into the affected cell with the outcome of either protecting the targeted cell or healing the disease or abnormalities present.

When healing a disease, the introduced gene will affect a specific part of the underlying disease mechanism (39).

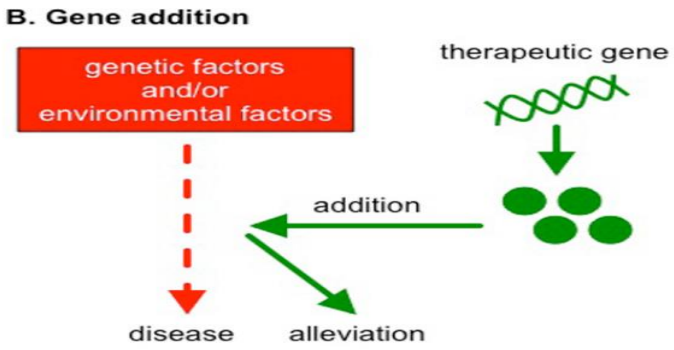

Figure 8. Gene addition process (39): through the addition of a therapeutic gene a disease can be prevented

Gene replacement can be achieved by either gene correction or knock-in. Targeted knock- in consists in the insertion of a cDNA of the therapeutic gene into the affected cell. Hereby the genetic regulation of the gene is not affected. The gene correction therapy is based on the replacement of a short defective sequence by introducing a new corrected sequence into the affected cell (40).

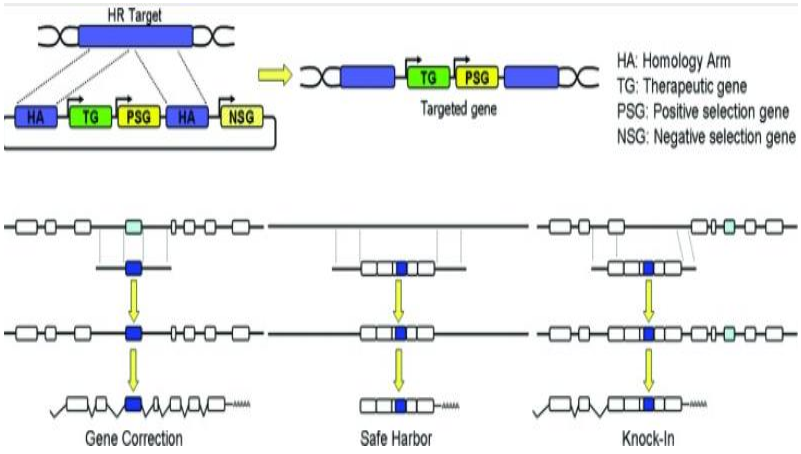

Figure 9. Gene replacement through gene correction (replacement short defective sequence) or knock-in technique (inserted cDNA) (40).

Suppression or gene excision therapy is based on the concept of decreasing or eliminating the expression of specific genes within the affected cells (41).

And finally, in general, within gene therapy, there are two main modalities, the targeted and non- targeted gene therapy. In the targeted gene therapy, the genetic material is directed towards a specific part of the genome. In the non-targeted gene therapy, it is not directed towards a specific location. Classical gene therapy is a non-targeted gene therapy as previous tools could not be so specific to modify the genetic material exactly (37).

1.4.3. Molecular Tools used in GT

In order to be able to transfer the therapeutic gene into the required cell, a specific tool needs to be used which is called carrier or vector (33). It exists two different types of vectors, the viral and non-viral vectors. Both **Viral vectors** and **Non-viral vectors** could be introduced by different techniques. It is referred to transduction for viral vectors and transfection for non-viral ones. A detailed description of them is given.

- A **viral vector** is a virus that has been attenuated, reducing its infectious risk, and therefore making it safe to use for gene therapy. It has the ability to enter into the required host cell and hereby insert the therapeutic genetic material.

The ideal characteristics of a viral vector include the high success rate of gene transfer as well as a safe transfer without causing an immune response.

A variety of viruses are used nowadays including adenovirus, adeno associated virus and retrovirus (42). Each viral vector has their own advantages and disadvantages. Adenovirus vectors show a high success in gene transfer and producing them is an easy procedure, while

it can lead to an intense immune response. Adeno-associated vectors also show a high success in gene transfer, a constant expression of the therapeutic gene and a low immune response, however the gene to be transferred needs to be of a small size. Retroviral vectors show a low level of immune response and constant gene expression; however, the transfer success is lower (42).

The use of a viral vectors leads in general to an increased risk of immune response and therefore infectious risk, even though the viruses are attenuated before using them for gene therapy, by removing a sequence of the genome fragment. This makes the treatment approach with viral vectors more susceptible to unwanted effects (43).

- **Non-viral vectors** are naked DNA, both particle and chemical based. They can be delivered to the target cells as naked genetic material (oligonucleotides, DNA, RNAS, transposons) (30,33) or in association with different compounds such as lipoplexes or complex polypeptides, oligosaccharides, gelatine or polyamine nanospheres.

Different physicochemical methods can be used for transfection. Hereby examples **include Gene gun transfer, Sonoporation, Electroporation and Magnetofection** (44).

Each non-viral vector has their advantages and disadvantages.

The **gene gun** shows high levels of safety and is commonly used for ex vivo gene transfer, while it cannot be used for cells located deep in the tissue (44).

Magnetofection is a technique which is mainly used in case of cells which are more complicated to transfer, therefore the capability of transfer is not very high. At the same time, it is a safe technique, not damaging the cells (44).

Electroporation indicates for a successful transfer efficiency as well as the possibility to transport proteins, while it leads to possible damage in cells and tissues. Furthermore, this method cannot be used for reaching deeper areas inside the body (42, 44).

In general, it can be stated that, despite their safety, ability to avoid the immune response and ability to carry large amounts of DNA, they have a poor transfer efficiency, and a short time of expression.

Below are summarized the main advantages and disadvantages of both methods in **Table 3**, as well as a **Figure 10** explaining in a simple way the procedure of gene therapy with viral and non-viral vectors.

Type of Vector	Advantages	Disadvantages
Viral (42)	• High success rate of gene transfer as well as a constant and stable expression in the majority of viral vectors (Adeno and Adeno associated vector)	• Lower level of safety due to higher risk of infection and immune response to the virus • Inability to reach deeper tissue (gene gun and electroporation)
Non-Viral (44)	• High level of safety of gene transfer • Low risk of immune response (adeno associated and retrovirus)	• Lower success rate of gene transfer (magnetofection) • Lower stability of gene expression

Table 3. Summary of the main characteristics of viral and non-viral vectors used in GT.

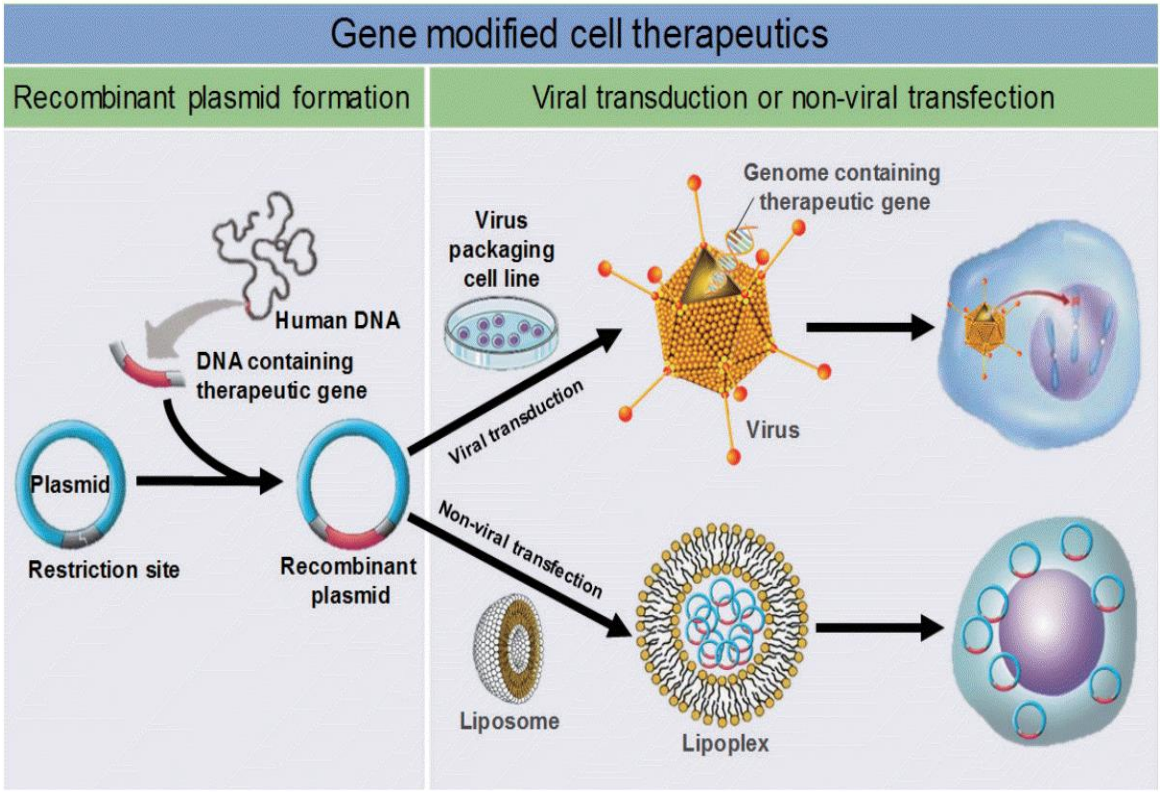

Figure 10. Gene modification using Viral and Non-viral vector (42). In the first phase DNA with a therapeutic gene is inserted into a plasmid, forming a recombinant plasmid. The second phase depends on the use of viral or non-viral vectors. In case of a viral vector a transduction takes place forming a genome with the therapeutic gene. In case of non-viral vector, a transfection through a liposome occurs, forming a lipoplex.

2. Objectives

Main Objective: To analyse the effectiveness of gene therapy in the treatment of oral squamous cell carcinoma in comparison to other treatment options.

Secondary objectives

1) To establish the effectiveness rate of gene therapy in the treatment of OSCC.
2) To assess the effectiveness rate of surgery in the treatment of OSCC
3) To establish the effectiveness rate of chemo and radiotherapy in the treatment of OSCC
4) To measure the effectiveness rate of Immunotherapy in the treatment of OSCC
5) To analyse the effectiveness rate of Photodynamic therapy in the treatment of OSCC
6) To evaluate the effectiveness rate of COX-2 Inhibition therapy in the treatment of OSCC

3. Methodology

Literature Search and Research Methods

The purpose of this paper consists in the analysis of gene therapy as a treatment option for OSCC based on recent research.

For the literature search the Biblioteca Crai Dulce Chacon was accessed, including the use of electronic databases, relevant publications, abstracts, and books. The databases accessed included Medline, PubMed, and Academic Search Ultimate. For further research, publications within Google Scholar were considered.

Concerning the applied search strategy key words were used to narrow down the available information. These included “Oral squamous cell carcinoma”, “Gene therapy”, “Oral cancer”,

“Treatment”, “Diagnosis”, “Viral/Non-Viral Vector”, “Chemotherapy”, “Radiotherapy”, “COX-2 Inhibitor”, “Photodynamic therapy”.

Inclusion criteria included relevant publications, studies, and books with a publication date mostly within the last 20 years. However also older publications were included in order to be able to compare how the treatment approaches for OSCC have developed in the past decades. The language of the chosen publications was set to be English, Spanish, or German.

Exclusion criteria included articles before the year of 2000, as treatment options have developed significantly in the past decades and hence this information is not up to date anymore. In vitro and animal studies were excluded, as they are not relevant for studying OSCC within a human population.

It was considered a wide range of studies, including clinical trials, meta-analysis, systemic reviews, and case studies. The most relevant studies researched, were selected to be included in this review. Hereby it was considered the heterogeneity of results in different meta-analysis and systematic reviews as well as the decreased internal validity of case studies, due to their lack in reproducibility and decreased number of participants.

This work could be the basis for a more in-depth research about this topic for a future publication in a scientific journal.

4. 1 Results

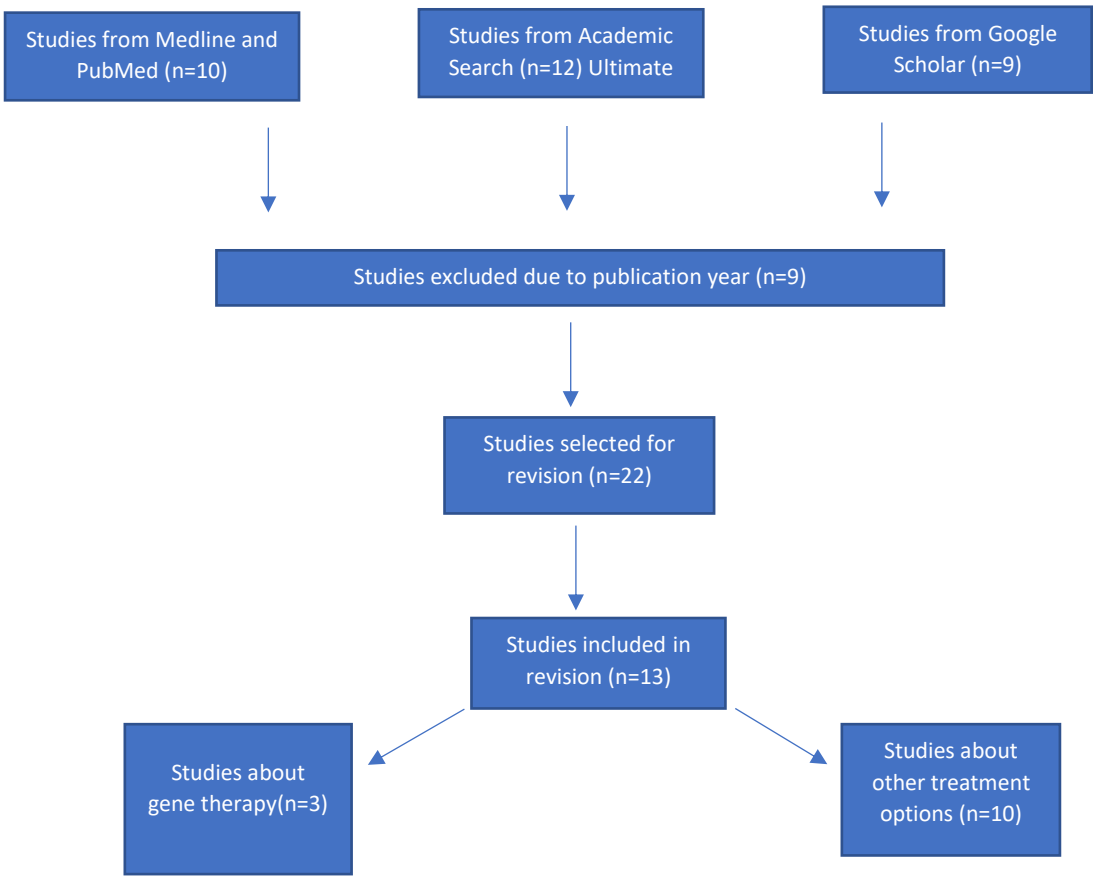

Figure 11. Graphic indicating based on which criteria the included studies have been chosen (out of 31 studies which have been considered, 13 were selected based on publication year and relevance to the topic)

Due to the wide variability and constant changes and improvements of treatment options for OSCC it is of high importance to understand each treatment option, their applications, advantages and disadvantages and the comparison between all of them, to be able to choose the most appropriate approach for each patient.

Surgical Approach for the treatment of OSCC

The sole use of surgical excision is often used for the removal of the primary tumour, especially in cases with an early diagnosis and hence a decreased tumour size. However, it is also a suitable method for the excision of metastasis in lymph nodes (38).

Several studies have investigated the effect of surgical removal of OSCC on its own, as well as in combination with other treatment approaches like radiotherapy or additional more invasive surgical approaches like the neck resection.

The following table summarizes the studies about the surgical approach considered for this revision.

Reference and Year	**Number of patients**	**Sex**	**Objective**	**Significant Findings**
Yookyeong et al. (2019) (46)	67 patients	Male: 45 Female: 22	Survival rate of OSCC after surgical intervention	Survival rate in stage I and II is 100% Overall survival rate is 71.6%
Hutchinson et al. (2019) (47)	250 patients	Female and male patients	Survival rate of OSCC after tumour resection in comparison with additional neck resection surgical intervention	Overall survival rate with only tumour resection was 67.6% compared to 75.8% in the group with additional neck resection

Table 4. Studies taken into account about the surgical approach in OSCC

Chemotherapy approach to treat OSCC

Chemotherapy is the treatment approach which is most used in combination with other treatments such as radiotherapy and/or surgery. Especially in cases where the resection margins around the cancerous lesion are small or possible affection of the vascular or lymph tissue the use of chemotherapy is very beneficial (22).

The following table summarizes the studies about the chemotherapy approach considered for this revision.

Reference and Year	**Number of patients**	**Sex**	**Objective**	**Significant Findings**
Hayashi et al. (2017) (48)	31 patients	Male: 12 Female:19	Survival rate of OSCC after chemotherapy treatment	Complete response in 81% Partial Response in 19% 78% survival rate
Bossi et al. (2014) (49)	198 patients	Female and male patients	Survival rate of OSCC after chemotherapy treatment and local relapse	Survival rate higher with chemotherapy (48.5%) than only surgery (36%)
Licitra et al. (2003) (50)	195 patients	Female and male patients	Survival rate of OSCC after chemotherapy treatment	Survival rate shows no difference Decreased need for severe follow up treatment after chemotherapy

Table 5. Studies taken into account about chemotherapy in OSCC

Radiotherapy as a treatment approach for OSCC

As it happens with chemotherapy, radiotherapy is mainly used as an additional treatment approach and is normally not used on its own. Only in case of stage I and II, radiotherapy alone can be the sole treatment option. It is not indicated in more advanced stages as more invasive options are required. Most commonly hereby is Brachytherapy, which can be divided into Low-dose radiation (LDR) or High dose radiation (HDR) (51).

The following table summarizes the study about the radiotherapy approach considered for this revision.

Reference and Year	**Number of patients**	**Sex**	**Objective**	**Significant Findings**
Zhenxing et al. (2014) (51)	420 patients	Female and male patients	Survival rate of OSCC after radiotherapy (comparison HDR to LDR)	Slight difference between HDR (71.9%) and LDR (67.4 %) survival rate

Table 6. Studies taken into account about radiotherapy in OSCC

Immunotherapy as a treatment approach for OSCC

Immunotherapy is a more recent treatment approach which has been developed in the past years. It could be an option in combination with other treatments as for example surgery, chemotherapy and/or radiotherapy. It is based on the underlying concept that the immune system can attack the cancerous cells present. The applied immune therapy leads to a strengthened immune system, increasing its ability to fight against the cancerous cells (52, 53). The following table summarizes the studies about the immunotherapy approach considered for this revision.

Reference and Year	Number of patients	Sex	Objective	Significant Findings
Timur et al. (2005) (54)	39 patients	Female and male patients	Pathological (size) and Histopathological (microscopical) response of OSCC to immunotherapy	General response rate to the treatment 42% Infiltration of mononuclear cells (increased infection resistance)
Xiong et al. (2020) (55)	10 patients	Female and male patients	The immunological effect of anti-PD-1	CD4+ levels are decreased (while T-cells are slightly increased) and increased of CD8+ cells No overall immune effect

Table 7. Studies taken into account about immunotherapy in OSCC

Photodynamic Therapy (PDT) as a treatment approach for OSCC

Photodynamic therapy is considered as an additional option for the treatment of OSCC. It is hereby based on its ability to lead to a cytotoxic effect, destruction of the vascularity of the tumour cells as well as an inflammation reaction (56).

The following table summarizes the studies about the PTD approach considered for this revision.

Reference and Year	Number of patients	Sex	Objective	Significant Findings
Schweitzer (2001) (57)	10 patients	Female and male patients	Response rate of OSCC to PDT (reduction in size)	8 patients with complete response and 2 patients with partial response
Biel et al. (2006) (57)	113 patients	Female and male patients	Response rate of OSCC to PDT (reduction in size)	107 patients with complete response and 6 patients with partial response

Table 8. Studies taken into account about PDT in OSCC

COX-2 Inhibitor therapy as a treatment approach for OSCC

COX-2 inhibition therapy is based on the abnormally increased levels of COX-2 in OSCC, this has been confirmed in a clinical study, where a 100 times higher COX-2 level was found in patient with OSCC. COX-2 Inhibitor as a treatment approach however has mainly been used in the prevention of the development of the OSCC (28).

Gene therapy as a treatment approach for OSCC

There are different types of gene therapy approaches available for the treatment of OSCC.In case of the presence of OSCC, the most common mutation can be found in p53. Therefore, most of the approaches of gene therapy are focused on the modification of the gene p53 (36). The following table summarizes the studies about the gene therapy approach considered for this revision.

Reference and Year	**Number of patients**	**Sex**	**Objective**	**Significant Findings**
Yi Li et al. (2014) (58)	99 patients	38 female and 61 males	Response rate (reduction in size) of OSCC to gene therapy and survival rate	Highest response rate in group 1 with gene therapy (48.5%) as well as highest survival rate with 54.3%
Liu et al. (2013) (58)	107 patients	Female and male patients	Recurrence rate of OSCC after gene therapy	Higher recurrence rate in group 2 (32%) without gene therapy in comparison to group 1 (7%)
Zhang et al. (2001-2003) (59)	69 patients	Female and male patients	Response rate of OSCC to gene therapy (reduction in size) and adverse effects	Tumour size was more than 2 times bigger in group 2 (without gene therapy) Only light side effect

Table 9. Studies about gene therapy in OSCC

4. 2 Discussion

The most important factor to reach a successful treatment outcome is the early diagnosis of the cancerous lesion within the dental office. Depending on the progression and therefore stage of OSCC different treatment options are available, leading to a more or less successful outcome. Therefore, it is of high important to choose the most suitable treatment option.

In context of the surgical approach authors including **Yookjyeeong et al. (2019)** confirmed that it is a highly successful option for early stages of OSCC (stage I and II) with a 100% response rate, while it is less successful for advanced stage (stage III and IV). The same was concluded by **Hutchinson et al. (2019)** who additionally concluded that not only the primary resection, but also especially the resection of the lymph nodes is essential for complete response independent of the stage of OSCC (46,47).

The studies have been chosen due to their recent publication date, being therefore up-to-date and since they focus on the surgical removal as a sole approach. Due to their relative high sample size, the results are indicative (46,47).

Surgery can be used as a sole treatment approach with high efficiency without the need of supplementary treatment like radiotherapy, however this is only applicable to the early stages of OSCC and require an early diagnosis (46,47).

However, it needs to be taken into account that post operational complications can occur due to the invasive nature of the approach. These include a decreased aesthetic as well as functional alterations, as for example problems in speaking, swallowing, and chewing of food. Herby it can be indicated that especially for older patients, surgical excision can bear increased risks, as for example the development of pneumonia after the surgery due to a weakened

immune system. The more invasive approach of neck dissection can lead to more severe post-operative complications but also reduces the chances of metastasis and therefore survival.

It needs to be decided based on the individual case, if the risk of the surgery can be justified by the benefit of increased survival rate (46,47).

Another treatment approach is the chemotherapy, hereby authors including **Hayashi et al (2017)** and **Bossi et al (2014)** both indicate a positive corelation between the chemotherapy and its effect on OSCC (response rate and survival rate) in different stages of cancer. However, this is seen in contrast to the study conducted by **Licitera et al (2003)** where no increased survival rate could be identified through the addition of chemotherapy. Hereby indicating that surgery on its own shows the same effectiveness (48,49,50).

Especially **Hayashi et al (2017)** identifies that chemotherapy as a sole approach, shows the same response as surgery in advanced stages (stage III and IV) concluded by **Yookjyeeong et al (2019).** This could be an essential advantage in older patients, where surgery would be too invasive or in case of anatomical limitations, where a surgical excision with an appropriate safety margin is not possible or in case of surgery being too dangerous (due to damage of blood vessels). However, it is also connected to side effects, including the development of induced toxicity due to the drugs given as part of chemotherapy.

The contradicting result could be related to the possible advance in pharmaceutics within 10 years between **Licitera (2003) and Hayashi and Bossi (2017 and 2014)** (48,49,50).

The studies have been chosen, due to their wide span in publication year, indicating the development of the treatment as well as their relatively big sample size.

Apart from that there is the treatment approach of radiotherapy, **Zhenxing et al (2013)** argument that between the different types of radiation therapy (HDR and LDR) there is no significant difference in survival rate. For the general survival rate in radiation therapy, it can be said that it is relatively high but only when used in early stages (I and II) of OSCC (51).

This can be seen in contradiction to other studies including **Licitra et al (2003)** who only indicate radiotherapy as an additional approach to chemotherapy or surgery and never as a sole approach (50).

Therefore, radiotherapy is a good treatment approach in connection with chemotherapy or surgery. However, it is not often used on its own and could lead to the development of osteonecrosis as a side effect.

The study has been chosen due to the big sample size and it is focusing only on radiotherapy without other approaches like chemotherapy affecting the results.

Immunotherapy is another treatment approach, **Timar et al (2005)** developed that the use of immunotherapy leads to a general low response rate in terms of size reduction and microscopical change of the OSCC, but to an increased resistance against possible infections (increased CD4+/CD8+ ratio). These results are applicable to stage II and III of OSCC (54).

These results are in contrast to **Xiong et al (2020**) who also identified an effect on the immune system, however this effect is not general but rather selective. Hereby an increased CD8+ count could be identified, while the CD4+ count was decreased (55).

Therefore, it could be recognized that immunotherapy has an unspecific effect on the immune system, while the effect on tumour reduction is relatively low. However, the use of

immunotherapy also leads to much fewer side effects as for example cytotoxic effects through chemotherapy (55).

The described studies have been chosen due to their wide span in publication date, allowing a comparison of the progression of the treatment over time as well as there exist only few studies concerning this field.

The treatment approach of PTD has been investigated by authors including **Schweitzer and Biel,** whereby both used the same medication (Photoforin) with different sized study samples, however with similar results. Therefore, valuable conclusions can be drawn on the effectiveness of PDT. The authors conclude that the use of PTD shows the best results in early stages (I and II) of OSCC. In comparison to immunotherapy PDT has a much higher response rate in reduction of tumour size (80%) and is therefore a more reliable approach (57).

It is argumented that PTD is a non-invasive option, and is especially suitable in margins of cancerous lesions, which cannot be treated by surgery.

However, it can lead to side effects including irritations of the skin, discomfort or infections. It can only be applied in areas, where the light reaches the lesions. Posterior areas in the oral cavity or anatomical limitations can hinder the correct application of PDT (57).

The described studies have been chosen as there exist only few studies concerning the field of PTD treatment in vivo.

Concerning the approach of COX-2 therapy very few studies investigating the effect on OSCC have been conducted, however from the available sources it could be argument that it is

rather a preventative than a curative approach and therefore always requires additional options (28).

In relation to gene therapy **Yi Li et al (2014), Liu et al (2013) and Zhang et al (2001-2003)** describet that gene therapy is effective in the treatment of advanced stages of OSCC (III and IV), however only in combination with chemo-/radiotherapy or surgery. The effect of gene therapy on its own has hereby not been proven. The relatively low survival rate of 55% can hereby be linked to the fact, that all patients suffered more advanced stages, in comparison to other studies, where patients with early stages were included. These results could be identified in all three studies, which consisted of varying sample sizes, and can therefore be generalized (58, 59).

Zhang et al (2001-2003) concluded that especially the use of radiotherapy on its own, does not lead to a high response and hence survival rate. This can also be confirmed **by Licitra et al (2003)** mentioning that radiotherapy as a sole approach is not sufficient. However, the added use of gene therapy can improve the outcome (59).

It can be analysed that gene therapy is a non-invasive treatment approach as it does not lead to any long-term damages as for example surgery with functional and aesthetic consequences. A possible side effect can include the immune response caused by the use of a viral vector to administer the treatment. However, this cannot be identified in all patients, and most commonly present in terms of flu like symptoms, and therefore does not cause damage.

It needs to be taken into account that in all the evaluated studies, gene therapy is not administered on its own, but in combination with chemotherapy, radiotherapy or surgery.

Furthermore, gene therapy seems to be a treatment approach more applicable in advanced stages of OSCC, and not as much in early stages. This could be another reason why gene therapy is most often used in combination, as practitioners do not want to rely on a single treatment approach (58,59).

They included studies have been chosen due to their wide range in publication years, ensuring that a progression of this approach can be identified. Furthermore, they include a relatively big sample size and all focus on gene p53 as a point of reference for the success of the treatment approach.

5. Conclusion

Main Objective

Gene therapy can be stated as a more recently developed approach, especially useful for advanced stages of OSCC (stage III and IV). It increases both the response and survival rate of the patients significantly. Therefore, it can be concluded that gene therapy is a treatment approach suitable in the treatment of OSCC. However, it can be identified that most effective results are in combination with other treatments as for example together with chemotherapy/radiotherapy or surgery, approaching the cancer from different aspects. Even though it will lead to increased side effects (immune responses), the advantage, a possible survival weights significantly more than the associated risks. Until now very little research about the effectiveness of gene therapy for OSCC has been conducted, so that more research will be necessary to have more representing results.

Secondary objectives

1. **Gene therapy** shows a survival rate of approximately 55%, being hereby mainly used in advanced stages III and IV.
2. **Surgery** shows a very high survival rate between 70 to 100% depending on the research consulted. It could be identified the highest effectiveness in early stages (I and II) of OSCC.
3. **Radiotherapy** indicates a survival rate between 67 to 71%, while **chemotherapy i**ndicates a survival rate between 48% and 78% depending on the research consulted, both being used in early and advanced stages of OSCC (I, II, III, IV).

4. **Immunotherapy** indicates a response rate of approximately 40% and is mainly used as a supportive treatment in early to medium stages (I, II, III) and to improve the immune response.
5. **Photodynamic therapy** indicates a complete response between 80-90% depending on the bibliography considered, whereby it is only applicable in early stages (I and II).
6. **COX-2 Inhibition therapy** has been mainly used as a preventive measure for OSCC, rather than as a treatment approach.

6. Social Responsibility

The presented work **“Could Gene therapy be a treatment approach for OSCC?”** indicates the importance of **environmental responsibility**. It is hereby based on the revision of several different approaches suitable for the treatment of OSCC and focuses on finding the best option depending on the individual case. Hereby the optimisation of the choice of treatment can improve both the length and quality of life of the patient. Furthermore, it also addresses the importance of **economic responsibility.** The early diagnosis of OSCC, as well as being aware about all existing treatment approaches, makes it possible for the clinician to choose less time and cost consuming treatment approaches. Moreover, the research and development of new and effective approaches, like gene therapy, can be essential for having access to a wider variety of economic options for the treatment of OSCC.

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8. Annexes

Annex 1

Glossary

Abbreviations

AML- Acute myeloid leukemia

COCE- Carcinoma oral de células escamosas

COX- Cyclooxygenase

DC- Dyskeratosis congenita

FA- Fanconi anaemia

GT-Gene therapy

HDR-High-dose radiation

HNSCC-Head and neck oral squamous cell carcinoma

HPV- Human Papillomavirus

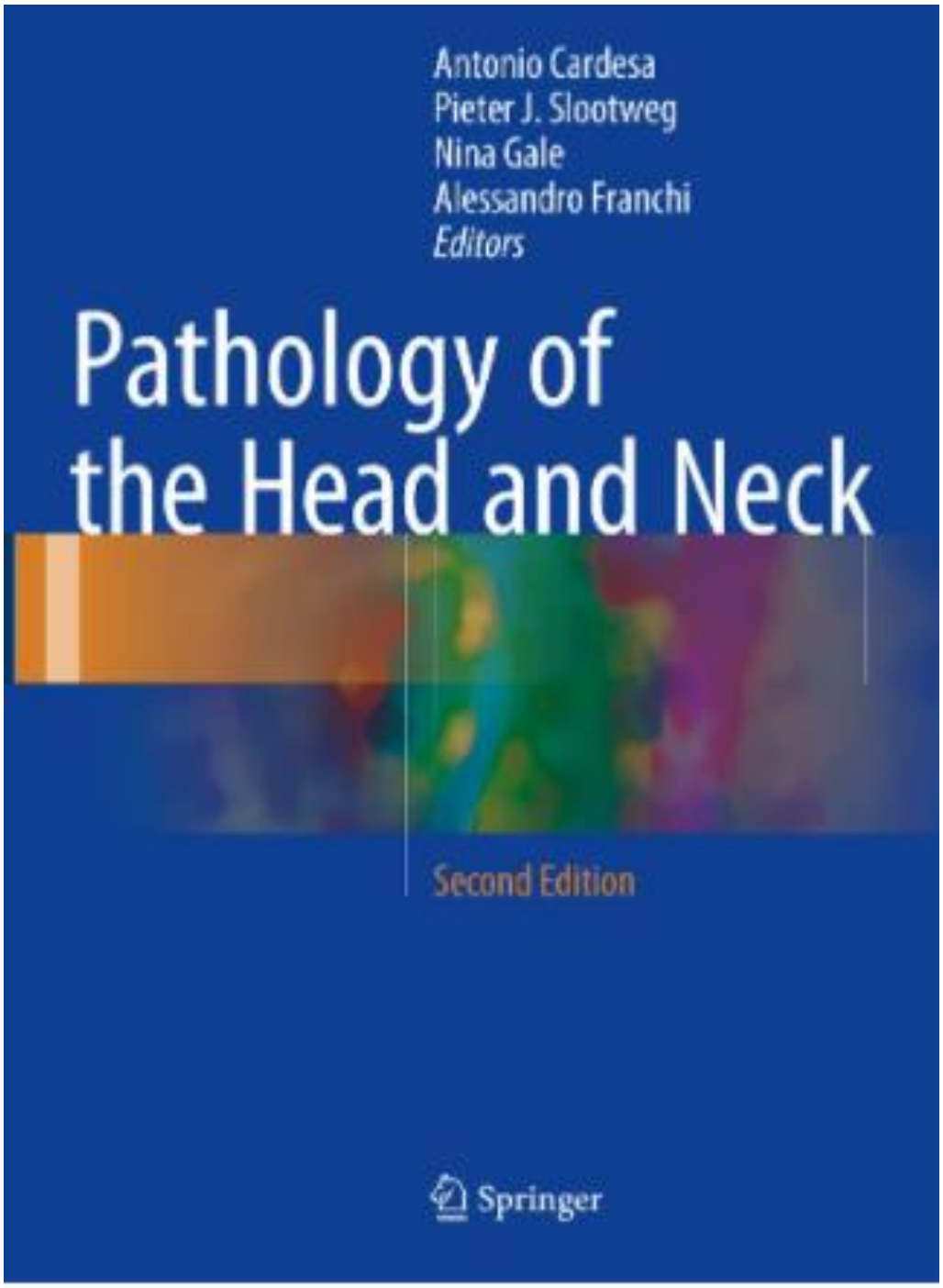
LDR- Low-dose radiation

OSCC- Oral Squamous Cell Carcinoma

PDT-Photodynamic Therapy

PET- Positron Emission Tomography

Annex 2


Antonio Cardesa
Pieter J. Slootweg
Nina Gale
Alessandro Franchi
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Annex 3

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A Systematic Review of Gene Therapy as Treatment of Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) represents about 90% of all malignant neoplasms of the oral cavity. Oral cancer is the cause of death among children ages 1-14 years. Gene therapy provides modern medicine and had great potential as a recent therapeutic modality. Gene therapy can potentially attack cancerous cells while respecting normal tissue. It may be useful as treatment for recurrence disease such as oral squamous cell carcinoma.
Objective: To review materials about gene therapy as a treatment for oral squamous cell carcinoma.
Methods: Scientific evidence and clinical cases were drawn from the literature to support this review and information about the gene therapy as a treatment of oral squamous cell carcinoma was collected.
Results: Gene therapy is introducing new genetic material into target cells while causing no damage to surrounding healthy cells and tissue. It has been defined as the genetic modification of cells of a patient in order to fight a disease. Gene therapy includes both the transfer of new genetic material and the manipulation of existing genetic material.
Conclusion: Gene therapy has potential as a recent treatment in cases of oral squamous cell carcinoma (OSCC).

Keywords: Gene therapy, Oral squamous cell carcinoma (OSCC)

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INTRODUCTION

Oral squamous cell carcinoma represents about 90 of all malignant neoplasms of the oral cavity in human.[1] Oral cancer is the 6[th] most common malignancy worldwide. Patients passed away because of cancer of the oral cavity and lip as much one hundred and forty five thousand patients.[2] According to histological data, oral squamous cell carcinoma accounts for majority of all oral cancer in the world.[3] Oral squamous cell carcinoma is rare in pediatric patients which defined by the American Academy of Pediatrics as patients under age 21. Many clinicians believe that this disease is associated with poorer survival compared to adults.[4]

The most risk factors of oral squamous cell carcinoma (OSCC) are tobacco and alcohol abuse. According to the International Agency for Research on Cancer that cigarettes smoke contains more than 60 chemical carcinogens.[5] Alteration of taste, pain, difficulty in eating, bacterial and fungal infections, mucosal ulceration, increased thickness of saliva, discoloration skin, and edema of the skin are the major side effects.[5,6]

Gene therapy provides modern medicine and had great potential as a recent therapeutic modality. Clinical application of this technique in the treatment of oral cancer and precancer requires optimization of viral vectors and improvement of transfection effectiveness.[7,8] Here we use systematic review and meta-analysis to describe more deeply about gene therapy as a treatment of oral squamous cell carcinoma.

Oral Squamous Cell Carcinoma (OSCC)

Oral cancer is the 6[th] most common malignancy worldwide. Patients passed away from cancer of the oral cavity and lip as much one hundred and forty five thousand.[2,3] The etiology of oral squamous cell carcinoma there are:

a. Tobacco

More than 80% consumption of tobacco with smoking greatly increases the risks of oral squamous cell carcinoma. The strongest being tobacco-specific *N*-nitrosamines is several carcinogens in tobacco.[10,11,12]

b. Alcohol

The combined use of alcohol and tobacco has a multiplicative effect on oral cancer risk. The various pathways by which alcohol may exert carcinogenic influence include topical exposure leading to a direct effect on cell membranes.[10,11]

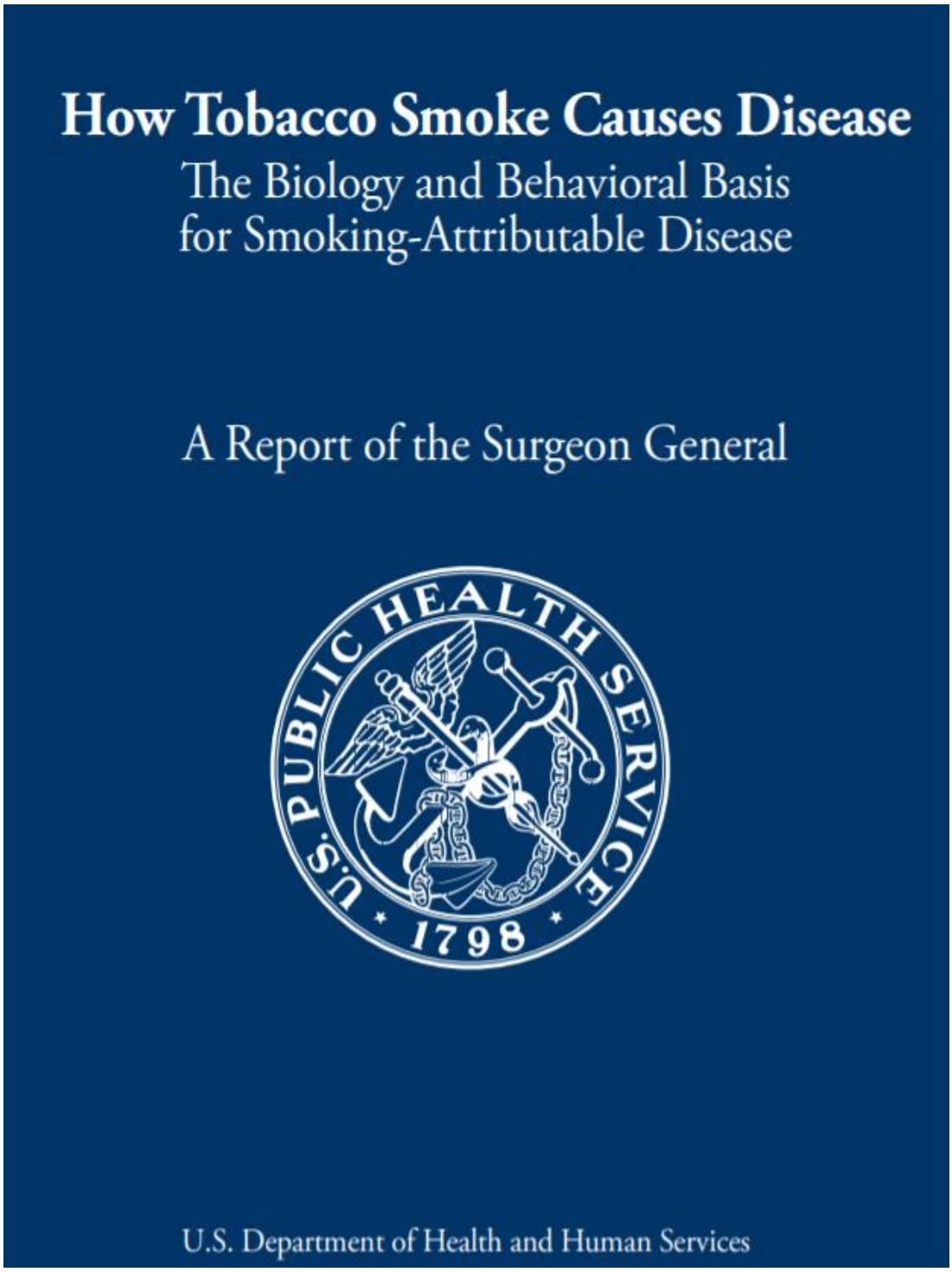
c. Genetic

Cytochrome p450 system is most carcinogens which metabolized in the liver. Individuals with the fast metabolizing version of alcohol dehydrogenase have a greater risk of developing oral cancer.[10,13,14]

Potential symptoms of the oral squamous cell carcinoma are:[3]

a. Non healing ulcer with or without induration
b. White patch with firm consistency

Annex 4


How Tobacco Smoke Causes Disease
The Biology and Behavioral Basis for Smoking-Attributable Disease
A Report of the Surgeon General
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Annex 5

J. Maxillofac. Oral Surg. (Apr-June 2011) 10(2):132–137
DOI 10.1007/s12663-011-0195-z

REVIEW

Oral Cancer: Risk Factors and Molecular Pathogenesis

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Abstract

Introduction Oral cancer is one of the most common cancers and it constitutes a major health problem particularly in developing countries. It is one of the leading causes of death. Tobacco and alcohol consumption appears to be the major determinants of oral cancer.

Materials and methods The literature search was carried out in NCBI Pubmed database using keywords "oral cancer", "risk factor", "epidemiology" and "patho*". Some basic information was also obtained from textbook and medical university websites.

Results Several risk factors have been well characterized to be associated with oral cancer with substantial evidences. The development of oral cancer is a multistep process involving the accumulation of genetic and epigenetic alterations in key regulatory genes. Experimental pathological studies of oral cancer in animal models and direct molecular genetic analysis of oral cancer subjects in recent times have revealed a substantial amount of knowledge on specific gene alterations or other genetic mechanisms involved in initiation and subsequent progression.

Conclusion Considering known risk factors, oral cancer appears to be to a certain extent, a preventable disease. Recent development of molecular picture of pathoprogression and molecular genetic tools opens the avenue for easier diagnosis, better prognostication and efficient therapeutic management.

Keywords Oral cancer · Epidemiology · Molecular biology · Risk factor

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Introduction

Oral cancer (OC) is the commonest cancer in India, accounting for 50–70% of total cancer mortality and accounts for highest incidence among Asian countries [1]. OC is the sixth most common cancer worldwide [2]. It affects anterior tongue, cheek, floor of mouth, gingiva or any other part of the oral cavity. Worldwide, there is a great variation in the incidence of cancer of the oral cavity. It accounts for less than 5% of all cancers in United States, Western Europe and Australia. India, few pockets in France, Brazil, central and eastern Europe have few of the highest rates of cancer of the oral cavity in the world. The differing social customs are likely to be responsible for regional variations in the disease incidence. The high rate of OC in France and Eastern Europe has historically been linked to the heavy consumption of alcohol and tobacco in these countries. The habit of chewing betel nut leaves rolled with lime and tobacco, a mixture known as pan, results in prolonged contact of the carcinogen with the

Annex 6

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Research Paper

Dietary score and the risk of oral cancer: a case-control study in southeast China

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Keywords: *oral cancer, dietary score, tobacco smoking, alcohol drinking, case-control study*

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ABSTRACT

This study aims to develop a simple dietary score to comprehensively evaluate the role of diet in the risk of oral cancer. A case-control study including 930 oral cancer cases and 2667 frequency-matched controls was performed in Fujian, China. Unconditional logistic regression model was used to estimate the effects of dietary factors on oral cancer. After adjustment for potential confounders, less intake of domestic meat (< 3 times per week), fish (< 3 times per week), seafood (< 3 times per week), leafy vegetables (< 1 time per day), other vegetables (< 1 time per day), fruits (< 3 times per week), milk and dairy products (< 1 time per week) and eggs (< 5 times per week) were significant risk factors for oral cancer. Then these variables were incorporated to establish dietary risk score. Assessed by the receiver operating characteristic curve, the score showed a satisfactory discriminatory capacity, with an area under the curve of 0.682 (95% CI: 0.662–0.702). Moreover, the score was positively associated with the risk of oral cancer as quartiles, and the association was apparently stronger in tobacco smokers or alcohol drinkers. Additionally, there were significant multiplicative interactions between the score and tobacco smoking or alcohol drinking for oral cancer. In the present study, a convenient dietary score with satisfactory discriminatory capacity was developed to assess the collected effect of dietary factors on oral cancer, which could provide a new strategy for the prevention of oral cancer through changing in dietary habits.

INTRODUCTION

Oral cancer is the eighth most common cancer in the world, with approximately 90% of cases being squamous cell carcinoma (SCC) [1, 2]. In China, it has been reported that there are 21,413 new cases and 11,333 deaths of oral cancer in 2012 [3]. Given that the disfigurement consequence and social communication difficulties after treatment and a permanent reduction in quality of life, therefore, much attention should be paid to the prevention of oral cancer.

Although tobacco smoking and alcohol drinking are known to be main risk factors for oral cancer [4, 5], diet also plays an important role in the etiology of oral cancer. It was well established that high consumption of fresh vegetables, fruits, fish and seafood could protect against oral cancer [6–8]. Several epidemiological studies indicated that high intake of red and processed meat were associated with increased risk of oral cancer [9, 10]. Whereas, there is limited epidemiologic data on the collective effect of diet factors on oral cancer risk.

Annex 7

OPINION

Impact of race/ethnicity on molecular pathways in human cancer

John K. Wiencke

Understanding the molecular circuitry of the cancer cell is within the grasp of the basic scientist; however, harnessing this knowledge to predict cancer risk requires integration of molecular and population sciences. But, what role, if any, does race/ethnicity have in cancer research and, more specifically, in the nature of genetic and epigenetic alterations that programme the malignant behaviour of the cancer cell?

Race, as it is used in common discourse, is a subdivision of a species formed by a group of individuals that share common biological characteristics that distinguish them from other groups[1]. The concept of ethnicity emphasizes cultural, socioeconomic, religious and political qualities of human groups, including language, diet, dress, kinship relation systems and historical or territorial identity[2]. The United States Census and biomedical researchers collect both types of data to categorize populations. There is abundant epidemiological evidence that self-identified race/ethnicity is associated with differences in cancer incidence and mortality. For example, over the 5-year period ending in the year 2000, national cancer statistics from the United States show an average annual prostate cancer incidence of 277 per 100,000 for African-American men compared with 168 per 100,000 among Caucasians. Racial differences in death rates for the disease were even more evident. An average of 73 prostate cancer deaths per 100,000 for African-American men compared with 30 per 100,000 for Caucasians were recorded[3].

Another example is early-onset breast cancer, which is more common among African-American compared with Caucasian women, and breast cancer mortality is higher among African-American women in all age groups[4–6]. By contrast, certain minority populations have reduced risks of developing some types of cancer. Primary brain tumours are more common in Caucasians, compared with minority non-whites[8]. African Americans were reported to have lower survival rates after diagnosis of primary brain tumour compared with Caucasians[10], whereas another study reported a higher incidence of survival among African Americans[11]. To address the complex issues regarding cancer risk, race and ethnicity, data are commonly collected by health researchers. This information can be used to obtain information about social class, possible environmental exposures and genotype.

There is far from a consensus on the value of racial information in cancer research. It has been argued that racial categories are no longer useful in aetiological research because they are too vague and imprecise[12]. Others point to the use of such classification schemes for epidemiological and clinical investigations[13,14]. Moreover, the political ramifications of collecting racial data continue to be intensely debated. In California's special recall election that was held on 7 October 2003, voters rejected the Racial Privacy Initiative (Proposition 54), which sought to ban the state from collecting racial data in all but a few exempted cases. Sixty-four percent of voters voted against the proposal, reflecting the concern that limiting the collection of racial information would slow the progress of cancer research.

Ancestry and racial categories

To help answer the question of whether there is a valid biological meaning to racial categories and whether these categories might help to explain the molecular features and aetiological heterogeneity of cancer in different populations, we can turn to the work of evolutionary biologists and population geneticists. Studies that use molecular-marker analysis show that human populations worldwide can be subdivided into groups that are consistent with race, based on ancestry within one of five continents[15]. These groups include African, Caucasian (European and Middle Eastern), Asian, Pacific Islander and Native American. DNA markers, including short tandem repeats (minisatellites) and single-nucleotide polymorphisms, have been used to determine relatedness and lineage within human populations (FIG. 1).

An example of such a marker is the Duffy-blood-group antigen, a glycosylated protein that was first recognized as the erythrocyte receptor for the human malaria parasite *Plasmodium knowlesi*[16]. A point mutation within the gene locus for Duffy (*FY*), which is located at 1q21–1q22, leads to lack of expression of the Duffy antigen in red blood cells. This mutation is very rare in most racial groups, but is present in 100% of

Figure 1 | **Classification of major racial/ethnic groups, based on the migration of modern *Homo sapiens*.** Genetic differentiation of humans according to their migration patterns and establishment of genetically isolated populations over time provides the basis for racial categories according to continental ancestry. The scheme that is outlined above begins with a radiation from east Africa to the rest of Africa about 100,000 years ago, and is followed by an expansion from the same area to Asia — probably by two routes, southern and northern — between 60,000 and 40,000 years ago. Oceania, Europe and America were settled from Asia in that order. Genetic divergence is brought about by relative isolation of groups in different environments and through the actions of genetic drift and differential natural selection. Figure adapted from REF. 15. © (2003) Nature Publishing Group.

Annex 8

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Oral squamous cell carcinoma: clinicopathological features from 346 cases from a single Oral Pathology service during an 8-year period

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ABSTRACT

Epidemiological data from oral squamous cell carcinoma (OSCC) is mostly derived from North American, European and East Asian populations. Objective: The aim of this study was to report the demographic and clinicopathological features from OSCC diagnosed in an Oral Pathology service in southeastern Brazil in an 8-year period. Material and Methods: All OSCC diagnosed from 2005 to 2012 were reviewed, including histological analysis of all hematoxylin and eosin stained slides and review of all demographic and clinical information from the laboratory records. Results: A total of 346 OSCC was retrieved and males represented 67% of the sample. Mean age of the patients was 62.3 years-old and females were affected a decade older than males ($p<0.001$). Mean time of complaint with the tumors was 10 months and site distribution showed that the border of the tongue (37%), alveolar mucosa/gingiva (20%) and floor of mouth/ventral tongue (19%) were the most common affected sites. Mean size of the tumors was 3.4 cm, with no differences for males and females ($p=0.091$) and males reported both tobacco and alcohol consumption more frequently than females. Histological grade of the tumors revealed that 27%, 40% and 21% of the tumors were, respectively, classified as well-, moderately- and poorly-differentiated OSCC, 26 cases (7.5%) were microinvasive OSCC and 17 cases were OSCC variants. OSCC in males mostly affected the border of tongue, floor of mouth/ventral tongue and alveolar mucosa/gingival, while they were more frequent on the border of tongue, alveolar mucosa/gingival and buccal mucosa/buccal sulcus in females ($p=0.004$). Conclusions: The present data reflect the epidemiological characteristics of OSCC diagnosed in a public Oral Pathology laboratory in southeastern Brazil and have highlighted several differences in clinicopathological features when comparing male and female OSCC-affected patients.

Keywords: Squamous cell carcinoma. Oral cancer. Mouth. South America. Epidemiology.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80-90% of all malignant neoplasms of the oral cavity[11]. Although oral cancer incidence is highly variable worldwide, it is accepted that oral cavity ranges from the 6th to the 9th most common anatomical location for cancer, depending mostly on the country (and even specific region in some countries) and gender of the patients[11]. Despite this mean incidence, it can represent the most common location for cancer in some specific regions, especially in southeastern Asia[11]. Major etiological and predisposing factors for OSCC include mostly smoking and drinking habits, and ultraviolet radiation (specifically for lip cancer), but several other factors such as human papillomavirus (HPV) and *Candida* infections, nutritional deficiencies and genetic predisposition have been also associated[11,18]. OSCC is a disease of adults and elderly and its most common clinical aspect is an ulcerated lesion with necrotic central area surrounded by elevated rolled borders[28].

Annex 9

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Squamous Cell Carcinomas in Patients with Fanconi Anemia and Dyskeratosis Congenita: A Search for Human Papillomavirus

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Abstract

Patients with Fanconi anemia (FA) and dyskeratosis congenita (DC) are at high risk of head and neck and anogenital squamous cell carcinomas (SCC). In the general population, these sites (particularly oropharyngeal SCC) may be associated with infection with human papillomavirus (HPV). In FA and DC, however, the majority of HNSCC occur in the oral cavity. We investigated the HPV status of HNSCC and vulvar SCC from 9 patients with FA and 4 with DC using a very sensitive PCR assay, and found HPV16 DNA in only a single vulvar tumor from one FA patient, and in none of the HNSCC. These results suggest that HPV may not be the cause of SCC in patients with FA or DC, and that vaccination may not reduce the incidence of HNSCC in these patients.

Keywords

Fanconi anemia; dyskeratosis congenita; squamous cell carcinoma; human papillomavirus

Fanconi anemia (FA) is primarily an autosomal recessive DNA repair disorder characterized in many patients by congenital abnormalities, bone marrow failure, acute myeloid leukemia, and solid tumors. The relative risk of tumors is ~50-fold higher than in the general population, ~800-fold for head and neck squamous cell carcinomas (HNSCC) and ~3000-fold for vulvar cancer. Dyskeratosis congenita (DC) is a clinically similar inherited bone marrow failure syndrome, due to abnormalities in telomere biology, with a relative risk of ~8-fold for solid tumors, and ~1100-fold for HNSCC (1).

In the general population, almost all cervical, and up to half of vulvar, cancers are associated with human papillomavirus (HPV) infection, particularly the high-risk types HPV16 and HPV18 (2). In the U.S., HPV16 or 18 is also associated with ~25% of HNSCC, particularly >50% of oropharynx rather than oral cavity (<10%) (3–5). A possible common feature relating the specific gynecologic and head and neck sites is a tissue transformation zone (6). Gillison *et al.* indicated that a prophylactic vaccine has potential to prevent cervical as well

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There have been two contradictory reports with regard to the role of human papillomavirus (HPV) in head and neck and gynecologic squamous cell carcinoma (SCC) in patients with Fanconi anemia. We studied tumors from patients with Fanconi anemia or dyskeratosis congenita, and did not find evidence for causality by HPV. We predict that vaccination with current HPV vaccines may not reduce the incidence of SCC in these patients.

Annex 10

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Fanconi's anaemia and related bone marrow failure syndromes

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The inherited bone marrow (BM) failure syndromes are a heterogeneous group of disorders characterized by BM failure, usually in association with one or more somatic abnormalities. The BM failure may present at birth or at a variable time thereafter including in adulthood in some cases. Over the last decade, there have been significant advances in the genetics of these syndromes particularly Fanconi's anaemia (FA) and dyskeratosis congenita (DC). These advances are beginning to provide a better understanding of normal haemopoiesis and of the pathophysiology of some cases of idiopathic aplastic anaemia (AA). They have also provided important insights into some aspects of DNA repair (FA/BRCA pathway) and telomere maintenance (DC-related genes), two pathways critical in the maintenance of genomic stability. These advances are already facilitating better diagnosis of patients with these disorders. It is hoped that they will also form the basis for developing new treatments.

Keywords: bone marrow failure; DNA repair; dyskeratosis congenita; Fanconi's anaemia; telomerase

Introduction

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Bone marrow (BM) failure syndromes are characterized by the inability of the BM to produce an adequate number of circulating blood cells. They are associated with significant mortality due to bleeding or infection. The vast majority (~70%) of these cases are classified as idiopathic as their primary aetiology remains unexplained (Table 1). In a subset (~15% of cases), a drug or infection can be identified that precipitates the BM failure/aplastic anaemia (AA), although it is not clear why only some individuals are susceptible. In ~10–20% of patients (the focus of this review), the disease is constitutional/inherited, where the disease is familial and/or presents with one or more other somatic abnormalities [1] (Table 2). The features of some of the classical syndromes are summarized in Table 3. The precise incidence/prevalence of these remains

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Annex 11

Oncology Research and Treatment

Editorial

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Head and Neck Cancer – New Insights into a Heterogeneous Disease

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The large majority of head and neck cancers are squamous cell carcinomas (HNSCC) that arise at the mucosal linings of the upper aerodigestive tract, suggesting a homogeneous disease. However, HNSCC are remarkably heterogeneous. They include several subclassifications in relation to anatomic location, etiology, and molecular findings. In Germany, 4,532 and 12,992 new HNSCC cases in women and men, respectively, were counted in 2013. These HNSCC developed at different locations, i.e., oral cavity, pharynx, and larynx [1]. The 5-year overall survival for all locations is still poor and is estimated at 51% for men and 61% for women in Germany. Historically, HNSCC have been linked to tobacco and alcohol consumption. Over the past decades, the overall incidence of HNSCC has been decreasing. In contrast to that, the incidence of oropharyngeal cancers is rising, which is related to a strong increase of human papillomavirus(HPV)-associated cancers in western countries. The reason for this increase might be a sexual transmission of HPV decades before cancer development. These cancers are now identified as a distinct tumor entity, with many differences in comparison to HPV-negative HNSCC regarding their cellular, biological, and clinical characteristics. The identification of HPV-related HNSCC by molecular biology and recently omics approaches has disclosed the heterogeneity of HNSCC and thus has contributed to a new classification of these tumors. Strikingly, it became evident that HPV-related oropharyngeal cancers showed a significantly better response to established treatment modalities resulting in an improved overall survival. Therefore, the HPV status can be regarded as one of the most accurate prognostic biomarkers in HNSCC, and p16-immunostaining became a reliable surrogate marker for the HPV status. In line with this, the latest version of the 'AJCC Cancer Staging Manual' [2] includes new staging rules for HPV-positive cancer.

Despite improvements in diagnosis and therapy, morbidity and mortality have remained high and appropriate treatment of patients still is a major challenge. Most cases are diagnosed with locally advanced disease, and multimodal treatment consists of concomitant chemoradiotherapy or surgery followed by risk-adapted radio(chemo)therapy. Although it is conceivable that distinct patient subgroups might benefit from treatment de-escalation (e.g., HPV-related HNSCC) or targeted therapy, effective personalized treatment options are not yet in clinical use for HNSCC.

The aim of this issue of Oncology Research and Treatment is to highlight new insights into the biology of HNSCC, to discuss their possible implications for established treatment modalities, to point to future therapeutic developments, and to call attention to open questions which should be addressed by clinical trials. Jou and Hess [3] focused on recent data derived from integrative genomics analysis and multi-scale modeling approaches regarding the biological and clinical diversity of HNSCC. Wagner et al. [4] gave an overview of the newly recognized tumor entity 'HPV-related HNSCC'. Semrau [5] discussed modern treatment technologies and new developments of radiation oncology. In their original article, Wuerdemann et al. [6] compared the outcome of patient cohorts with HPV-related and -unrelated oropharyngeal cancers treated with primary surgery. Schuler at al. [7] outlined novel treatment options for HNSCC, especially in the field of immunotherapy.

Disclosure Statement

The author declares no conflict of interest.

Annex 12

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Oral epithelial dysplasia: Classifications and clinical relevance in risk assessment of oral potentially malignant disorders

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ABSTRACT

After more than a decade, the World Health Organization (WHO) published the revised grading system for oral epithelial dysplasia in 2017. The revised classification has changes reflecting our evolution of understanding of the dysplastic process. Although the WHO 2017 three-tier grading system is the gold standard for histological diagnosis of oral potentially malignant disorders, it has certain limitations. Suggestions to overcome these limitations include the use of clinical determinants and molecular markers to supplement the grading system. It has also been suggested that a two-tier system may be more reproducible and clinically translatable for better management. These advances in the understanding of epithelial dysplasia are very important globally and for us in the Indian subcontinent, given the prevalence of habits (tobacco/areca nut) and burden of oral cancer in this part of the world. The following review traces the evolution of the grading system of dysplasia, its relevance and clinical utility.

Keywords: Cancer, epithelial, grading, histopathology, oral dysplasia, potentially malignant, precancer

INTRODUCTION

Oral cancer is the 11th most common cancer in the world, with an estimated 300,000 new cases and 145,000 deaths in 2012.[1,2] In India, 20/100,000 population are affected by oral cancer, which accounts for around 30% of all types of cancer.[3] Most cases of oral cancer are associated with habits (tobacco/areca nut) and are preceded by asymptomatic clinical lesions collectively referred to as oral potentially malignant disorder (OPMD).[4] OPMDs include leukoplakia, erythroplakia, reverse smoker's palate, erosive lichen planus, oral submucous fibrosis, lupus erythematosus and actinic keratosis.[5,6]

The worldwide prevalence rate of OPMDs ranges from 1% to 5%. Estimates provided by individual studies vary depending on the country, the population under investigation, the pattern of tobacco use and the clinical definition used for leukoplakia. One of the early epidemiological studies assessing the risk of OPMDs in India reported that 80% of oral cancers were preceded by OPMDs.[7] The global prevalence (1986 to 2002) of leukoplakia was estimated to be 1.49% to 2.60%.[8,9] The prevalence of erythroplakia among populations in India and Malaysia is estimated to be 0.02%.[10,11,12,13] Reported incidence rates of OPMDs in the Indian subcontinent have ranged between 0.6/1000 and 30.2/1000 with a regional variation in prevalence from 0.2% in Bihar state in the north to 4.9% in Andhra Pradesh in the east. This difference in the range is attributed to the prevalence and type of tobacco use.[14,15]

OPMD is a clinical diagnosis for which the histological diagnosis may be hyperplasia, hyperkeratosis, oral epithelial dysplasia (OED) or oral squamous cell carcinoma (OSCC). OED is characterized by cytological and architectural alterations reflecting the loss of normal maturation and stratification pattern of surface epithelium.[16,17]

This review aims to discuss the different classifications of OED, their limitations and relevance in determining the risk of malignant transformation. Understanding this relation between the clinical diagnosis of OPMD and histopathological diagnosis of OED is essential for early diagnosis and clinical management.

MALIGNANT TRANSFORMATION OF ORAL POTENTIALLY MALIGNANT DISORDER/ORAL EPITHELIAL DYSPLASIA TO ORAL SQUAMOUS CELL CARCINOMA

Oral leukoplakia is reported to carry up to 2-fold increased risk of developing oral cancer depending on the site and habits.[18] The MTR of leukoplakia varies from 1.4% to 7%.[19,20,21,22] A recent systematic review of the observational studies in OPMD reported an MTR of 0.13%–34% across 24 studies.[23] MTR of epithelial dysplasia ranges between 1.4% and 36% (Ho *et al.*: 1.4%–7.62% per year; Lumerman *et al.* 6.6%–36%).[16,21] The variation in rates between studies is attributed to differences in follow-up times, study group definition and selection and tobacco habits.[24]

Oral epithelial dysplasia – Terminology

The word dysplasia denotes abnormal growth.[25] The dysplastic alterations may revert to normal when the underlying inciting stimulus is removed. Dysplastic features in stratified squamous epithelium are characterized by cellular atypia and loss of normal maturation and stratification.[17] The World Health Organization (WHO) monograph on head and neck tumors (2005) uses the term "epithelial precursor lesions" and defines it as "altered epithelium with an increased likelihood for progression to squamous cell carcinoma."[26]

Annex 13

PRIMER

Head and neck squamous cell carcinoma

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Abstract | Most head and neck cancers are derived from the mucosal epithelium in the oral cavity, pharynx and larynx and are known collectively as head and neck squamous cell carcinoma (HNSCC). Oral cavity and larynx cancers are generally associated with tobacco consumption, alcohol abuse or both, whereas pharynx cancers are increasingly attributed to infection with human papillomavirus (HPV), primarily HPV-16. Thus, HNSCC can be separated into HPV-negative and HPV-positive HNSCC. Despite evidence of histological progression from cellular atypia through various degrees of dysplasia, ultimately leading to invasive HNSCC, most patients are diagnosed with late-stage HNSCC without a clinically evident antecedent pre-malignant lesion. Traditional staging of HNSCC using the tumour–node–metastasis system has been supplemented by the 2017 AJCC/UICC staging system, which incorporates additional information relevant to HPV-positive disease. Treatment is generally multimodal, consisting of surgery followed by chemoradiotherapy (CRT) for oral cavity cancers and primary CRT for pharynx and larynx cancers. The EGFR monoclonal antibody cetuximab is generally used in combination with radiation in HPV-negative HNSCC where comorbidities prevent the use of cytotoxic chemotherapy. The FDA approved the immune checkpoint inhibitors pembrolizumab and nivolumab for treatment of recurrent or metastatic HNSCC and pembrolizumab as primary treatment for unresectable disease. Elucidation of the molecular genetic landscape of HNSCC over the past decade has revealed new opportunities for therapeutic intervention. Ongoing efforts aim to integrate our understanding of HNSCC biology and immunobiology to identify predictive biomarkers that will enable delivery of the most effective, least-toxic therapies.

Head and neck squamous cell carcinomas (HNSCCs) develop from the mucosal epithelium in the oral cavity, pharynx and larynx and are the most common malignancies that arise in the head and neck (FIG. 1). The burden of HNSCC varies across countries/regions and has generally been correlated with exposure to tobacco-derived carcinogens, excessive alcohol consumption, or both. Increasingly, tumours that arise in the oropharynx are linked to prior infection with oncogenic strains of human papillomavirus (HPV), primarily HPV-16, and, to a lesser extent, HPV-18 and other strains[1–3]. As the most common oncogenic HPVs, HPV-16 and HPV-18, are covered by FDA-approved HPV vaccines, it is feasible that HPV-positive HNSCC could be prevented by successful vaccination campaigns worldwide. HNSCCs of the oral cavity and larynx are still primarily associated with smoking and are now collectively referred to as HPV-negative HNSCC. No screening strategy has proved to be effective, and careful physical examination remains the primary approach for early detection.

Although a proportion of oral pre-malignant lesions (OPLs), which present as leukoplakia (white patches) or erythroplakia (red patches), progress to invasive cancer, the majority of patients present with advanced-stage HNSCC without a clinical history of a pre-malignancy. HNSCC of the oral cavity is generally treated with surgical resection, followed by adjuvant radiation or chemotherapy plus radiation (known as chemoradiation or chemoradiotherapy (CRT)) depending on the disease stage. CRT has been the primary approach to treat cancers that arise in the pharynx or larynx. HPV-positive HNSCC generally has a more favourable prognosis than HPV-negative HNSCC, and ongoing studies are testing the efficacy of therapeutic dose reduction (of both radiation and chemotherapy) in HPV-positive disease treatment. With the exception of early-stage oral cavity cancers (which are treated with surgery alone) or larynx cancers (which are amenable to surgery or radiation alone), treatment of the majority of patients with HNSCC requires multimodality approaches and

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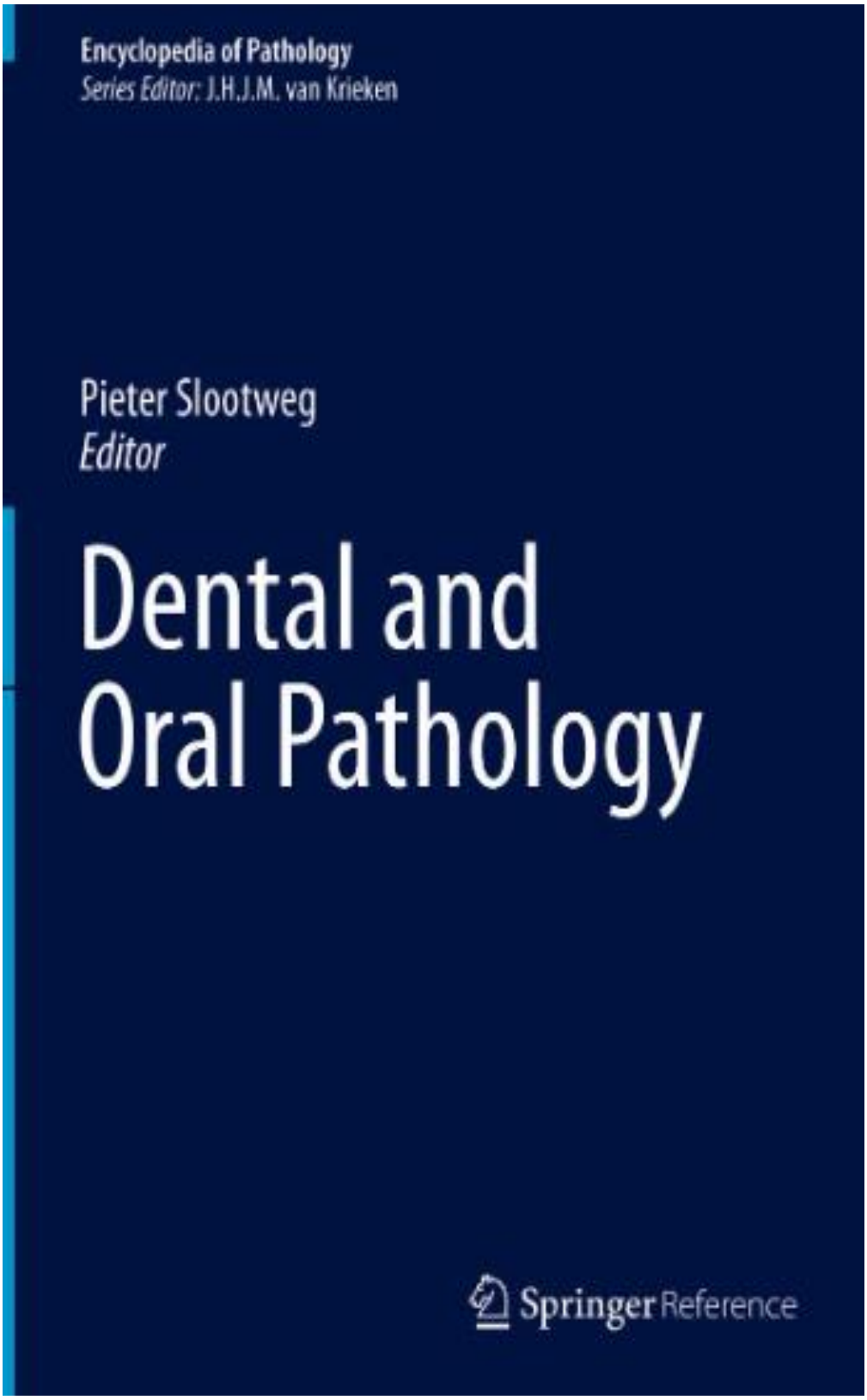
Annex 14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Annex 15

Special Topic: Potentially premalignant oral epithelial lesions (PPOEL)

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Clinical features and presentation of oral potentially malignant disorders

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Oral potentially malignant disorders (OPMDs) are conditions that precede the onset of invasive cancers of the oral cavity. The term embraces precancerous lesions and conditions referred to in earlier World Health Organization (WHO) definitions. Leukoplakia is the most common OPMD; erythroplakia, although rare, is more serious. Several variants of leukoplakia are recognized, and clinical subtyping may help determine the prognosis to a limited extent. Biopsy is essential to confirm the provisional clinical diagnosis, and timely referral to a specialist is indicated. Certain OPMDs, such as oral submucous fibrosis, are encountered particularly in population groups from Asia with specific lifestyle habits. This review provides clinical descriptions of the wide range of potentially malignant disorders encountered in the oral cavity as a prelude to the topics discussed in this focus issue. (Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:582-590)

A range of oral mucosal disorders with an increased risk of malignancy has been described in the literature, and these disorders are listed under the umbrella term *oral potentially malignant disorders* (OPMDs). The spectrum of OPMDs include oral leukoplakia, erythroplakia, erythroleukoplakia, oral submucous fibrosis (OSF), palatal lesions in reverse smokers, oral lichen planus, oral lichenoid reactions, graft-versus-host disease (GvHD), oral lupus erythematosus, and some hereditary conditions, such as dyskeratosis congenita and epidemolysis bullosa. Actinic cheilitis of the lower lip is also associated with an increased risk of lip cancer. The majority of these disorders may be asymptomatic in the early stages of their evolution and may be detected by dental practitioners on routine oral examination. It is essential, therefore, that health professionals are knowledgeable about the clinical features and diagnostic aspects of OPMDs to further investigate and, where appropriate, make referrals to specialists for treatment.

It has been known for over a century that oral cancer may develop in areas of pre-existing mucosal pathology in the oral cavity. In the literature, these lesions have been referred to by such terms as "pre-cancer," "precancerous/premalignant lesions," and "intraepithelial neoplasia." A more precise term, "potentially malignant disorders," was adopted by the WHO Collaborating Center because there is no certainty that all precancerous lesions will eventually develop into oral cancer.[1] The term also embraces precancerous lesions and conditions that were included in the previous WHO classifications.[2] In this focus issue, it is proposed to introduce a new term "potentially premalignant oral epithelial lesions [PPOELs]" (see Editorial). The underlying concept is that these lesions have the potential to become malignant, so in their current state, that is, before malignant transformation, they are still (potentially) premalignant.

Since the 2007 publication characterizing OPMDs,[1] new evidence has emerged to support the inclusion of oral lichenoid lesions and oral lesions of GvHD as potentially malignant disorders. Brief descriptions of these conditions are also included here.

LEUKOPLAKIA

To precisely diagnose oral leukoplakia, it is important to consider its definition.[3] Historically, the term *leukoplakia* was used clinically to denote any adherent white patch or plaque (keratosis). Over several decades, clinicians realized that all white patches arising in the oral cavity should not be labeled oral leukoplakia. Several definitions of oral leukoplakia have been put forth in the past few decades. The most recent definition in use refers to leukoplakia as "predominantly white plaques of questionable risk, having excluded (other) known diseases or disorders that carry no increased risk for cancer."[3] Examples of other benign white lesions that should be excluded to arrive at the diagnosis of oral leukoplakia are frictional keratosis (cheek biting), alveolar ridge keratosis, leukoedema, white sponge nevus, and Fordyce granules, which are usually buff colored.

Oral leukoplakia may be asymptomatic or display a benign clinical appearance making it difficult for the

Statement of Clinical Relevance

Potentially malignant disorders (e.g., leukoplakia and erythroplakia) encountered during a routine oral mucosal examination represent the most significant clinical findings, except in the case of a malignancy, in a dental practice. Early diagnosis, referral to a specialist, and appropriate intervention may reduce the rate of progression of these conditions to invasive cancer.

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Annex 16

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TP53 Mutations in Head and Neck Squamous Cell Carcinoma and Their Impact on Disease Progression and Treatment Response

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Abstract

Recent studies describing the mutational landscape of head and neck squamous cell carcinoma (HNSCC) on a genomic scale by our group and others, including The Cancer Genome Atlas, have provided unprecedented perspective for understanding the molecular pathogenesis of HNSCC progression and response to treatment. These studies confirmed that mutations of the *TP53* tumor suppressor gene were the most frequent of all somatic genomic alterations in HNSCC, alluding to the importance of the *TP53* gene in suppressing the development and progression of this disease. Clinically, *TP53* mutations are significantly associated with short survival time and tumor resistance to radiotherapy and chemotherapy in HNSCC patients, which makes the *TP53* mutation status a potentially useful molecular factor for risk stratification and predictor of clinical response in these patients. In addition to loss of wild-type p53 function and the dominant-negative effect on the remaining wild-type p53, some p53 mutants often gain oncogenic functions to promote tumorigenesis and progression. Different p53 mutants may possess different gain-of-function properties. Therefore, mutant p53 is not just one protein but actually a variety of proteins that contribute to an exceptionally vast network of tumor-promoting processes. Herein we review the most up-to-date information about *TP53* mutations available via The Cancer Genome Atlas-based analysis of HNSCC and discuss our current understanding of the potential tumor-suppressive role of p53, focusing on gain-of-function activities of p53 mutations. We also summarize our knowledge regarding use of the *TP53* mutation status as a potential evaluation or stratification biomarker for prognosis and a predictor of clinical response to radiotherapy and chemotherapy in HNSCC patients. Finally, we discuss possible strategies for targeting HNSCCs bearing *TP53* mutations.

Keywords

Head and neck squamous cell carcinomas; HNSCC; p53; P53 mutation; Gain-of-function

Head and neck squamous cell carcinoma (HNSCC) is the major form of head and neck cancer and the sixth most common cancer worldwide. The global number of new HNSCC cases annually is estimated to be more than 600,000, with about 355,000 deaths observed

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Annex 17

The early diagnosis of small-sized oral squamous cell carcinoma: a challenge for the clinician. Report of two cases and literature review

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KEYWORDS

Early diagnosis, Oral cancer, Squamous Cell Carcinoma.

ABSTRACT

Aim Oral Squamous Cell Carcinoma (SCC) has different clinical presentations, depending on its location, evolution time, precancerous lesions and risk factors. It is apparent that the high mortality due to this malignancy is caused by its detection in advanced stages, despite easy accessibility of the oral cavity for routine clinical examination. This is mainly because these lesions are in most cases asymptomatic in the early stages (pain appears because of nerve/muscles damage or local traumas) and their size does not increase for a long time. The stage of advancement of oral SCC at the time of diagnosis is the most important prognostic factor, even if the course of oral SCC is unpredictable. The purpose of this study is to stress the issue that small-sized oral squamous cell carcinomas are very difficult to distinguish from benign simulants and premalignant lesions, and careful intra and extra-oral examinations should be performed, in order to decrease the risk of malignant transformation.

Case report Two cases are reported, in order show how difficult it can be to establish a differential diagnosis and the importance of biopsy in case of doubt.

Introduction

Oral cancer refers to cancer occurring between the vermilion border of the lips and the junction of the hard and soft palates or the posterior third of the tongue.

Oral squamous cell carcinoma (SCC) is nowadays the sixth most common cancer in the world with two-thirds of cases localized in developing countries (1) and with an incidence of about 275,000 new cases every year, it is an increasing healthy emergency worldwide with lethal effects in over 50% of the cases diagnosed. It affects more frequently males than females (M:F=1,5:1), probably because of the major risk factors exposure.

Oral SCC has different clinical presentations, depending on its location, evolution time, precancerous lesions and risk factors. In most cases it appears as a leukoplastic, erythroplastic or leukoerythroplastic lesion, but it could also resemble a verrucous leukoplakia or may present superficial eroded areas. It sometimes appears as a necrotic ulcer, with irregular margins and raised indurated borders, and some other times it is exophytic and smooth. When increased consistency on palpation is observed, chorion infiltration must be suspected.

It is apparent that the high mortality due to this malignancy is caused by a detection of the disease in advanced stages, despite the easy accessibility of the oral cavity for regular clinical examination, mainly because these lesions are in most cases asymptomatic in the early stages (pain appears because of nerve/muscles damage or local traumas) and their size does not increase for a long time. As result many oral SCCs are diagnosed at an advanced stage and the prognosis is therefore poorer than it could have been if diagnosed earlier (2).

In the recent literature, the role of early oral SCC diagnosis in avoiding more extensive and costly treatments, greater morbidity and poorer survival has been widely highlighted (3, 4, 5). Onizawa et al. (6) reported that extended oral SCC are diagnosed more easily, while there is a later referral of patients affected by T1 tumors, because of difficulties in obtaining an early clear clinical diagnosis of small mucosal neoplastic lesions.

In clinical practice the tumor stage is defined by TNM staging system of oral cavity cancer (designed by the American Joint Committee on Cancer) and tumor dimensions (T) are divided into 4 categories (T1: ≤2

Annex 18

Journal section: Oral Medicine and Pathology
Publication Types: Review

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Techniques for early diagnosis of oral squamous cell carcinoma: Systematic review

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Abstract

Background and objectives: The diagnosis of early oral potentially malignant disorders (OPMD) and oral squamous cell carcinoma (OSCC) is of paramount clinical importance given the mortality rate of late stage disease. The aim of this study is to review the literature to assess the current situation and progress in this area.

Material and Methods: A search in Cochrane and PubMed (January 2006 to December 2013) has been used with the key words *"squamous cell carcinoma", "early diagnosis" "oral cavity", "Potentially Malignant Disorders" y "premalignant lesions"*. The inclusion criteria were the use of techniques for early diagnosis of OSCC and OPMD, 7 years aged articles and publications written in English, French or Spanish. The exclusion criteria were case reports and studies in other languages.

Results: Out of the 89 studies obtained initially from the search 60 articles were selected to be included in the systematic review: 1 metaanalysis, 17 systematic reviews, 35 prospective studies, 5 retrospective studies, 1 consensus and 1 semi-structured interviews.

Conclusions: The best diagnostic technique is that which we have sufficient experience and training. Definitely tissue biopsy and histopathological examination should remain the gold standard for oral cancer diagnose. In this systematic review it has not been found sufficient scientific evidence on the majority of proposed techniques for early diagnosis of OSCC, therefore more extensive and exhaustive studies are needed.

Key words: *Squamous cell carcinoma, early diagnosis, oral cavity, potentially malignant disorders, premalignant lesions.*

Annex 19

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Guidelines

Guidelines for the Surgical Management of Oral Cancer: Korean Society of Thyroid-Head and Neck Surgery

Korean Society of Thyroid-Head and Neck Surgery Guideline Task Force

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Korean Society of Thyroid-Head and Neck Surgery appointed a Task Force to provide guidance on the implementation of a surgical treatment of oral cancer. MEDLINE databases were searched for articles on subjects related to "surgical management of oral cancer" published in English. Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies. The quality of evidence was rated with use RoBANS (Risk of Bias Assessment Tool for Nonrandomized Studies) and AMSTAR (A Measurement Tool to Assess the Methodological Quality of Systematic Reviews). Evidence-based recommendations for practice were ranked according to the American College of Physicians grading system. Additional directives are provided as expert opinions and Delphi questionnaire when insufficient evidence existed. The Committee developed 68 evidence-based recommendations in 34 categories intended to assist clinicians and patients and counselors, and health policy-makers. Proper surgical treatment selection for oral cancer, which is directed by patient- and subsite-specific factors, remains the greatest predictor of successful treatment outcomes. These guidelines are intended for use in conjunction with the individual patient's treatment goals.

Keywords. *Mouth Neoplasms; Squamous Cell Carcinoma; Surgery; Guideline; Republic of Korea*

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Annex 20

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Oral cancer: Current role of radiotherapy and chemotherapy

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Abstract

The term oral cavity cancer (OSCC) constitutes cancers of the mucosal surfaces of the lips, floor of mouth, oral tongue, buccal mucosa, lower and upper gingiva, hard palate and retromolar trigone. Treatment approaches for OSCC include single management with surgery, radiotherapy [external beam radiotherapy (EBRT) and/or brachytherapy], as well as adjuvant systemic therapy (chemotherapy and/or target agents); various combinations of these modalities may also be used depending on the disease presentation and pathological findings. The selection of sole or combined modality is based on various considerations that include disease control probability, the anticipated functional and cosmetic outcomes, tumor resectability, patient general condition, and availability of resources and expertise. For resectable OSCC, the mainstay of treatment is surgery, though same practitioners may advocate for the use of radiotherapy alone in selected "early" disease presentations or combined with chemotherapy in more locally advanced stage disease. In general, the latter is more commonly reserved for cases where surgery may be problematic. Thus, primary radiotherapy ± chemotherapy is usually reserved for patients unable to tolerate or who are otherwise unsuited for surgery. On the other hand, brachytherapy may be considered as a sole modality for early small primary tumor. It also has a role as an adjuvant to surgery in the setting of inadequate pathologically assessed resection margins, as does postoperative external beam radiotherapy ± chemotherapy, which is usually reserved for those with unfavorable pathological features. Brachytherapy can also be especially useful in the re-irradiation setting for persistent or recurrent disease or for a second primary arising within a previous radiation field. Biological agents targeting the epithelial growth factor receptor (EGFR) have emerged as a potential modality in combination with radiotherapy or chemoradiotherpy and are currently under evaluation in clinical trials.

Key words: *Radiotherapy, chemoradiotherapy, oral cavity cancer, treatment.*

Annex 21

Docetaxel in the treatment of squamous cell carcinoma of the head and neck

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Abstract: Squamous cell carcinoma of the head and neck (SCCHN) presents at a locally advanced (LA) stage in many patients. Chemotherapy has been successfully integrated into first-line treatment programs, either during or prior to radiotherapy (RT) – the cornerstone modality for local disease control of inoperable disease or when organ preservation is desired. Concomitant chemoradiotherapy (CCRT) provides an absolute survival benefit when compared with other types of locoregional therapy that exclude chemotherapy. Nonetheless, distant metastases still represent the most common cause of treatment failure. Consequently, adding induction chemotherapy (ICT) to definitive non-surgical local therapies with a curative intent has been vigorously explored in LA SCCHN. Recently, it has been shown that ICT using the combination of the taxane docetaxel with cisplatin–5-fluorouracil provides significant survival benefit over cisplatin–5-FU, when used before either definitive RT (TAX323 trial) or carboplatin-based CCRT (TAX324 trial). Docetaxel is also being investigated in metastatic or recurrent (M/R) disease, with promising initial results. It is very likely that the future management strategies of SCCHN will incorporate biologic agents as an add-on to docetaxel-containing schemas, administered either as ICT prior to CCRT in the LA setting or for the management of M/R disease.

Keywords: chemoradiotherapy, chemotherapy, docetaxel, head and neck carcinoma, induction, locally advanced, taxane

Introduction

With a global annual incidence of approximately 500,000 cases, squamous cell carcinoma of the head and neck (SCCHN) – which includes carcinomas of the oral cavity, floor of mouth, tongue, tonsils and juxtatonsillar fossae, larynx, and pharynx (oropharynx, epipharynx, and hypopharynx) – is the fifth most common cancer worldwide (Parkin et al 2005). Even in the US, where the incidence of SCCHN is relatively low, this type of cancer accounts for up to 3% of all malignant neoplasms (National Cancer Institute 2008a). SCCHN, an aggressive epithelial malignancy, has historically been associated with poor prognosis. Moreover, as the majority of SCCHN cases are associated with tobacco consumption and alcohol abuse, many patients present with notable comorbidities linked to lifestyle, a factor that limits the delivery of effective antitumor therapy. Indeed, up until the mid-1990s, 5-year survival rates had been reported to be as low as 30% or below for stage IVa/b (M0) disease (Vokes et al 1993), and 40% for stage III disease (Laramore et al 1992). However, over the last decade, overall mortality rates for cancers of the oral cavity, pharynx, and larynx have been modestly decreasing in the general population (National Cancer Institute 2008b, c). This is the case not only in low stage cancers, an effect mostly attributable to progressively earlier detection of curable tumors over time, but also in locally advanced (LA) disease. The latter effect is due to an interplay of numerous factors, including the continuous advances in oncologic supportive care, refinement of the manner of administration of complex chemotherapy regimens, technical advances in the delivery

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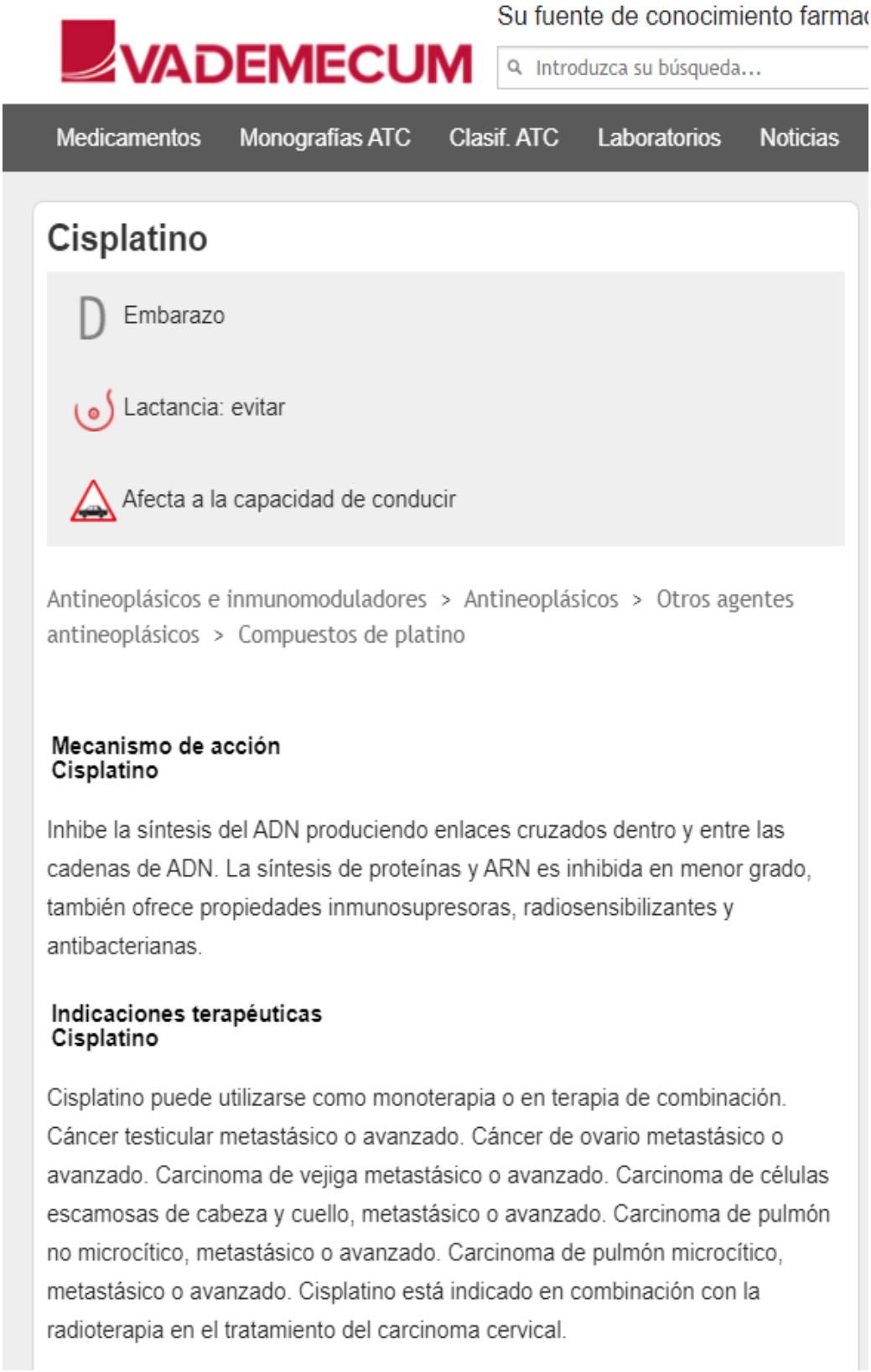
Annex 22


VADEMECUM
Su fuente de conocimiento farmac
Introduzca su búsqueda...
Medicamentos
Monografías ATC
Clasif. ATC
Laboratorios
Noticias
Cisplatino
D Embarazo
Lactancia: evitar
Afecta a la capacidad de conducir
Antineoplásicos e inmunomoduladores > Antineoplásicos > Otros agentes antineoplásicos > Compuestos de platino
Mecanismo de acción
Cisplatino
Inhibe la síntesis del ADN produciendo enlaces cruzados dentro y entre las cadenas de ADN. La síntesis de proteínas y ARN es inhibida en menor grado, también ofrece propiedades inmunosupresoras, radiosensibilizantes y antibacterianas.
Indicaciones terapéuticas
Cisplatino
Cisplatino puede utilizarse como monoterapia o en terapia de combinación. Cáncer testicular metastásico o avanzado. Cáncer de ovario metastásico o avanzado. Carcinoma de vejiga metastásico o avanzado. Carcinoma de células escamosas de cabeza y cuello, metastásico o avanzado. Carcinoma de pulmón no microcítico, metastásico o avanzado. Carcinoma de pulmón microcítico, metastásico o avanzado. Cisplatino está indicado en combinación con la radioterapia en el tratamiento del carcinoma cervical.

Annex 23

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Cisplatin in cancer therapy: molecular mechanisms of action

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Abstract

Cisplatin, cisplatinum, or *cis*-diamminedichloroplatinum (II), is a well-known chemotherapeutic drug. It has been used for treatment of numerous human cancers including bladder, head and neck, lung, ovarian, and testicular cancers. It is effective against various types of cancers, including carcinomas, germ cell tumors, lymphomas, and sarcomas. Its mode of action has been linked to its ability to crosslink with the purine bases on the DNA; interfering with DNA repair mechanisms, causing DNA damage, and subsequently inducing apoptosis in cancer cells. However, because of drug resistance and numerous undesirable side effects such as severe kidney problems, allergic reactions, decrease immunity to infections, gastrointestinal disorders, hemorrhage, and hearing loss especially in younger patients, other platinum-containing anti-cancer drugs such as carboplatin, oxaliplatin and others, have also been used. Furthermore, combination therapies of cisplatin with other drugs have been highly considered to overcome drug-resistance and reduce toxicity. This comprehensive review highlights the physicochemical properties of cisplatin and related platinum-based drugs, and discusses its uses (either alone or in combination with other drugs) for the treatment of various human cancers. A special attention is given to its molecular mechanisms of action, and its undesirable side effects.

Keywords

Cisplatin; Platinum-based drugs; Mechanisms of action; Cancer treatment

1. Introduction

Cisplatin (CAS No. 15663-27-1, MF-$Cl_2H_6N_2Pt$; NCF-119875), cisplatinum, also called *cis*-diamminedichloroplatinum(II), is a metallic (platinum) coordination compound with a square planar geometry. It is a white or deep yellow to yellow-orange crystalline powder at room temperature. It is slightly soluble in water and soluble in dimethylprimanide and *N,N*-dimethylformamide. Cisplatin is stable under normal temperatures and pressures, but may

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Annex 24

5-FLUOROURACIL: MECHANISMS OF ACTION AND CLINICAL STRATEGIES

Daniel B. Longley, D. Paul Harkin and Patrick G. Johnston

5-Fluorouracil (5-FU) is widely used in the treatment of cancer. Over the past 20 years, increased understanding of the mechanism of action of 5-FU has led to the development of strategies that increase its anticancer activity. Despite these advances, drug resistance remains a significant limitation to the clinical use of 5-FU. Emerging technologies, such as DNA microarray profiling, have the potential to identify novel genes that are involved in mediating resistance to 5-FU. Such target genes might prove to be therapeutically valuable as new targets for chemotherapy, or as predictive biomarkers of response to 5-FU-based chemotherapy.

FLUOROPYRIMIDINES
Antimetabolite drugs such as 5-fluorouracil that are fluorinated derivatives of pyrimidines.

IRINOTECAN
An anticancer drug that inhibits DNA topoisomerase I. It is used in the treatment of advanced colorectal cancer.

OXALIPLATIN
A platinum-based DNA-damaging anticancer drug that is used in the treatment of advanced colorectal cancer.

DNA MICROARRAY
A technique that allows global changes in gene expression to be assessed.

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Antimetabolite drugs work by inhibiting essential biosynthetic processes, or by being incorporated into macromolecules, such as DNA and RNA, and inhibiting their normal function. The fluoropyrimidine 5-fluorouracil (5-FU) does both. FLUOROPYRIMIDINES were developed in the 1950s following the observation that rat hepatomas used the pyrimidine uracil — one of the four bases found in RNA — more rapidly than normal tissues, indicating that uracil metabolism was a potential target for antimetabolite chemotherapy[1]. The mechanism of cytotoxicity of 5-FU has been ascribed to the misincorporation of fluoronucleotides into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme thymidylate synthase (TS).

5-FU is widely used in the treatment of a range of cancers, including colorectal and breast cancers, and cancers of the aerodigestive tract. Although 5-FU in combination with other chemotherapeutic agents improves response rates and survival in breast and head and neck cancers, it is in colorectal cancer that 5-FU has had the greatest impact. 5-FU-based chemotherapy improves overall and disease-free survival of patients with resected stage III colorectal cancer[2]. Nonetheless, response rates for 5-FU-based chemotherapy as a first-line treatment for advanced colorectal cancer are only 10–15% (REF. 3). The combination of 5-FU with newer chemotherapies such as IRINOTECAN and OXALIPLATIN has improved the response rates for advanced colorectal cancer to 40–50% (REFS 4,5). However, despite these improvements, new therapeutic strategies are urgently needed.

Understanding the mechanisms by which 5-FU causes cell death and by which tumours become resistant to 5-FU is an essential step towards predicting or overcoming that resistance. So, what do we know about the mechanism of action of 5-FU and what strategies have been used to enhance its activity? DNA MICROARRAY technology has the potential to identify novel genes that have key roles in mediating resistance to 5-FU-based chemotherapy. Such genes might be therapeutically valuable as PREDICTIVE BIOMARKERS of 5-FU chemosensitivity and/or provide new molecular targets that overcome drug resistance. How have pre-clinical studies impacted on the clinical use of 5-FU and how might DNA microarray profiling affect its future clinical application?

Mechanism of action of 5-FU

5-FU is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen (FIG. 1). It rapidly enters the cell using the same facilitated transport mechanism as uracil[6]. 5-FU is converted intracellularly to several active metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) (FIG. 1) — these active metabolites disrupt RNA synthesis and the action of TS. The rate-limiting enzyme in 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD), which converts 5-FU to dihydrofluorouracil (DHFU)[7]. More than 80% of administered 5-FU is normally catabolized primarily in the liver, where DPD is abundantly expressed[7].

Annex 25

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A DISCUSSION ON CHEMOPREVENTION OF ORAL CANCER BY SELECTIVE CYCLOOXYGENASE-2 (COX-2) INHIBITORS

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Oral cancers are potentially fatal diseases, have a high mortality rate and because of this it is highly challenging for the clinicians. Cyclooxygenase (COX), the key enzyme in prostaglandin cascade, is expressed in two isoform: the constitutive COX-1 and inducible COX-2. COX-2 expression extensively up regulated in oral cancer, oral premalignant lesion and seemed to be enhanced specifically in high-risk oral lesions. In recent studies it has been found that Zinc regulates COX-2 expression in vivo, in animal model may lead to prevention or therapeutic possibilities for upper aerodigestive tract cancer. The data in recent literatures strongly indicate that COX-2 expression is extensively up-regulated in oral cancer and it is believed that COX-2 inhibition strongly suppressed the oral lesion therefore; selective COX-2 inhibitor should be investigated as new chemopreventive agents for patient who are at high risk for developing oral cancer.

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Keywords: Cyclooxygenase, Selective COX-2 inhibitors, Oral cancer, Chemoprevention

1. Introduction

Oral malignancy, (OSCC), is a major health problem worldwide (400,000 cases per year) [1]. Sixteen million new cases of cancer are estimated every year by 2020 [2]. Its incidence and mortality in the United States is increasing in recent years especially among young males [3] and approximately 28260 new cases and 7230 deaths were expected in 2004 [4]. The five year survival rate remains about 50% inspite of advances in chemotherapy and Radiotherapy. The surviving patients are also left with severe functional compromise and may develop a second cancer within a few years [4, 5]. It is important to recognize preventive strategies for this disease. The advent of genomics has provided insight into the mechanisms by which normal cells become cancerous [6].

The arachidonic acid metabolism has been suggested to play an important role in oral carcinogenesis [7]. Cyclooxygenase (COX), the key enzyme required for the conversion of arachidonic acid to prostaglandins was first identified over 20 years ago. Drugs like aspirin that inhibit cyclooxygenase activity have been available to the public for about 100 years. Two-cyclooxygenase isoform have been identified and are referred to as COX-1 and COX-2. Under many circumstances the COX-1 enzyme is produced constitutively (i.e. gastric mucosa) where as COX-2 are inducible (i.e. site of inflammation) [8]. Cyclooxygenase-2 (COX-2) was barely detectable in the normal epithelium but u-pregulated in hyperplasia and squamous cell carcinoma [9]. The development of resistance to variety of chemotherapeutic agents is one of the major challenges in effective cancer treatment [10]. COX-2 inhibitor prevent the growth of human oral

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Annex 26

Review Article

Photodynamic Therapy as a Treatment Option for Oral Cancer and Dysplasia

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Abstract

The challenge in cancer management is to find a balance between the intended therapeutic outcomes whilst preserving and maintaining function and aesthetics. This dilemma initiated ongoing efforts focusing on PDT which is now considered a historical re-utilized technique showing promising results but with many limitations. PDT has emerged as a successful and clinically acceptable therapeutic approach to the management of malignant and benign diseases. It is important to understand that PDT cannot completely replace other treatment modalities but can be used as a useful adjunct or as additional treatment.

Keywords: Dysplasia; Photodynamic therapy; Carcinoma; Treatment

Introduction

In order to successfully manage head and neck squamous cell carcinomas (HNSCC), it is imperative that function and aesthetics are maintained as much as possible. Improving loco-regional disease control rates has been somewhat the focus of research in the last 30-40 years. Non-surgical treatment approaches such as PDT can be applied to keep speech and swallowing function but with less than optimal improvements in survival.[1,2]

Nevertheless, it is immediately clear that advances in the management came at the expense of altering function and changing aesthetics. This was so that the ultimate goal of loco-regional control of the disease was achieved.[3,4]

The challenge in cancer management is to find a balance between the intended therapeutic outcomes whilst preserving and maintaining function and aesthetics. This dilemma initiated ongoing efforts focusing on PDT which is now considered a historical re-utilized technique showing promising results but with many limitations. Photodynamic therapy (PDT) is a treatment modality that is minimally invasive. Unlike ionizing radiation, PDT has the advantage that it can be applied repeatedly at the same site. PDT utilization in medicine had been widely accepted and it is an appealing option in oncology because the use of chemotherapy, ionizing radiation, or surgery does not preclude the use of PDT, and all of these approaches can be used in a patient who has received PDT.

PDT is not an alternative approach to the common used modalities however, there are certain circumstances were PDT can be used as an alternative approach. Although PDT is still emerging, it is already a successful and clinically approved therapeutic modality used for the management of neoplastic and nonmalignant diseases.[5]

PDT structure

PDT utilizes three components to achieve the intended effect on tumor cells and these essential components are: a photosensitizer (PS), light, and oxygen. The essential components are ineffective on tumor cells when exposed to the cells as single modalities. However, when they join together a highly reactive product, single, is formed causing significant toxicity resulting in cell death via apoptosis or necrosis.[6,7]

There are three mechanisms that cause the anti-tumor effects of PDT. These include the direct cytotoxic effects on tumor cells, damage to the tumor vascularity, and an inflammatory reaction leading to the development of systemic immunity.[8]

The therapeutic effect of PDT production of singlet oxygen

The basics of the photochemical reaction which initiates the process of tumor cell destruction is dependent on the excitation of the tissue oxygen located in the body by the injected

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Annex 27

Review Article

Gene therapy in oral cancer – An update

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ABSTRACT

Gene therapy is the use of DNA as an agent to treat genetic disorders. Gene therapy aims at the insertion of a therapeutic gene into the cells of a patient for the correction of an inborn error of metabolism, to alter or repair an acquired genetic abnormality. Today, most of the gene therapy studies are aimed at cancer and hereditary diseases which are linked to genetic defects. Cancer usually occurs due to the production of multiple mutations in a single cell which cause it to proliferate out of control. Several methods such as surgery, radiation therapy, and chemotherapy have been used widely to treat cancers, but recurrence is common in approximately one third of patients. To improve the treatment modality and to increase the survival rate, gene therapy can be used as an adjunct to other therapies for cancer patients. The purpose of this article is to review the concepts and technique, with an insight into the current research on its applications in oral squamous cell carcinoma (OSCC).

Key words: Gene gun, gene therapy, oral cancer, vectors

INTRODUCTION

OSCC is the most common malignancy in the oral cavity and is the 6th most common cancer world-wide.[1] Oral cancer is associated with genetic mutations resulting from exposure to tobacco, alcohol, betel quid, etc.[2] The current treatment strategies for OSCC include a combination of surgery, radiation therapy, and chemotherapy. Surgical resection of tumors cause significant functional (speech and swallowing) and cosmetic defects. Chemotherapy is associated with toxicity and has not been proved to possess an impact on the survival of patients. Recurrence is common in approximately one third of the patients despite definitive treatment. Gene therapy which involves replacement of the defective gene by a therapeutic gene has emerged as a promising treatment modality in the field of biomedicine.[3] This alternate treatment option has been proven to increase survival rates of OSCC patients. This review highlights current research methods available for combating OSCC.

THE HISTORY OF GENE THERAPY

Joshua Lederberg and Edward Tatum laid out the fundamentals for gene therapy.[4] Michael *et al.* succeeded in transferring a gene (TK gene, which codes for thymidine kinase) into mammalian cells in 1977.[5,6] In the year 1990, the first approved gene therapy clinical trial took place when Ashanthi De Silva, a 4-year-old girl with Adenosine Deaminase (ADA)-deficiency/Severe Combined Immunodeficiency (SCID) syndrome, was given her own T cells engineered with a retroviral vector carrying a normal ADA gene by the NIH (National

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Annex 28

BOLETÍN OFICIAL DEL ESTADO
Núm. 177 Sábado 25 de julio de 2015 Sec. I. Pág. 62935

I. DISPOSICIONES GENERALES

MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD

8343 *Real Decreto Legislativo 1/2015, de 24 de julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios.*

I

La disposición final cuarta de la Ley 10/2013, de 24 de julio, por la que se incorporan al ordenamiento jurídico español las Directivas 2010/84/UE del Parlamento Europeo y del Consejo, de 15 de diciembre de 2010, sobre farmacovigilancia, y 2011/62/UE del Parlamento Europeo y del Consejo, de 8 de junio de 2011, sobre prevención de la entrada de medicamentos falsificados en la cadena de suministro legal, y se modifica la Ley 29/2006, de 26 de julio, de garantías y uso racional de los medicamentos y productos sanitarios, autoriza al Gobierno para elaborar un texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios. Esta autorización, por un plazo de dos años a partir de la entrada en vigor del texto legal habilitante, tiene por objeto consolidar, en un texto único, las sucesivas modificaciones que se han ido incorporando, desde su entrada en vigor, en la citada ley e incluye la facultad de regularizar, aclarar y armonizar los textos legales que deben ser refundidos.

La autorización que da cobertura al presente texto refundido tiene su razón de ser en la necesidad de dotar de una mayor seguridad jurídica a una regulación que se ha caracterizado por una continua sucesión de normas que han completado o modificado, de forma muy dispar, el texto original de la Ley 29/2006, de 26 de julio, lo que aconseja la aprobación de un texto único en el que se incluyan, debidamente armonizadas, todas las disposiciones aplicables en el ámbito de esta ley. El texto resultante debería tener, así, una vocación de estabilidad, una vez que se han culminado con éxito los necesarios procesos de consolidación y adaptación imprescindibles para asegurar la continuidad de la prestación pública sanitaria y mejorado los mecanismos de farmacovigilancia y de protección de la cadena de suministro.

La Ley 29/2006, de 26 de julio, pretendió, al igual que la Ley 25/1990, de 20 de diciembre, del Medicamento, derogada por ella, dotar a la sociedad española de un instrumento institucional que permitiera que los problemas relativos a los medicamentos fueran abordados por cuantos agentes sociales se vieran involucrados en su manejo, en la perspectiva del perfeccionamiento de la atención a la salud. El tiempo transcurrido desde la aprobación de ambos textos legales permite afirmar que se ha alcanzado en gran parte el objetivo pretendido consagrándose la prestación farmacéutica como una prestación universal.

La prestación farmacéutica comprende los medicamentos y los productos sanitarios, así como el conjunto de actuaciones encaminadas a que los pacientes los reciban y los utilicen de forma adecuada a sus necesidades clínicas y en las dosis precisas según sus requerimientos individuales, durante el período de tiempo adecuado, con la información necesaria para su correcto uso y al menor coste posible.

Es necesario hacer una valoración positiva de lo que son y de lo que representan los medicamentos y los productos sanitarios para el Sistema Nacional de Salud por lo que la política farmacéutica desarrollada en las últimas décadas se ha orientado en la dirección de asegurar su disponibilidad para cubrir las necesidades de los pacientes.

En este aspecto, el papel de los profesionales del sector ha sido fundamental para alcanzar estos logros. El médico es una figura central en las estrategias de impulso de la calidad en la prestación farmacéutica dado el papel que se le atribuye en el cuidado de la salud del paciente y, por tanto, en la prevención y en el diagnóstico de la enfermedad, así como en la prescripción, en su caso, del tratamiento con medicamentos. El trabajo que los

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CONCISE REVIEW
Clinical

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Gene Therapy for the Treatment of Oral Squamous Cell Carcinoma

ABSTRACT

Despite advances in surgery, radiotherapy, and chemotherapy, the survival of patients with oral squamous cell carcinoma has not significantly improved over the past several decades. Treatment options for recurrent or refractory oral cancers are limited. Gene therapy for oral cancer is currently under investigation in clinical trials. The goal of cancer gene therapy is to introduce new genetic material into target cells without toxicity to non-target tissues. This review discusses the techniques used in cancer gene therapy for oral squamous cell carcinoma and summarizes the ongoing strategies that are being evaluated in clinical trials.

KEY WORDS: gene therapy, oral cancer, oral squamous cell carcinoma.

INTRODUCTION

Modifications of traditional cancer therapies, including surgery, radiotherapy, and chemotherapy, have not improved the survival rates of patients with mucosal squamous cell carcinoma. Local and/or regional tumor recurrence develops in approximately one-third of patients, despite definitive treatment (Schwartz *et al.*, 2000). The patient with recurrent or metastastic cancer is often considered incurable. A variety of chemotherapeutic agents has been used alone, and in combination, for the treatment of recurrent oral squamous cell carcinoma. However, chemotherapy is associated with well-known toxicities and has demonstrated no clear impact on survival in patients with recurrent oral cancer (Schrijvers *et al.*, 1998). Patients with recurrent oral cancer that is refractory to chemotherapy and/or radiation therapy have a median life expectancy of several months, and the response rate to second- or third-line chemotherapeutic regimens is approximately 15%. Two-thirds of patients dying of this disease have no evidence of symptomatic distant metastases. Therefore, local and regional disease control is paramount, underscoring an urgent need for more effective therapies. Gene therapy has the potential to target cancer cells while sparing normal tissues. Such a strategy may be useful for recurrent disease as well as in the adjuvant setting (*i.e.*, at the resected tumor margins). However, the clinical application of gene therapy for treatment of oral cancer will require optimization of gene delivery in conjunction with determinations of transfection efficiency.

DEFINITION OF GENE THERAPY

Gene therapy can be defined as gene transfer for the purpose of treating human disease (Cusack and Tanabe, 1998). This includes the transfer of new genetic material as well as the manipulation of existing genetic material. This holds true especially for cancer cells, where dominantly activated oncogenes can be targeted. The transfer of genetic material may occur *in vivo* (where the gene is introduced into the body) or *ex vivo* (where a tumor is removed, the genetic materials delivered, and the cells are then re-introduced into the patient). The *ex vivo* approach has not been utilized in oral cancer because superficial lesions usually lend themselves to the direct injection of genetic material.

STRATEGIES FOR GENE THERAPY

Potential uses of gene therapy in oral cancer include the treatment of recurrent disease and adjuvant treatment—for example, at surgically resected margins. Localized distant metastatic disease is another potential target of gene therapy in patients with oral cancer. Although systemic administration is theoretically desirable to address metastatic disease, gene therapy has not yet been shown to be suitable for systemic delivery in cancer patients. Due to the requirement for direct injection, oral cancer is a

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Annex 30

COMMENTARY

Phosphatases reverse p53-mediated cell cycle checkpoints

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The Cancer Genome Atlas and other large-scale cancer genome sequencing projects have identified an impressive number of significantly mutated genes that are likely drivers of cancer progression (1). These studies have definitively confirmed that the gene encoding the p53 tumor suppressor is the most frequently mutated gene in human cancers. A major component of the p53 anticancer response is its ability to arrest cell division at different stages of the cell cycle. In PNAS, a study by Shaltiel et al. provides additional insights into the mechanisms of reversal of the p53-mediated cell cycle arrest (2). Such insights may provide new cancer therapeutic opportunities.

In the normal cell, the p53 protein functions as a stress integrator that becomes activated in response to myriad dysfunctional states (e.g., damaged DNA, aberrant oncogenic signaling, and hypoxia). Once activated, p53 can initiate stress response programs to eliminate the dysfunctional state, such as repair of DNA damage. If the stressed cell is dividing, p53 can enact any one of several antiproliferative programs. Under low to moderate stress conditions, the dividing cell may be transiently arrested. Alternatively, under conditions of high stress, the cell may be permanently arrested (senescence) or eliminated completely in an ordered manner (apoptosis). In those cases where cell division is merely arrested, time is allowed for the damage or dysfunction to be repaired without being transmitted to progeny cells. Thus, p53 has been called the "guardian of the genome" (3). Given the importance of p53 as a cellular failsafe mechanism, it is not surprising that its inactivation is a highly selected event in cancer progression.

Activated p53 can halt cell division in both the G1 and G2 phases of the cell division cycle. G1 is the preparation phase of the cell before replication of its DNA and G2 prepares the cell for mitosis. Arrest and repair of cells before DNA replication or mitosis are likely to prevent damage-induced mutational events or abnormal chromosome segregation events, respectively. Once repair of damage is achieved, the cell uses pathways to relieve the p53-mediated cell cycle arrest and return to a normal dividing state.

Much of the p53-mediated cell cycle arrest is effected by kinases that phosphorylate p53 (Fig. 1). Therefore, phosphatases acting on the p53 phosphorylation sites are natural candidates to reverse cell cycle arrest. Unfortunately, our knowledge of phosphatases that remove phosphate groups from p53 (about seven identified thus far) is less advanced than our knowledge of the kinases that phosphorylate it (at least 33 identified) (4). In 2005, my laboratory reported that the serine/threonine phosphatase WIP1 (*PPM1D*) could dephosphorylate p53 at serine 15, the same site phosphorylated by the p53-activating kinase ATM (5), and act to reverse the G2/M cell cycle arrest caused by DNA damage. The Medema laboratory later showed that WIP1 was able to relieve G2 arrest by interfering with the ability of p53 to repress expression of key G2/M progression factors such as cyclin B1 (6) (Fig. 1). Interestingly, WIP1 had earlier been discovered as a p53 transcriptional target gene (7). Thus, WIP1 is likely to operate as part of a negative feedback regulatory loop with p53 in which damage-activated p53 up-regulates WIP1 that accumulates and inactivates p53 once damage is repaired. The suppressor (WIP1) of a tumor

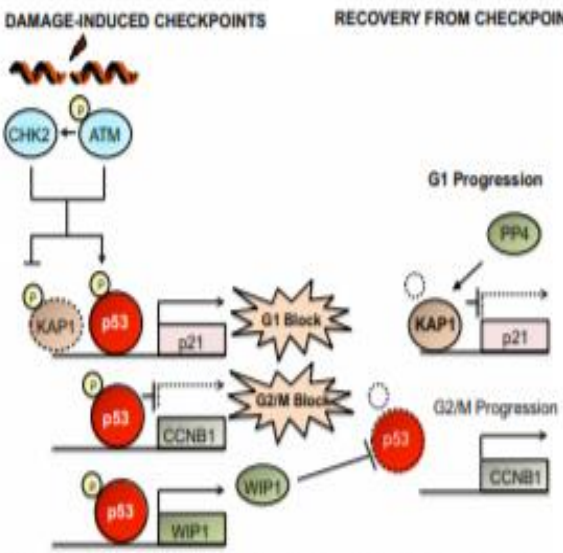

Fig. 1. Phosphatases PP4 and WIP1 reverse G1 and G2/M checkpoints. Following DNA damage, ATM and CHK2 kinases phosphorylate p53 and KAP1. Phosphorylation of p53 stimulates its transcriptional activation of the p21 gene, resulting in a G1 cell cycle arrest. ATM and CHK2 phosphorylation of KAP1 prevents its normal repression of p21 RNA expression. In addition, p53 directly binds to genes that mediate G2/M progression such as cyclin B1 (CCNB1) and inhibits their expression, enhancing G2/M cell cycle arrest. The phosphatase WIP1 is up-regulated by p53 and participates in a negative feedback loop that relieves the G2/M block through dephosphorylation of p53 (facilitating degradation of p53). The G1 block is relieved by PP4 dephosphorylation of KAP1, resulting in its reactivation and suppression of p21 RNA transcription.

Author contributions: L.A.D. wrote the paper.

The author declares no conflict of interest.

See companion article on page 7313.

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Annex 31

Indian Journal of Dental Research

REVIEW ARTICLE
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Gene therapy for oral squamous cell carcinoma: An overview

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Abstract

A potential approach to the treatment of genetic disorders is gene therapy. The goal of gene therapy is to introduce therapeutic genetic material into the target cell to exert the intended therapeutic effect. Gene therapy has already shown promising results for the treatment of monogenic disorders such as severe combined immunodeficiency and haemophilia. Now the procedure has been extended to the level of treating malignant conditions such as cancer of the lungs, breast, colon etc. The prevalence of tumours of the larynx and oral cavity has increased in both developed and developing countries. This increase underscores the need for a novel therapeutic modality that would decrease or completely terminate the proliferation of malignant cells. This review highlights various types of gene therapy procedures with respect to oral squamous cell carcinoma.

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Available from: https://www.ijdr.in/text.asp?2007/18/3/120/33787

Full Text

The current treatment strategies for oral squamous cell carcinoma (OSCC) include a combination of surgery, radiation therapy and chemotherapy. However, surgical resection of tumours frequently causes profound defects in oral functions such as speech and swallowing as well as in cosmetic aspects. [1] Chemotherapy is associated with well-known toxicity and has demonstrated no clear impact on the survival of patients with recurrent oral cancer. Recurrence develops in approximately one third of the patients despite definitive treatment. [2] Two thirds of the patients dying of this disease have no evidence of symptomatic distant metastasis, therefore, local and regional disease control is paramount, underscoring an urgent need for more effective therapy.

Several reports have indicated that the combination of radiation and gene therapies has synergistic suppressive effects on various cancer cells, including colorectal, ovarian, nasopharyngeal and head / neck cancer cells. [3] Gene therapy can also be used as an adjuvant to surgery (at the resected tumour margins). This review highlights various gene therapy methods that are available for combating OSCC.

Annex 32

The Cell-Cycle Arrest and Apoptotic Functions of p53 in Tumor Initiation and Progression

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P53 is a transcription factor highly inducible by many stress signals such as DNA damage, oncogene activation, and nutrient deprivation. Cell-cycle arrest and apoptosis are the most prominent outcomes of p53 activation. Many studies showed that p53 cell-cycle and apoptosis functions are important for preventing tumor development. p53 also regulates many cellular processes including metabolism, antioxidant response, and DNA repair. Emerging evidence suggests that these noncanonical p53 activities may also have potent antitumor effects within certain context. This review focuses on the cell-cycle arrest and apoptosis functions of p53, their roles in tumor suppression, and the regulation of cell fate decision after p53 activation.

Cell-cycle arrest and apoptosis are the most noticeable biological outcomes of p53 activation in cell culture and animal experiments. The seminal finding of p53 as an inhibitor of oncogene-mediated transformation in foci formation is likely the result of its cell-cycle arrest or apoptosis activities (Finlay et al. 1989). The mammalian p53 DNA-binding domain has marginal thermostability, which facilitates the identification of temperature-sensitive mutants and provides a powerful tool for controlling p53 function. The ability of activated p53 to induce cell-cycle arrest in rat embryo fibroblasts and apoptosis in a leukemia cell line were discovered using temperature-sensitive p53–135V mutant (Martinez et al. 1991; Yonish-Rouach et al. 1991). Subsequent work using viral vector-mediated gene delivery and inducible expression validated these as the most prominent effects of p53 activation in cell culture.

Given the ability of p53 to induce both cell-cycle arrest and cell death, the regulation of cell fate decision has been the focus of numerous studies (Vousden and Prives 2009). This is a topic with significant clinical relevance because p53-mediated apoptosis in normal tissues are involved in chemotherapy toxicity, ischemia, and neurodegenerative diseases such as Alzheimer's and Parkinson's (Checler and Alves da Costa 2014). Induction of p53-mediated apoptosis in tumor cells is considered a desirable outcome of cancer therapy, whereas induction of cell-cycle arrest may interfere with drugs that target mitosis and reduce the efficacy of DNA-damaging drugs. Cell fate decision in re-

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Additional Perspectives on The p53 Protein available at www.perspectivesinmedicine.org

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Annex 33

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Review Article

Dentistry Section

Gene Therapy Applications in Dentistry: A Review

DINESH FRANCIS SWAMY[1], SAPNA SADA RAUT DESSAI[2], ELAINE SAVIA BARRETTO[3], KATHLEEN MANUELA DSOUZA[4]

ABSTRACT

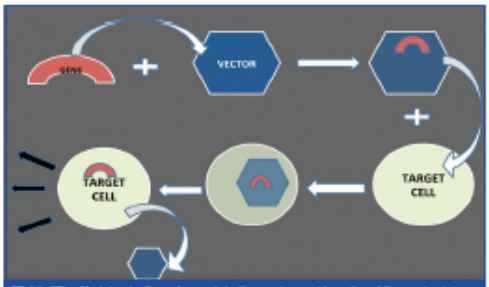
Gene therapy involves transfer of new genetic material or manipulation of the existing material for the purpose of treating human disease. It involves transfer of genetic material – ex vivo and in vivo and may conceivably revolutionize clinical practice in the coming years. Although progress has chiefly been in the medical domain, research has moved to various dental conditions as well. This review is presented to highlight various applications of gene therapy in dentistry in the areas such as salivary gland disorders, chronic pain, DNA vaccines, bone repair, implantology, head and neck cancer, orthodontic therapy, periodontal repair and tooth regrowth.

Keywords: Bone Repair, Gene transfer techniques, Growth factor, Neoplasms

INTRODUCTION

Gene therapy is a field of biomedicine that involves replacing or repairing defective genes in the diseased cell genome to restore normal cell function without causing any toxicity to non-target tissues. Since the first attempt at human gene therapy in 1980 [1], scientists and clinicians have been constantly researching and conducting trials [2].

Gene therapy can be somatic gene therapy or germ line gene therapy. Somatic gene therapy involves alteration of the genes in selected cells that are not gametes or undifferentiated cells. The effects of these changes are restricted to treated individuals. On the other hand, germ line gene therapy introduces permanent, inheritable changes to the genome of the individual, by targeting gametes. The state of gene therapy research is confined for ethical and technical reasons almost in its entirety to somatic cell gene therapy [3]. Typically, therapeutic genes are identified, isolated and cloned and then introduced into a vector. A vector is a vehicle that is used to deliver the gene of interest to the target tissue [Table/Fig-1]. A vector should deliver precise amount of material into each target cell. Vectors that have been experimented and used include viral and non-viral vectors. Viruses provide the most efficient form of gene transfer. Various viral vectors in experiment today are retroviruses, adenoviruses, adeno-associated viruses and herpes viruses. Non-viral vectors include electroporation, microinjection, use of ballistic particles, calcium vectors, lipid vectors and protein complexes [3].



[Table/Fig-1]: Introduction of gene into the vector and transfer of the vector into the target cell.

Although considered largely experimental, research is moving to clinical applications, with several commercial gene therapies now available for certain genetic conditions. Many dental conditions and dental applications are also being researched presently all over the world [4, 5]. Following is a brief review of gene therapy in dentistry.

Gene Therapy for Bone Repair

Bone loss may be a consequence of several oral conditions such as periodontal disease, trauma, neoplastic pathology, reconstructive surgery or may be deficient as a result of congenital defects. Currently, efforts at addressing such bone defects revolve primarily around using substitute materials that are either synthetic or harvested. The ideal goal would be to have directed regeneration of bone to meet the necessary needs. This would require manipulation of the critical aspects of bone physiology i.e., osteoinduction, differentiation of osteoblasts and the production of osteoid matrix, osteoconduction and mechanical stimulation [4].

This is where members of the Transformation Growth Factor (TGF) super-family such as Platelet Derived Growth Factors (PDGFs), Insulin-like Growth Factors (IGFs), Transforming Growth Factor-β (TGF-βs) and Bone Morphogenic Proteins (BMPs) have been employed for tissue engineering. When introduced to a site in sufficient concentration, they induce repair by creating molecular cues for native Mesenchymal Stem Cells (MSCs) to migrate, proliferate, differentiate and begin the cascade of osseous extracellular matrix production [6].

While most of these molecules act with overlapping roles, it is the group of BMPs (specifically BMP-2, BMP-4 and BMP-7) that have shown the maximum promise in their direct ability to induce bone formation, both when delivered in in vitro cultures [7] as well to local sites in vivo [8]. However, a problem encountered is that BMPs are degraded and have a short half-life and require sustained high dose delivery. Gene therapy has shown the ability to overcome these issues. The two basic gene therapy strategies employing BMPs are: (1) the delivery of BMP-related genes directly into the area of interest in vivo and (2) the seeding of ex vivo genetically-modified cells into a scaffold which is then implanted [3]. Both approaches permit the sustained delivery of elevated levels of BMPs to target tissues [6].

Studies have shown the ability of Ad-BMP-7 adenovirus vectors to transfer genes encoding for BMP-7 directly into tissues (direct gene transfer) and have monitored the expression of the transduced gene products from the neighbouring vector-inoculated cells [9, 10]. Similarly, the in vivo applicability of BMP-2 gene delivery to tissues of mandibular defects by means of an adenoviral vector has been tested successfully [8].

The ex vivo methods have been validated in several studies where gene transfer is accomplished in harvested cells (typically stem cells of mesenchymal origin, bone marrow derived, adipose derived or

Annex 34

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Gene Therapy for Duchenne muscular dystrophy

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Abstract

Introduction—Duchenne muscular dystrophy (DMD) is a relatively common inherited disorder caused by defective expression of the protein dystrophin. The most direct approach to treating this disease would be to restore dystrophin production in muscle. Recent progress has greatly increased the prospects for successful gene therapy of DMD, and here we summarize the most promising developments.

Areas Covered—Gene transfer using vectors derived from adeno-associated virus (AAV) has emerged as a promising method to restore dystrophin production in muscles bodywide, and represents a treatment option applicable to all DMD patients. Using information gleaned from PubMed searches of the literature, attendance at scientific conferences and results from our own lab, we provide an overview of the potential for gene therapy of DMD using AAV vectors including a summary of promising developments and issues that need to be resolved prior to large-scale therapeutic implementation.

Expert Opinion—Of the many approaches being pursued to treat DMD and BMD, gene therapy based on AAV-mediated delivery of microdystrophin is the most direct and promising method to treat the cause of the disorder. The major challenges to this approach are ensuring that microdystrophin can be delivered safely and efficiently without eliciting an immune response.

Keywords

adeno-associated viral (AAV) vectors; Becker muscular dystrophy (BMD); Duchenne muscular dystrophy (DMD); Dystrophin; gene therapy; immune response; micro-dystrophin; micro-utrophin; tolerization

1. Introduction

Mutations in the 2.1 MB dystrophin gene lead to Duchenne and Becker muscular dystrophy (DMD/BMD). The disorder is inherited in an X-linked, recessive pattern and affects approximately one in 3,500–4,000 newborn males worldwide, making it the most common form of muscular dystrophy [1]. While DMD typically results from null-allele mutations that prevent expression of the dystrophin protein, BMD results from mutations that lower expression or that lead to accumulation of an internally truncated protein with reduced stability or functional capacity. Dystrophin deficiency leads to progressive skeletal muscle wasting accompanied by increased fatty and connective tissue, while cardiac muscle displays increasing fibrosis and a progressive dilated cardiomyopathy. Most DMD patients succumb to respiratory or cardiac failure in their 3rd to 4th decade of life. BMD has a later age of onset and slower progression [1]. The recessive nature and slow progression of

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STATE-OF-THE-ART HUMAN GENE THERAPY: PART II. GENE THERAPY STRATEGIES AND APPLICATIONS

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Abstract

In Part I of this Review, we introduced recent advances in gene delivery technologies and explained how they have powered some of the current human gene therapy applications. In Part II, we expand the discussion on gene therapy applications, focusing on some of the most exciting clinical uses. To help readers to grasp the essence and to better organize the diverse applications, we categorize them under four gene therapy strategies: (1) gene replacement therapy for monogenic diseases, (2) gene addition for complex disorders and infectious diseases, (3) gene expression alteration targeting RNA, and (4) gene editing to introduce targeted changes in host genome. Human gene therapy started with the simple idea that replacing a faulty gene with a functional copy can cure a disease. It has been a long and bumpy road to finally translate this seemingly straightforward concept into reality. As many disease mechanisms unraveled, gene therapists have employed a gene addition strategy backed by a deep knowledge of what goes wrong in diseases and how to harness host cellular machinery to battle against diseases. Breakthroughs in other biotechnologies, such as RNA interference and genome editing by chimeric nucleases, have the potential to be integrated into gene therapy. Although clinical trials utilizing these new technologies are currently sparse, these innovations are expected to greatly broaden the scope of gene therapy in the near future.

1. Introduction

Human genetic disorders arise from mutations in the DNA genome, which in many cases abrogate the normal function of genes. To tackle these "loss-of-function" diseases, recombinant DNA technology fostered the hope that delivering the normal copy of a mutated gene to the patient will cure the disease. This "gene replacement" approach to

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DISCLOSURE

G. Gao is a founder of Voyager Therapeutics and holds equity in the company. G. Gao is an inventor on patents with potential royalties licensed to Voyager Therapeutics.

Annex 36

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DOI: 10.1089/hum.2012.251

Review

New Frontier in Regenerative Medicine: Site-Specific Gene Correction in Patient-Specific Induced Pluripotent Stem Cells

Zita Garate,[1] Brian R. Davis,[2] Oscar Quintana-Bustamante,[1] and Jose C. Segovia[1]

Abstract

Advances in cell and gene therapy are opening up new avenues for regenerative medicine. Because of their acquired pluripotency, human induced pluripotent stem cells (hiPSCs) are a promising source of autologous cells for regenerative medicine. They show unlimited self-renewal while retaining the ability, in principle, to differentiate into any cell type of the human body. Since Yamanaka and colleagues first reported the generation of hiPSCs in 2007, significant efforts have been made to understand the reprogramming process and to generate hiPSCs with potential for clinical use. On the other hand, the development of gene-editing platforms to increase homologous recombination efficiency, namely DNA nucleases (zinc finger nucleases, TAL effector nucleases, and meganucleases), is making the application of locus-specific gene therapy in human cells an achievable goal. The generation of patient-specific hiPSC, together with gene correction by homologous recombination, will potentially allow for their clinical application in the near future. In fact, reports have shown targeted gene correction through DNA-Nucleases in patient-specific hiPSCs. Various technologies have been described to reprogram patient cells and to correct these patient hiPSCs. However, no approach has been clearly more efficient and safer than the others. In addition, there are still significant challenges for the clinical application of these technologies, such as inefficient differentiation protocols, genetic instability resulting from the reprogramming process and hiPSC culture itself, the efficacy and specificity of the engineered DNA nucleases, and the overall homologous recombination efficiency. To summarize advances in the generation of gene corrected patient-specific hiPSCs, this review focuses on the available technological platforms, including their strengths and limitations regarding future therapeutic use of gene-corrected hiPSCs.

Introduction: Regenerative Medicine—Cell Plus Gene Therapy

Regenerative medicine aims to replace and/or to regenerate damaged cells, organs, or tissues in order to restore normal function. Cell therapy is an important regenerative medicine approach, in which either differentiated cells or stem cells capable of differentiation are transplanted into an individual with the objective of yielding specific cell types present in the damaged tissue and consequently restoring its function. The most successful example of cell therapy is bone marrow (BM) transplantation, in which the transplanted hematopoietic stem cells (HSCs) are able to regenerate the patient's blood. BM transplantation started in the 1950s and now is a widely established procedure for many hematopoietic diseases (Thomas *et al.*, 1977). Cell therapies for other tissues then followed in the footsteps of the hematopoietic experience. Nowadays, there are numerous ongoing clinical trials using various types of stem cells and some of them are U.S. Food and Drug Administration (FDA)-approved cell-based products (www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/default.htm).

Cell replacement can be done with autologous or allogeneic stem cells. When performing allogeneic cell therapy, the risk of immune rejection usually requires the use of immunosuppressive drugs, which can induce toxicity and increase the risk of infections and cancer, which could be life-threatening.

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Annex 37

Ghosh and Tabrizi *Alzheimer's Research & Therapy*
DOI 10.1186/s13195-017-0307-1

Alzheimer's Research & Therapy

REVIEW Open Access

Gene suppression approaches to neurodegeneration

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Abstract

Gene suppression approaches have emerged over the last 20 years as a novel therapeutic approach for the treatment of neurodegenerative diseases. These include RNA interference and anti-sense oligonucleotides, both of which act at the post-transcriptional level, and genome-editing techniques, which aim to repair the responsible mutant gene. All serve to inhibit the expression of disease-causing proteins, leading to the potential prevention or even reversal of the disease phenotype. In this review we summarise the main developments in gene suppression strategies, using examples from Huntington's disease and other inherited causes of neurodegeneration, and explore how these might illuminate a path to tackle other proteinopathy-associated dementias in the future.

Keywords: Gene suppression, RNA interference, Anti-sense oligonucleotides, Zinc-finger proteins, CRISPR/Cas9, Therapeutics, Huntington's disease, Dementia

Background

Gene suppression approaches refer to targeted molecular genetic therapies that serve to lower the expression of specific genes. The term "gene silencing" has also been used to describe these methods; however, this term should be considered a misnomer as complete gene inactivation does not occur, and might not be desirable. Such techniques have made enormous progress over the last 20 years, and show great promise for the treatment of inherited neurodegenerative diseases arising from a known genetic mutation.

Neurodegenerative diseases that result from the toxic gain-of-function of a mutant protein or non-coding RNA that are without a significant loss-of-function are ideal candidates for gene suppression approaches. There are many strategies to lower the amount of toxic disease proteins that result from single gene mutations, including both post-transcriptional inhibition such as RNA interference (RNAi), anti-sense oligonucleotides (ASOs) and catalytic nucleic acids, and genome editing techniques such as zinc finger proteins (ZFPs) and CRISPR/Cas9. The great benefit of interventions at the gene suppression level, as opposed to interventions aimed at the toxic protein itself, is that the plethora of potentially negative downstream cellular pathogenic effects that may arise from the abnormal functioning of a single protein are all reduced as a consequence of treatment.

In this review we will use examples from Huntington's disease (HD), in respect of which a worldwide collaborative research effort has led to the swift progression of gene suppression approaches, from in-vitro and in-vivo development through to a clinical trial of a Huntingtin-lowering therapy that is currently in progress [1]. Huntington's disease is an autosomal dominant, neurodegenerative condition caused by a CAG expansion in exon 1 of the gene encoding the Huntingtin (HTT) protein. The presence of the mutant gene leads to the adult onset of chorea, psychiatric symptoms and cognitive decline, and is fatal after 15–20 years [2]. In addition, examples of gene suppression approaches in amyotrophic lateral sclerosis (ALS) [3] will be discussed. However, lessons learnt from development of gene suppression technologies in these conditions could equally be applied to any dementia or neurodegenerative condition in which the responsible disease protein is known. Indeed the potential of lowering tau protein for the treatment of Alzheimer's disease (AD) and other tauopathies has been demonstrated recently [4], as well as targeting alpha-synuclein (SNCA) [5] and leucine-rich-repeat kinase 2 (LRRK2) [6] for Parkinson's disease (PD), and ataxin-2 for the treatment of spinocerebellar ataxia type 2 (SCA2) [7], sporadic ALS and frontotemporal dementia (FTD) [8].

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Annex 38

REVIEW ARTICLE

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PRECISION AND FUTURE MEDICINE

Current advances in combining stem cell and gene therapy for neurodegenerative diseases

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ABSTRACT

Neuronal death is the common final pathologic pathway of various neurodegenerative diseases (NDs). Although central nervous system has little regenerative potential, it is expected that damaged neural tissue can be recovered by exogenous supplementation of stem cells; however, stem cell therapy cannot modulate specific causes of NDs, such as accumulation of extracellular amyloid peptides in Alzheimer's disease. In contrast, gene therapy can deliver therapeutic genes to specific ND targets. Therefore, combining stem cell and gene therapy would have dual treatment mechanisms (regenerating damaged neural tissue and modifying specific causes of NDs) and lead to better clinical outcomes. In this review, we discuss various therapeutic genes that can be used to develop stem cell gene therapy for various NDs and the techniques for how therapeutic genes can be integrated into stem cells.

Keywords: Genetic therapy; Neurodegenerative diseases; Stem cells

INTRODUCTION

Neurodegenerative diseases (NDs) are various incurable conditions in the central nervous system (CNS). Despite the different locations and symptoms of NDs, their common final pathological pathway is neuronal dysfunction and loss [1]. The human CNS has poor ability to repair damage because neurons cannot spontaneously regenerate from residual nervous tissues [2]. NDs result in permanent and progressive losses of various CNS functions such as cognition, memory, and/or motor functions. Accordingly, there are few effective regenerative treatments for NDs; the current focus is only on delaying their progress [3].

Stem cells have self-renewal and multi-lineage differentiation capacities [4]. Based on their abilities, they come into the spotlight as novel candidates for regenerative treatment of NDs.

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Original Russian Text © A. N. Lukashev, A. A. Zamyatnin, Jr., 2016, published in Biokhimiya, 2016, Vol. 81, No. 7, pp. 926-936.

REVIEW

Viral Vectors for Gene Therapy: Current State and Clinical Perspectives

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Abstract—Gene therapy is the straightforward approach for the application of recent advances in molecular biology into clinical practice. One of the major obstacles in the development of gene therapy is the delivery of the effector to and into the target cell. Unfortunately, most methods commonly used in laboratory practice are poorly suited for clinical use. Viral vectors are one of the most promising methods for gene therapy delivery. Millions of years of evolution of viruses have resulted in the development of various molecular mechanisms for entry into cells, long-term survival within cells, and activation, inhibition, or modification of the host defense mechanisms at all levels. The relatively simple organization of viruses, small genome size, and evolutionary plasticity allow modifying them to create effective instruments for gene therapy approaches. This review summarizes the latest trends in the development of gene therapy, in particular, various aspects and prospects of the development of clinical products based on viral delivery systems.

DOI: 10.1134/S0006297916070063

Key words: DNA, RNA, viral vector, molecular targeting, smart drugs

During the recent decades, gene therapy (GT) has become one of the most actively developing and most promising branches of medicine. Gene therapy has a number of potential applications, such as:

– treatment of hereditary genetic diseases, primarily monogenic ones;
– anticancer therapy;
– treatment of infectious diseases;
– treatment of common therapeutic diseases.

Moreover, recombinant vaccines can also be regarded as gene therapy, because the way they are developed and produced is closer to GT approaches rather than to the development of classical vaccines.

Abbreviations: AAV, adeno-associated virus; GT, gene therapy; HIV, human immunodeficiency virus; HSC, hematopoietic stem cells; MLV, murine leukemia virus; MVA, modified vaccinia virus Ankara.

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METHODS OF GENE THERAPY

A characteristic feature of a gene therapy is the use of a nucleic acid to provide a specific effect in a cell. Generally, therapeutic effect is achieved through the expression of a gene encoded by this nucleic acid. It can code either for a protein, or for RNA, initiating RNA interference. Moreover, it should be noted that functions of nucleic acids are not limited only to storage and expression of genetic information. Newly developed "smart drugs" can be nucleic acids that not only contain genetic information, but also have special 3D-structures and enzymatic activities. Such nucleic acids are able to change their conformation in response to certain external stimuli, activating a ribozyme that excises, for example, microRNA, which initiates RNA interference [1-3].

Discovery of CRISPR-Cas systems allowing targeted changing (editing) of a genome [4] stimulated studies aimed at optimization of the delivery of CRISPR-Cas

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Review Article

Genetics Section

Non Viral Vectors in Gene Therapy- An Overview

MURALI RAMAMOORTH[1], APARNA NARVEKAR[2]

ABSTRACT

Non-viral vectors are simple in theory but complex in practice. Apart from intra cellular and extracellular barriers, number of other challenges also needs to be overcome in order to increase the effectiveness of non-viral gene transfer. These barriers are categorized as production, formulation and storage. No one-size-fits-all solution to gene delivery, which is why in spite of various developments in liposome, polymer formulation and optimization, new compounds are constantly being proposed and investigated. In this review, we will see in detail about various types of non-viral vectors highlighting promising development and recent advances that had improved the non-viral gene transfer efficiency of translating from "Bench to bedside".

Keywords: Biological vectors, Engineered vectors, Gene transfer, Transfection

INTRODUCTION

Gene therapy is defined as the procedure used to treat or improve the health condition of the patient by modifying the patient's cells genetically [1]. It provides an unique approach to treat both inherited and acquired diseases by delivering a therapeutic gene material and its associated regulatory elements into the nucleus; in order to correct the loss of function caused by mutation or to express the deficient gene product at physiologic levels [2]. It is well documented that almost all human diseases occur due to defect in either a single gene or set of genes due to mutation.

Gene therapy is considered as an alternative for enzyme /protein replacement therapy. The disadvantages like in vivo clearance and manufacturing cost faced by the replacement therapy makes gene therapy a potential alternative for various rare genetic disorders.

In spite of various methods or types of gene therapy, the therapy starts with the identification of mutant gene which is responsible for the cause of the disease. The next step is cloning the identical healthy gene. This is called therapeutic gene or transgene. The therapeutic gene is tailored to the need i.e. to augment or suppress or repair. Once the therapeutic gene is produced it is loaded in a vehicle called vector. The function of the vector is to deliver the therapeutic gene to the patient target cell. After the vector reaches the target cell, it delivers the genetic material to the nucleus. In the nucleus the genetic material gets integrated into DNA and corrects the defective or mutated gene. The most critical step in achieving gene therapy is choosing the vectors. Sequential key steps in gene therapy are shown in [Table/Fig-1].

Vectors are vehicles that ferry the genetic material into a wide variety of cells, tissues and whole organs. The optimal vector and delivery system depends on the target cells and its characteristics, duration of expression and the size of the genetic material to be incorporated in the vector [3,4].The present vectors used for gene therapy are broadly classified as Viral vectors, Non-viral vectors and engineered vectors. The non-viral vectors are Naked DNA, particle based and chemical based. They are administered by direct administration (plasmid DNA/Naked DNA)/ chemical /physical. Most of cardiovascular clinical trials use non-viral vectors as a mode of gene transfer.

RATIONALE FOR USING NON-VIRAL VECTORS

The efficiency of transfecting host cells is relatively high with viral vectors compared to non-viral methods. The main drawbacks of using virus vectors are its immunogenicity and cytotoxicity. The first related fatality of gene therapy clinical trial was related to the inflammatory reaction to the viral vector (Adenovirus). Additional cause of concern over using viral gene transfer vehicle is the phenomenon known as insertional mutagenesis i.e. ectopic chromosomal integration of viral DNA disrupts the expression of tumour suppression gene or activates oncogene leading to the malignant transformation of cells. Due to its demonstrated reduced pathogenicity, low cost and ease of production, non-viral vectors have important safety advantage over viral approaches. The major advantage of using non-viral vectors is its bio-safety. However the application of non-viral gene transfer have been ignored for a long time in past because of their poor efficiency of delivery thereby low transient expression of their transgenes [2]. Non-viral vectors have drawn significant attention due to its less immunotoxicity. Use of non-viral vectors in clinical trials increased from 2004 to 2013 while that of viral vector saw significant decrease. Advances in efficiency, specificity, gene expression duration and safety led to an increased number of non-viral vector products entering clinical trials.

Unfortunately none of the currently available non-viral vectors fulfills ideal vector properties. This has led to research focus on suitable ideal vector delivery system.

Technical challenges and limitations to successful Non-Viral Gene transfer

The major technical limitations or critical steps in attaining a successful gene therapy are categorized into [5-8]: efficiency of vector transport and unloading into target cells, perseverance, activity, immune response, regulatory issues and ethical concerns and commercialization. These different stages pose a big challenge to gene therapy to be efficiently treating the disease. The cost of gene therapy creates an image that it is meant for the affluent. This was clearly evident with the first commercialized gene therapy Alipogene tiparvovec for Lipoprotein Lipase deficiency in November 2013. The

[Table/Fig-1]: Schematic illustration of key steps in gene therapy

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Cochrane Library

Cochrane Database of Systematic Reviews

Interventions for the treatment of oral and oropharyngeal cancers: surgical treatment (Review)

Bulsara VM, Worthington HV, Glenny AM, Clarkson JE, Conway DI, Macluskey M

Bulsara VM, Worthington HV, Glenny AM, Clarkson JE, Conway DI, Macluskey M.
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Annex 42

ORIGINAL ARTICLE

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Overall and disease-specific survival outcomes following primary surgery for oral squamous cell carcinoma: analysis of consecutive 67 patients

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Abstract (J Korean Assoc Oral Maxillofac Surg 2019;45:83-90)

Objectives: This study evaluated the predictive factors for survival of patients with oral squamous cell carcinoma (OSCC) and investigated the overall and disease-specific survival (DSS) outcomes.

Materials and Methods: A total of 67 consecutive patients who underwent surgery for OSCC from January 2006 to November 2014 were included in this study. Patients were classified according to age, sex, pTNM stages, primary sites, smoking and alcohol drinking habits, depth of invasion, perineural and lymphovascular invasion, cell differentiation and postoperative radiotherapy. Kaplan-Meier methods were used to estimate the survival categorized by patient groups. Cox regression methods were used to investigate the main independent predictors of survival.

Results: Nineteen patients died of OSCC during follow-up periods. Another five patients died of other diseases including lung adenocarcinoma (n=1), cerebral infarction (n=1), general weakness (n=2), and pneumonia (n=1). The tongue (n=16) was the most common site for primary origin, followed by buccal mucosa (n=15), mandibular gingiva (n=15), maxillary gingiva (n=9), floor of mouth (n=9), retromolar trigone (n=2), and palate (n=1). Eleven patients had pTNM stage I disease, followed by stage II (n=22) and stage IV (n=34). No patients had pTNM stage III disease in this study. The overall survival of all patients was 64.2% and the DSS was 71.6%. DSS of patients with stage I and II disease was 100%. Stepwise Cox regression showed the two predictors for DSS were pTNM stage (P=0.0001, odds ratio=19.633) and presence of metastatic lymph nodes (P=0.0004, odds ratio=0.1039).

Conclusion: OSCC has been associated with poor prognosis; however, there were improved survival outcomes compared with past studies. Advanced-stage disease and presence of metastatic lymph nodes were associated with poorer survival compared with early-stage OSCC and absence of neck node metastasis. Stage I and II OSCC were associated with excellent survival results in this study.

Key words: Head and neck neoplasm, Survival, Lymphatic metastasis, Neoplasm staging

[paper submitted 2018. 2. 25 / revised 2018. 3. 16 / accepted 2018. 3. 22]

I. Introduction

Oral squamous cell carcinoma (OSCC) is a common malignancy worldwide, with regional variations in incidence and mortality[1-3]. The most common risk factors associated with OSCC are tobacco and alcohol abuse[4]; however, the incidence in younger than 40 years without tobacco and alcohol abuse has increased[5]. Survival outcomes of OSCC have improved over the last 20 years, but the prognosis is still relatively unfavorable, with 5-year overall survival (OS) and disease-free survival estimated to be 47% and 74%[6-8]. Prognosis is thought to be influenced by factors related to the host, surgeon, and tumor. Establishing the interaction between these factors and patient prognosis is important. The most well-known critical factors associated with survival are disease stage at initial diagnosis, neck metastasis, invasiveness of cancer cells, and tumor thickness[2,6,9-11]. If regional metastases have occurred, the 5-year survival of OSCC is halved; therefore, the single most important clinical predictor in determining survival is the existence of clinically positive lymph nodes[9]. Other clinical and histopathologic prognostic factors include the size of the primary tumor, site, grade of cell differentiation, depth of invasion, biologic tumor markers, perineural invasion,

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Annex 43

BJC British Journal of Cancer

www.nature.com/bjc

ARTICLE

Clinical Study

Nationwide randomised trial evaluating elective neck dissection for early stage oral cancer (SEND study) with meta-analysis and concurrent real-world cohort

Iain L. Hutchison[1,2], Fran Ridout[2], Sharon M. Y. Cheung[2], Neil Shah[3], Peter Hardee[1], Christian Surwald[4], Janavikulam Thiruchelvam[5], Leo Cheng[1], Tim K. Mellor[6], Peter A. Brennan[6], Andrew J. Baldwin[7], Richard J. Shaw[8], Wayne Halfpenny[9], Martin Danford[9], Simon Whitley[1], Graham Smith[10], Malcolm W. Bailey[9], Bob Woodwards[7], Manu Patel[11], Joseph McManners[12], Chi-Hwa Chan[13], Andrew Burns[14], Prav Praveen[15], Andrew C. Camilleri[16], Chris Avery[17], Graham Putnam[18], Keith Jones[19], Keith Webster[15], William P. Smith[20], Colin Edge[21], Iain McVicar[22], Nick Grew[23], Stuart Hislop[24], Nicholas Kalavrezos[25], Ian C. Martin[14] and Allan Hackshaw[26]

BACKGROUND: Guidelines remain unclear over whether patients with early stage oral cancer without overt neck disease benefit from upfront elective neck dissection (END), particularly those with the smallest tumours.

METHODS: We conducted a randomised trial of patients with stage T1/T2 N0 disease, who had their mouth tumour resected either with or without END. Data were also collected from a concurrent cohort of patients who had their preferred surgery. Endpoints included overall survival (OS) and disease-free survival (DFS). We conducted a meta-analysis of all six randomised trials.

RESULTS: Two hundred fifty randomised and 346 observational cohort patients were studied (27 hospitals). Occult neck disease was found in 19.1% (T1) and 34.7% (T2) patients respectively. Five-year intention-to-treat hazard ratios (HR) were: OS HR = 0.71 (p = 0.18), and DFS HR = 0.66 (p = 0.04). Corresponding per-protocol results were: OS HR = 0.59 (p = 0.054), and DFS HR = 0.56 (p = 0.007). END was effective for small tumours. END patients experienced more facial/neck nerve damage; QoL was largely unaffected. The observational cohort supported the randomised findings. The meta-analysis produced HR OS 0.64 and DFS 0.54 ($p < 0.001$).

CONCLUSION: SEND and the cumulative evidence show that within a generalisable setting oral cancer patients who have an upfront END have a lower risk of death/recurrence, even with small tumours.

CLINICAL TRIAL REGISTRATION: NIHR UK Clinical Research Network database ID number: UKCRN 2069 (registered on 17/02/2006), ISCRTN number: 65018995, ClinicalTrials.gov Identifier: NCT00571883.

British Journal of Cancer (2019) 121:827–836; https://doi.org/10.1038/s41416-019-0587-2

BACKGROUND

Oral squamous cell carcinoma (OSCC) is the eleventh most common cancer worldwide,[1] with 369 000 new cases annually and rising.[1,2] Patients with early stage disease (T1/T2) and no overt neck disease (N0) are usually treated surgically, but there has been uncertainty over the best management of the neck because of the presence of occult neck metastasis that are clinically and radiologically undetectable.[3,4] Some surgeons only resect the primary tumour, reserving neck dissection as salvage treatment for subsequent neck metastasis. Others perform elective neck dissection (END) simultaneous with the mouth tumour resection. The long-standing question remains whether delaying neck dissection until neck metastasis is clinically detectable undertreats the third[5,6] of patients with occult neck disease and prejudices their survival, or whether offering END upfront overtreats the two-thirds without occult neck disease, unnecessarily increasing morbidity and cost.

END requires a longer, more complex and expensive operation. However, those patients having primary cancer resection only who later develop neck metastases have an increased number of

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Annex 44

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Radiation Oncology

RESEARCH Open Access

Clinical outcomes of retrograde intra-arterial chemotherapy concurrent with radiotherapy for elderly oral squamous cell carcinoma patients aged over 80 years old

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Abstract

Background: The aim of this retrospective observational study was to evaluate toxicities, overall survival, and locoregional control in elderly oral squamous cell carcinoma patients who had undergone retrograde intra-arterial chemotherapy combined with radiotherapy.

Methods: Thirty-one elderly patients over 80 years old with oral squamous cell carcinoma were enrolled in present study. The treatment schedule consisted of intra- arterial chemotherapy (docetaxel, total 60 mg/m^2; cisplatin, total 150 mg/m^2) and daily concurrent radiotherapy (total, 60 Gy) for 6 weeks.

Results: The median patient age was 82.5 years old (range, 80–88 years). Of the 31 patients, six (19%) had stage II, 6 (19%) had stage III, 17 (55%) had stage IVA, and 2 (6%) had stage IVB. The median follow-up period for all patients was 37 months (range, 7–86 months). The 3-year overall survival and locoregional control rates were 78% and 81%, respectively. The major acute grade 3 adverse events were oral mucositis in 22 (71%) patients, neutropenia in 16 (52%), and dermatitis in 11 (35%). With respect to late toxicities, 1 patient (3%) developed grade 3 osteoradionecrosis of the jaw. No grade 4 or higher toxicities were observed during the treatment and follow-up periods.

Conclusions: Retrograde intra-arterial chemotherapy combined with radiotherapy was effective in improving overal survival and locoregional control even for elderly patients.

Keywords: Intra-arterial chemotherapy, Elderly patient, Head and neck cancer, Oral cancer, Radiotherapy

Introduction

The percentage of elderly patients with head and neck squamous cell carcinoma within the population is increasing as a result of the increased average age of the population. Almost 24% of patients with head and neck cancer are over the age of 70 years [1].

The majority of studies support appropriate surgical management of resectable head and neck malignancies (HNM) in elderly patients [2, 3]. With respect to the postoperative quality of life (QOL) of HNM patients, there were no significant differences between elderly and younger patients [4]. However, especially in oral cancer patients, it is obvious that some functions, such as speech, mastication, and swallowing, were more affected by the surgical intervention.

Several single-institutional studies have suggested reasonable rates of toxicities and excellent oncologic outcomes with the use of chemoradiotherapy (CRT) in elderly HNM patients. However, in such head and neck cancer clinical trials dealing with elderly patients, the age limit is often restricted to 65–75 years [5, 6]. Furthermore, in most large phase III trials evaluating concurrent CRT for HNM, the median age of enrolled

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Annex 45

Annals of Oncology

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Preoperative chemotherapy in advanced resectable OCSCC: long-term results of a randomized phase III trial

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Background: Data on preoperative chemotherapy in resectable oral cavity cancer are conflicting. We present the long-term results of a randomized trial of induction chemotherapy in resectable oral cavity cancer.

Patients and Methods: A randomized, parallel, multicentre trial evaluated the impact of three cycles of cisplatin 100 mg/m^2 and fluorouracil 1000 mg/m^2 (120-h infusion administered every 21 days) in stage T2–T4, N0–N2, previously untreated patients with advanced disease. Control group received upfront surgery. Postoperative radiation was offered to both arms when pathologic risk features were identified. The co-primary end points were the occurrence of locoregional or distant tumour relapse, and death.

Results: Among the 198 enrolled patients, with a median follow-up of 11.5 years, there was no difference in the incidence of locoregional relapse between chemotherapy and control group ($P = 0.6337$), nor in distant metastasis development ($P = 0.1527$). There was also no difference between groups in overall survival ($P = 0.3402$). Patients with a pathological complete response (pCR) had higher probability of survival than those without (10-year OS: 76.2% versus 41.3%, $P = 0.0004$). Late toxicities in patients with a minimum follow-up of 60 months (42 in each group) were similar between arms, except from fibrosis (cumulative incidence 40% versus 22% in chemotherapy arm) and grade 2 dysphagia (14% versus 5%).

Conclusions: Long-term follow-up of this randomized trial confirmed the absence of survival benefit with preoperative chemotherapy in oral cavity cancer. Late toxicity was similar in the two arms except for fibrosis and dysphagia, which were less in the chemotherapy arm. The survival benefit for patients achieving a pCR was maintained.

Key words: resectable oral cavity cancer, randomised trial, induction chemotherapy, cisplatin, fluorouracil, pathological complete response

introduction

The mainstay of locally advanced oral cavity cancer treatment lies on surgery followed by radiotherapy and chemotherapy in case of high-risk pathological features [1]. On the other side, primary chemotherapy and radiation are considered for patients whose cancer is technically or functionally unresectable [2].

Approximately half of the patients with locally advanced oral cavity cancer will not survive beyond 5 years from time of diagnosis, due to locoregional and/or distant metastases or a secondary primary tumour [3].

Moreover, surgery and radiation result in functional impairment, such as in eating, drinking and speaking difficulties, leading to a decrease in quality of life [4]. Therefore, investigation into ways to improve treatment and outcomes for patients with oral cavity cancer is warranted.

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The original trial by Licitra et al. [5], on which this article is based, focused on the use of a preoperative chemotherapy regimen and was conducted in a highly selected patient population. At that time, it was the first trial to focus solely on advanced resectable oral cavity tumours. This multicentre, randomized trial investigated the effects of induction chemotherapy on overall survival (OS) and disease relapse. Although the trial showed no survival benefit or reduction in probability of tumour relapse, it did reveal that fewer patients required a mandibulectomy and/or radiation, both of which have the potential to negatively affect patient's quality of life.

In line with the open debate about the role of induction chemotherapy, the long-term results of the above-mentioned trial of preoperative chemotherapy in patients with resectable oral cavity cancer are reported here.

patients and methods

The methodology of the original study has been fully published [5], therefore only a brief synopsis is provided here.

Annex 46

Primary Chemotherapy in Resectable Oral Cavity Squamous Cell Cancer: A Randomized Controlled Trial

By Lisa Licitra, Cesare Grandi, Marco Guzzo, Luigi Mariani, Salvatore Lo Vullo, Francesca Valvo, Pasquale Quattrone, Pinuccia Valagussa, Gianni Bonadonna, Roberto Molinari, and Giulio Cantù

Purpose: Prognosis of patients with advanced oral cavity cancer is worth improving. Chemotherapy has been reported to be especially active in oral cavity tumors. Here we repeat the results of a randomized, multicenter trial enrolling patients with a resectable, stage T2-T4 (> 3 cm), N0-N2, M0 untreated, squamous cell carcinoma of the oral cavity.

Patients and Methods: Patients were randomly assigned to three cycles of cisplatin and fluorouracil followed by surgery (chemotherapy arm) or surgery alone (control arm). In both arms, postoperative radiotherapy was reserved to high-risk patients, and surgery was modulated depending on the tumor's closeness to the mandible. Patients' accrual was opened in 1989 and closed in 1999. It included 195 patients.

Results: In the chemotherapy arm, three toxic deaths were recorded. No significant difference in overall survival was found. Five-year overall survival was, for both arms, 55%. Postoperative radiotherapy was administered in 33% of patients in the chemotherapy arm, versus 46% in the control arm. A mandible resection was performed in 52% of patients in the control arm, versus 31% in the chemotherapy arm.

Conclusion: The addition of primary chemotherapy to standard surgery was unable to improve survival. However, in this study, primary chemotherapy seemed to play a role in reducing the number of patients who needed to undergo mandibulectomy and/or radiation therapy. Variations in the criteria used to select patients for these treatment options may make it difficult to generalize these results, but there appears to be room for using preoperative chemotherapy to spare demolitive surgery and/or radiation therapy in patients with advanced, resectable oral cavity cancer.

J Clin Oncol 21:327-333.

STANDARD TREATMENT for advanced oral cavity cancer consists of a multidisciplinary approach, including surgery and radiation therapy. However, tumor control and survival are unsatisfactory, because only one half of these patients are cured.[1]

To date, the addition of chemotherapy has not been proven effective. The combination of cisplatin and fluorouracil was shown to give high response rates in untreated patients. Toxicity was mild, and feasibility of both surgery and postoperative radiation therapy was not precluded.[2,3] However, a positive effect on local regional control and survival was left to be demonstrated. Three meta-analyses, all published after this trial started, reviewed the use of chemotherapy in advanced head and neck cancer. All three concluded that, in general, chemotherapy was associated with a statistically significant advantage in survival, but that this benefit was small (4% to 6%).[4-6] It is interesting to observe that randomized trials seem to show marginal tumoricidal activity of chemotherapy at distant sites, possibly implying that the regimens used were not strong enough. In addition to suboptimal chemotherapy regimens, available data are also difficult to be interpreted for other reasons, such as relaxed selection criteria in terms of tumor site, tumor extension, and local-regional treatment, as well as low statistical power.[3,7,8] However, many clinicians do use preoperative chemotherapy in their practice, in the wake of its appealing high response rates.[9]

The present randomized trial, begun in 1989, focused on preoperative chemotherapy in a highly selected patient population, using a standard chemotherapy regimen. Indeed, randomized trials focusing on specific head and neck sites are very few, and to our knowledge, this is the first one that deals with advanced resectable oral cavity tumors only. The accrual for this multicenter randomized trial lasted 10 years, up to December 1999. The final results of the study are herein reported.

PATIENTS AND METHODS

Eligibility of Patients

The study was a randomized, multicenter, parallel group comparison trial, coordinated by Istituto Nazionale Tumori of Milan. A multidisciplinary team, including a medical oncologist, a head and neck surgeon, and a radiation oncologist, evaluated all eligible patients.

The study was open to patients who had a biopsy-proven, resectable, stage T2-T4, N0-N2, M0, previously untreated oral cavity squamous cell carcinoma. T2 lesions were included, if they were larger than 3 cm. Tumors extending into the oropharynx were accepted, provided that the lesion was contained within the oral cavity by more than 50%. Pretreatment tumor stage was categorized according to the Union Against Cancer TNM (tumor-lymph node-metastasis) Classification of Malignant Tumors (1987). Exclusion criteria were Karnofsky performance status less than 70, abnormal serum creatinine, white cell blood count ≤ 4,000 per cubic millimeters, platelet count ≤ 100,000 per cubic millimeters, and inadequate nutritional, pulmonary, and cardiac status. Other staging exams were chest x-ray, EKG, and panendoscopy.

From the Istituto Nazionale Tumori, Milan, Italy.

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Annex 47

Cochrane Library
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[Intervention Review]

High dose rate versus low dose rate intracavity brachytherapy for locally advanced uterine cervix cancer

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ABSTRACT

Background

This is an updated version of the original Cochrane review published in 2010 (Issue 7).

Carcinoma of the uterine cervix is the second most common cancer and the third leading cause of cancer death among women. Radiotherapy has been used successfully to treat cervical cancer for nearly a century. The combination of external beam radiotherapy (EBRT) and intracavity brachytherapy (ICBT) has become a standard treatment for cervical cancer. Whether high dose rate (HDR) or low dose rate (LDR) brachytherapy improves outcomes in terms of local control rates, survival and complications for women with cervical cancer remains controversial.

Objectives

To assess the efficacy and safety of HDR versus LDR ICBT in combination with EBRT for women with uterine cervical cancer.

Search methods

We searched the Cochrane Gynaecological Cancer Group Specialised Register and the Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2014, Issue 1), MEDLINE (1966 to March 2014), EMBASE (1974 to March 2014), and the Chinese Biomedical Literature Database (CBM) (1978 to March 2014) for relevant original, published trials.

Selection criteria

Randomised controlled trials (RCTs) and quasi-RCTs that compared HDR with LDR ICBT, combined with EBRT, for women with locally advanced uterine cervical cancer.

Data collection and analysis

Two authors independently extracted the data using standardised forms. Primary outcome measures included overall survival (OS), relapse-free survival (RFS) and pelvic control rate, while secondary outcomes included rates of recurrence and complications.

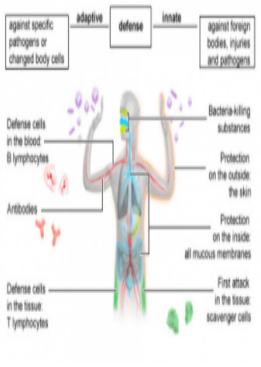
Annex 48

InformedHealth.org [Internet]. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG); 2006-.

The innate and adaptive immune systems

Last Update: July 30, 2020; Next update: 2023.

The immune system fights germs and foreign substances on the skin, in the tissues of the body and in bodily fluids such as blood. The immune system is made up of two parts: the innate (general) immune system and the adaptive (specialized) immune system. These two systems work closely together and take on different tasks.



The innate immune system: Fast and general effectiveness

The innate immune system is the body's first line of defense against germs entering the body. It responds in the same way to all germs and foreign substances, which is why it is sometimes referred to as the "nonspecific" immune system. It acts very quickly: For instance, it makes sure that bacteria that have entered the skin through a small wound are detected and destroyed on the spot within germs from spreading, though.

The innate immune system consists of

- Protection offered by the skin and mucous membranes
- Protection offered by the immune system cells (defense cells) and proteins

Protection offered by the skin and mucous membranes

All outer and inner surfaces of the human body a key part of the innate immune system. The closed surface of the skin and of all mucous membranes already forms a physical barrier against germs, which protects them from entering. Additionally, chemical substances like acid, enzymes or mucus prevent bacteria and viruses from gaining a foothold. Movements created, for example, by hair-swirling in the body. Tear fluid, sweat and urine (which flushes the organs of the urinary tract) have a similar effect.

Protection offered by the immune system cells (defense cells) and proteins

The innate immune system activates special immune system cells and proteins if germs get past the skin and mucous membranes and enter the body.

What happens during an inflammation?

When a part of the skin is infected, immune system cells move to the area or immune system cells that are already there are activated. Specific immune system cells release substances into the immediate area that make the blood vessels wider and more permeable. This causes the area around the infection to swell, heat up and redden, and inflammation results. A fever may develop as well. T cells arrive.

Certain proteins (enzymes) are also activated to help in the immune response (see below).

Scavenger cells: Neutralizing germs

Bacteria or viruses that enter the body can be stopped right away by scavenger cells (phagocytes). Scavenger cells are special kinds of white blood cells (leukocytes). These cells enclose germs and "digest" them. The remains of these germs move to the surface of the scavenger cells to be detected by the adaptive immune system.

There are also other types of immune system cells that release substances to kill bacteria and various germs. Both germs and body tissue and immune system cells die and decay during an immune system response. Their remains form pus, a yellowish fluid.

The role of proteins

Several proteins (enzymes) help the cells of the innate immune system. A total of nine different enzymes activate one another in a process similar to a chain reaction. One enzyme in the first stage alerts several enzymes of the second stage, each of which again activates several enzymes of the third stage, and so on. This allows immune system responses to escalate very quickly.

The tasks of these enzymes include:

- marking germs as targets for scavenger cells,
- attracting other immune system cells from the bloodstream,
- destroying bacteria cell walls to kill them, and
- fighting viruses by destroying the viral envelope (the outermost layer of a virus) or cells that have been infected with viruses.

Annex 49

REVIEW ARTICLE
Year : 2019 | Volume : 11 | Issue : 6 | Page : 107--111

Immunotherapy in oral cancer

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Abstract

Immunotherapy is one of the newer entities which is promising, at least can be very much helpful as an adjuvant therapy. This newer modality of the treatment in the field of cancer treatment may be the fourth pillar supporting surgery, chemotherapy, and radiotherapy. Careful selection of patient is the key for success of immunotherapy, which is based on patient's immunological contexture. This review aimed to present the fundamental aspects of tumor immunity and immunotherapy, focused on oral squamous cell carcinoma.

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Full Text

Introduction

Immune system is the guardian of our body; it detects and destroys abnormal cells that are found in milieu. Abnormal cells may be foreign bodies, microorganisms, and even cancer cells. Ehrlich proposed that the immune system can search and attack transformed cells before any clinical presentation. Though cancer cells originate in the body, their genetic heterogeneity and components make them noticeable to the immune system. William Coley in 1891 found regression in cancer when he injected inactivated bacterial toxin (Coley's toxin).[1] The immune system mainly comprises two arms, namely, innate and adaptive immunity.

The innate immunity encompasses macrophages, natural killer (NK) cells, dendritic cells, and eosinophils, and the adaptive immunity includes B and T lymphocytes, commonly known as B and T cells. B cells produce antibodies and T cells generate CD4+ and CD8+ cells. Cancer cells escape immune system by decreased expression of cell surface antigen, by secreting antigen that inactivates immune system, and by inducing microenvironment to secrete substances suppressing immune responses, thereby promoting tumor growth.[2]Immunotherapy involves the stimulation of specific components of immune system, thereby strengthening it to counteract the signals that suppress the immune system.

Immune Surveillanceand Immune Editing

The concept of immune surveillance was later discovered when the tumor-associated antigen was discovered in transplanted animal model.[3] The immune modulators such as levamisole were used for adjuvant therapy in colorectal cancers but had guarded results. Bacillus Calmette-Guérin (BCG), a well-known tuberculosis vaccine, has shown tumor regression in bladder cancer when injected intravesically.[4]

The immune surveillance has evolved into immune editing (a new concept put forward by Schreiber et al.[5]), which comprises three phases. The immunosurveillance or elimination phase is the first phase, in which the tumor growth is controlled by destruction of nascent cancer cells by T-cell activation via antigen presentation. Equilibrium phase is the second phase, which is characterized by tumor heterogeneity due to genetic instability of cells. In this phase, the tumor growth is in a steady state, either by growth enhancement or by inhibition. The escape phase is the final phase in which tumor cells escape or suppress immune system, thereby leading to tumor progression.[5],[6]

Tumor Microenvironment

Recently, understanding of oncogenesis has changed its dimension, apart from tumor cells. Tumor microenvironment (TME) also plays a major role in tumor progression and its immunology. TME constitutes fibroblasts, stromal cells, immune cells, and endothelial cells, which actively take part in oncogenesis and immunology of tumor. The immune cells have promoting and inhibitory functions on tumor: M2 macrophages, myeloid-derived suppressor cells (MDSCs), regulatory T cells (Treg), and CD4 type 2 helper T cells(TH2) are promoter cells whereas NK cells, CD8+ T cells, M1 macrophages, CD4 type 1 helper T cells (TH1), and dendritic cells inhibit tumor. The tumor milieu is maintained by balance in protumor and antitumor immune cells, which in turn is governed by specific chemokines and adhesion molecules.[7] The immune cells can be found within tumor core, invasive front, or in tertiary lymphoid structures (TLS). The high endothelial venules are specialized vascular structures that surround the TLS and help in recruiting immune cells.[8] The understanding of "immune contexture" is essential in immunotherapy, the terminology that refers to the immune cells distribution in the core of the tumor and its microenvironment.[7]

Immunotherapy

Immunotherapy can be broadly divided into active and passive.

Active immunotherapy involves attack of tumor cells by directing immune system (tumor as target). The immune cells were derived from blood or tumor of the patient, cultured in laboratory, and put back into the body, which in turn attack the tumor cells. In active immunotherapy, NK cells, dendritic cells, and cytotoxic T cells were commonly used.

Passive immunotherapy involves enhancement of immune system by targeting cell surface receptors, which in turn can form antibody-dependent cell-mediated (immunity) cytotoxicity (ADCC), for example, ipilimumab.

Annex 50

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Neoadjuvant Immunotherapy of Oral Squamous Cell Carcinoma Modulates Intratumoral CD4/CD8 Ratio and Tumor Microenvironment: A Multicenter Phase II Clinical Trial

József Tímár, Andrea Ladányi, Csaba Forster-Horváth, Júlia Lukits, Balázs Döme, Éva Remenár, Mária Gődény, Miklós Kásler, Beáta Bencsik, Gábor Répássy, György Szabó, Norbert Velich, Zsuzsa Suba, János Élő, Zsuzsa Balatoni, Károly Pócza, Béla Zemplén, Paul Chretien, and Eyal Talor

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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0732-183X/05/2315-3421/$20.00

DOI: 10.1200/JCO.2005.06.005

A B S T R A C T

Purpose
To investigate the clinicopathologic effects of local neoadjuvant Leukocyte Interleukin Injection (LI) regimen in oral squamous cell carcinoma (OSCC) patients. Treatment regimen included LI 800 IU/d as interleukin-2 (IL-2), administered half peritumorally and half perilymphatically five times per week for 3 weeks; low-dose cyclophosphamide; indomethacin; zinc; and multivitamins.

Patients and Methods
Thirty-nine patients diagnosed with T2-3N0-2M0 OSCC participated in the pathology portion of this phase II multicenter study (19 LI-treated patients and 20 historical controls). Clinical responses were determined by imaging. Paraffin-embedded tumor samples were obtained at surgery for all patients. Surgery for the LI-treated group was performed between days 14 and 54 after the end of treatment. Histologic evaluation, pathologic staging, necrosis, and American Joint Committee on Cancer grading were performed from hematoxylin and eosin sections. Immunohistochemistry and morphometry determined cellular infiltrate.

Results
Two pathologically complete, two major (> 50%), and four minor responses (> 30% but < 50%) resulted from LI treatment (overall response rate, 42%). Histopathology showed that the intratumoral $CD4^+$:$CD8^+$ ratio was low (< 1) in patients not treated with LI (controls). An increase in tumor-infiltrating $CD4^+$ and a decrease of $CD8^+$ T cells was observed in LI-treated patients, leading to a significantly ($P < .05$) higher intratumoral $CD4^+$:$CD8^+$ ratio (> 2.5). This was paralleled by dendritic cell transition from tumor surface toward stromal interface ($P < .05$), with macrophage decrease and neutrophil accumulation, multifocal microscopic necrosis, and significant ($P < .05$) increase in tumor stroma of LI-treated patients compared with controls.

Conclusion
LI-treated OSCC patients were characterized by a markedly altered composition of tumor-infiltrating mononuclear cells, increased $CD4^+$:$CD8^+$ ratio, and increased tumor stroma to epithelial ratio, all of which were distinct from controls.

J Clin Oncol 23:3421-3432. © 2005 by American Society of Clinical Oncology

INTRODUCTION

Tumor-host interaction is a complex feature of tumor progression and is an increasingly important target for anticancer strategies.[1] It involves cellular interactions between cancer cells, immune effector cells, and inflammatory cells, as well as cells of the tumor vasculature and the stroma. Because of this complex and multifaceted interaction, the therapeutic modulation of the function of individual members of this complex interactive network

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Xiong et al. BMC Cancer (2020) 20:229
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BMC Cancer

RESEARCH ARTICLE Open Access

Immunological effects of nivolumab immunotherapy in patients with oral cavity squamous cell carcinoma

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Abstract

Background: Although checkpoint blockades have become widely used, the immunological impact in cancer patients, especially those with oral cavity squamous cell carcinoma (OCSCC), has not been well studied.

Methods: The present study assessed the immunological impact of anti-PD-1 (nivolumab) treatment in 10 patients with OCSCC. This involved phenotypic analyses of peripheral blood T-cell subpopulations and their expression of immune mediators prior to and following nivolumab treatment. The focus was on immunological effects of treatment without regard to possible clinical responses.

Results: Nivolumab caused a decline in the frequency of blood $CD4^+$ cells but did not affect their expression of IFN-γ. However, nivolumab increased the proportion of $CD4^+$ cells expressing the Treg-supporting factor Foxp3. Nivolumab treatment caused an increase in the proportion of $CD8^+$ cells. While their expression of granzyme B increased, it did not attain significance. Analyses of $CD8^+$ cell subpopulations showed nivolumab caused an increase in levels of unconventional $CD8^{dim}CD3^+$ T-cells. It also caused an increase in expression of granzyme B by these unconventional T-cells as well as by the conventional $CD8^{hi}CD3^+$ cells. The $CD8^{hi}CD3^+$ subpopulation also had a near-significant increase in IFN-γ expression. Treatment with nivolumab had no effect on the levels of the NK containing $CD8^{dim}CD3^-$ subpopulation of cells or their expression of IFN-γ or granzyme B.

Conclusions: These results show nivolumab causes opposing effects on $CD4^+$ and $CD8^+$ cell populations, with $CD4^+$ cell levels declining but increasing the proportion of Treg cells, and unconventional $CD8^+$ T-cell levels increasing with increased expression of immune mediators by $CD8^+$ T-cell subpopulations.

Keywords: Anti-PD-1 antibody, CD4, CD8, Granzyme B, Immune, Interferon-γ, Nivolumab, T-cell

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Annex 52

Review Article

Photodynamic Therapy as a Treatment Option for Oral Cancer and Dysplasia

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Abstract

The challenge in cancer management is to find a balance between the intended therapeutic outcomes whilst preserving and maintaining function and aesthetics. This dilemma initiated ongoing efforts focusing on PDT which is now considered a historical re-utilized technique showing promising results but with many limitations. PDT has emerged as a successful and clinically acceptable therapeutic approach to the management of malignant and benign diseases. It is important to understand that PDT cannot completely replace other treatment modalities but can be used as a useful adjunct or as additional treatment.

Keywords: Dysplasia; Photodynamic therapy; Carcinoma; Treatment

Introduction

In order to successfully manage head and neck squamous cell carcinomas (HNSCC), it is imperative that function and aesthetics are maintained as much as possible. Improving loco-regional disease control rates has been somewhat the focus of research in the last 30-40 years. Non-surgical treatment approaches such as PDT can be applied to keep speech and swallowing function but with less than optimal improvements in survival. [1,2]

Nevertheless, it is immediately clear that advances in the management came at the expense of altering function and changing aesthetics. This was so that the ultimate goal of loco-regional control of the disease was achieved. [3,4]

The challenge in cancer management is to find a balance between the intended therapeutic outcomes whilst preserving and maintaining function and aesthetics. This dilemma initiated ongoing efforts focusing on PDT which is now considered a historical re-utilized technique showing promising results but with many limitations. Photodynamic therapy (PDT) is a treatment modality that is minimally invasive. Unlike ionizing radiation, PDT has the advantage that it can be applied repeatedly at the same site. PDT utilization in medicine had been widely accepted and it is an appealing option in oncology because the use of chemotherapy, ionizing radiation, or surgery does not preclude the use of PDT, and all of these approaches can be used in a patient who has received PDT.

PDT is not an alternative approach to the common used modalities however, there are certain circumstances were PDT can be used as an alternative approach. Although PDT is still emerging, it is already a successful and clinically approved therapeutic modality used for the management of neoplastic and nonmalignant diseases. [5]

PDT structure

PDT utilizes three components to achieve the intended effect on tumor cells and these essential components are: a photosensitizer (PS), light, and oxygen. The essential components are ineffective on tumor cells when exposed to the cells as single modalities. However, when they join together a highly reactive product, single, is formed causing significant toxicity resulting in cell death via apoptosis or necrosis. [6,7]

There are three mechanisms that cause the anti-tumor effects of PDT. These include the direct cytotoxic effects on tumor cells, damage to the tumor vascularity, and an inflammatory reaction leading to the development of systemic immunity. [8]

The therapeutic effect of PDT production of singlet oxygen

The basics of the photochemical reaction which initiates the process of tumor cell destruction is dependent on the excitation of the tissue oxygen located in the body by the injected

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Photodynamic therapy treatment of early oral and laryngeal cancers

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Abstract

Photodynamic therapy (PDT) is a nonsurgical, minimally invasive treatment that uses a light source to activate light-sensitive drugs or photosensitizers in the treatment of cancer and other diseases. PDT has been successfully employed to treat early carcinomas of the oral cavity and larynx preserving normal tissue and vital functions of speech and swallowing. Two hundred seventy-six patients with early carcinomas of the oral cavity and larynx were treated from 1990 to 2006. Cure rates with a single treatment for early laryngeal and oral cancers were 91% and 94%, respectively. PDT is an effective primary and alternative treatment modality for early oral cavity and laryngeal cancers.

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RESEARCH ARTICLE Open Access

Selective intra-arterial infusion of rAd-p53 with chemotherapy for advanced oral cancer: a randomized clinical trial

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Abstract

Background: In this study, a combination of recombinant adenoviral p53 (rAd-p53) gene therapy and intra-arterial delivery of chemotherapeutic agents for treatment of oral squamous cell carcinoma was evaluated.

Methods: In total, 99 patients with stage III or IV oral carcinoma who had refused or were ineligible for surgery were enrolled in a randomized, placebo-controlled, double-blind, phase III clinical trial. They were randomly assigned to group I (n = 35; intra-arterial infusion of rAd-p53 plus chemotherapy), group II (n = 33; intra-arterial infusion of rAd-p53 plus placebo chemotherapy), or group III (n = 31; intra-arterial infusion of placebo rAd-p53 plus chemotherapy).

Results: The median length of follow-up was 36 months (range, 3 to 86 months). During follow-up, 16 patients in group I, 20 in group II, and 22 in group III died. Group I (48.5%) had a higher complete response rate than groups II (16.7%) and III (17.2%) ($P = 0.006$). The rate of non-responders in group I was significantly lower than that in groups II and III ($P < 0.020$). A log-rank test for survival rate indicated that group I had a significantly higher survival rate than group III ($P = 0.019$). The survival rate of patients with stage III but not stage IV oral cancer was significantly higher in group I than in group III ($P = 0.015$, $P = 0.200$, respectively). The survival rate of patients with stage IV did not differ significantly among the three groups. Of the 99 patients, 63 patients experienced adverse events of either transient flu-like symptoms or bone marrow suppression, while 13 patients had both these conditions together. No replication-deficient virus was detected in patient serum, urine, or sputum. rAd-p53 treatment increased Bax expression in the primary tumor of 80% of patients, as shown by immunohistochemical staining.

Conclusions: Intra-arterial infusion of combined rAd-p53 and chemotherapy significantly increased the survival rate of patients with stage III but not stage IV oral cancer, compared with intra-arterial chemotherapy. Intra-arterial infusion of combined rAd-p53 and chemotherapy may represent a promising alternative treatment for oral squamous cell carcinoma.

Trial registration: ChiCTR-TRC-09000392 (Date of registration: 2009-05-18).

Keywords: Oral carcinoma, Gene therapy, Chemotherapy, Intra-arterial infusion, p53

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Annex 55

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ORIGINAL ARTICLE

Randomized, controlled phase II study of post-surgery radiotherapy combined with recombinant adenoviral human p53 gene therapy in treatment of oral cancer

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The aim of this study is to evaluate clinical benefits of recombinant adenoviral human p53 (*rAd-p53*) gene therapy combined with radiotherapy in prevention of oral cancer recurrence after a radical resection. A total of 51 patients with tongue cancer (TCa) and 56 patients with gingival carcinoma (GCa) satisfying the inclusion criteria were randomly assigned to two groups: the experiment group (EG) and the control group (CG). The EG group received multipoint injections of rAd-p53 into the surgical wound surface at a dose of 1×10^{12} viral particles after a radical resection. Patients in both EG and CG were given radiotherapy at a total dose of 60 Gy at 3 weeks after surgery. All these patients were followed up for at least 3 years. Two cases (2/27) of TCa and 2 (2/30) in GCa patients had a local recurrence in EG, but 8 (8/24) TCa and 8 (8/26) GCa patients in CG had a local recurrence. Both recurrent rates of TCa (33.3%) and GCa (30.8%) in CG are statistically significantly higher than those of TCa (7.4%) and GCa (6.7%) in EG, respectively. The overall recurrent rate in EG is 7%, which is also statistically significantly lower than that (32%) in CG. The 3-year overall survival (OS) rate and 3-year disease-free survival (DFS) rate of EG is 100% and 93%, respectively. The 3-year OS and DFS rates of CG are 94 and 68%, respectively. Mild or medium fever and flu-like symptoms were more frequently observed in EG and were considered to be associated with application of rAd-p53. Post-tumorectomy wound surface injection of rAd-p53 combining with radiotherapy is a safe and effective regimen for the patients with TCa or GCa.

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Keywords: oral cancer; recombinant adenoviral human p53

INTRODUCTION

The *p53* gene has been well characterized and is one of most important tumor suppressor genes. It has multiple antitumor functions, including blocking cell cycle, inducing cell apoptosis, inhibiting tumor angiogenesis and sensitizing tumor to chemotherapy and radiotherapy. p53 mutation occurs in up to 63% of oral squamous carcinoma.[1,2] Loss of normal functions of p53 is associated with tumorigenesis, chemotherapy and radiotherapy resistance.[3,4] Gendicine is the brand name of recombinant adenoviral human *p53* gene (*rAd-p53*), in which, a wild-type *p53* gene was inserted into a replication-defective type-V adenovirus. A phase II clinical trial of the *rAd-p53* gene therapy plus radiotherapy compared with radiotherapy alone in treatment of patients with advanced head and neck cancer showed that the combined therapy significantly improved the 5-year overall survival (OS) and progression-free survival.[5] Several reports demonstrated that rAd-p53 was effective for advanced lung, liver, breast and cervical cancers.[6–10]

Surgery is a mainstay of treatment for oral cancer. Radiotherapy with or without systemic chemotherapy is usually used for an adjuvant to surgery, or for cases unable to tolerate or unsuited for surgery, or as a salvage treatment.[11] The tumor stage, size, location and patient's general condition are factors that influence treatment options. Although progress has been made in treatment of oral squamous carcinoma, the 5-year OS rate is still kept around 50%.[12] Local recurrence is a main reason of treatment failure.[13–16] On the basis of favorable safety profile and synergistic antitumor effect with radiotherapy of rAd-p53,[5–10] we hypothesize that post-surgery *p53* gene therapy combined with radiotherapy can further reduce local recurrence and improve survival in oral cancer.

MATERIALS AND METHODS

The study was approved by the hospital ethics committee (Ethics Committee, 301 Military General Hospital, Beijing 100853, China), but was not registered in an international clinical trial registry platform. All the enrolled patients signed a consent form.

Patients

From January 2005 to April 2011, a total of 107 patients with tongue cancer (TCa) or gingival carcinoma (GCa) satisfying the inclusion criteria were randomly assigned to two groups: the experiment group (EG) and the control group (CG). The EG group included 57 patients, 27 of them with TCa and 30 with GCa, and the CG group included 50 patients, 24 with TCa and 26 with GCa. The randomization schedule was generated using the SAS PROC PLAN procedure (SAS Institute, Cary, NC, USA). The inclusion criteria are as follows: histopathologically diagnosed TCa or GCa; those carcinomas that were considered as resectable; patients over 18 years old; those with an Eastern Cooperative Oncology Group score of 0–2; those with normal hemogram, blood coagulation, liver and kidney function tests; those who understood and signed the informed consent form. The patients' characteristics and disease stage are summarized in Table 1 by the treatment group. The sixth edition of the tumor node metastasis-staging system for head and neck is used for staging tumors.[17]

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