GRADUATION PROJECT

Degree in Dentistry

Genetic biomarkers in head and neck immunotherapy

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ABSTRACT:

Introduction: Squamous cell carcinoma is known to be the type of head and neck cancer with the most prevalence. When managing these patients it is essential to understand what treatment option is preferred, one of which being immunotherapy. Within immunotherapy, the use of biomarkers and immunotherapeutic agents is vital' **Objectives**; Evaluate the epidemiological factors related with head and neck cancer and identify the genetic biomarkers of immunotherapy used in patients with head and neck cancer. Furthermore identify biomarkers used in treatments of immunotherapy such as PD-1/PDL-1and combination treatment; **Methodology**: Using the CBioPortal we studied a sample of 37 patients with oropharynx squamous cell carcinoma and used their tumoral mutational burden as a predictive biomarker. Within this sample it was also possible to study the genetic mutations of those with primary vs metastatic cancer and treated with PD-1/PDL1 or combination treatment; **Results**: Male population was affected greater than female population for oropharynx squamous cell carcinoma and the greatest age for diagnosis was 61-70 years old. A larger number of patients had metastasis rather than primary tumor site. For those patients that received PD1/PDL-1 therapy and were male who had a primary tumor site, the survival rate was higher compared with combination treatment. Patients who received combination treatment were male and were deceased. Certain mutated genes responded better to either treatment; **Conclusions**: The identification and analysis of genetic biomarkers have shown promising results and we used a TMB as the potential biomarker of choice using CBioPortal. This may aid in personalized treatment options for patients. The use of genetic biomarkers has the potential to improve the treatment outcome for cancer patients and it should be a focus area for immunologists in the future.

Keywords: Dentistry; Immunotherapy; head and neck cancer; genetic biomarkers; PD-1/PDL-1; combination

RESUMEN:

Introducción: El carcinoma de células escamosas se conoce como el tipo de cáncer de cabeza y cuello más prevalente. Al tratar a estos pacientes, es esencial entender cuál es la opción de tratamiento preferida, una de las cuales es la inmunoterapia. Dentro de la inmunoterapia, el uso de biomarcadores y agentes inmunoterapéuticos es vital; **Objetivos**: Evaluar los factores epidemiológicos relacionados con el cáncer de cabeza y cuello e identificar los biomarcadores genéticos de inmunoterapia utilizados en pacientes con cáncer de cabeza y cuello. Además, identificar biomarcadores utilizados en tratamientos de inmunoterapia como PD-1/PDL-1 y tratamiento combinado. **Metodología**: Usando el CBioPortal, estudiamos una muestra de 37 pacientes con carcinoma de células escamosas de orofaringe y utilizamos su carga mutacional tumoral como biomarcador predictivo. Dentro de esta muestra, también fue posible estudiar las mutaciones genéticas de quienes tenían cáncer primario vs metastásico y fueron tratados con PD-1/PDL1 o tratamiento combinado; **Resultados**: La población masculina se vio más afectada que la población femenina por el carcinoma de células escamosas de orofaringe y la mayor edad para el diagnóstico fue de 61-70 años. Un número mayor de pacientes tenía metástasis en lugar de tumor primario. Para aquellos pacientes que recibieron terapia PD1/PDL-1 y eran masculinos que tenían un sitio tumoral primario, la tasa de supervivencia fue mayor en comparación con el tratamiento combinado. Los pacientes que recibieron tratamiento combinado eran masculinos y fallecieron. Ciertos genes mutados respondieron mejor a uno o otro tratamiento; **Conclusiones**: La identificación y análisis de biomarcadores genéticos han mostrado resultados prometedores y utilizamos una TMB como el biomarcador potencial de elección utilizzando CBioPortal. Esto puede ayudar en opciones de tratamiento personalizadas para los pacientes. El uso de biomarcadores genéticos tiene el potencial de mejorar el resultado del tratamiento para pacientes con cáncer y debería ser un área de enfoque para los inmunólogos en el futuro.

Palabras clave: Odontología; inmunoterapia; cáncer de cabeza y cuello; biomarcadores genéticos; PD-1/PDL-1; combinado.

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1. **INTRODUCTION**.

1.1. Generalities and epidemiology

Cancer is the second most common cause of mortality worldwide with a great variety at tissue level, therefore causes major challenges for its specific diagnosis, and to determine the efficacy of treatments available. (3). Cancer or 'tumors' disrupt cellular reactions and cause a dysfunction of vital genes, thus affecting the bodily cell turnover, leading to an abnormal proliferation which is normally regulated by proto-oncogenes. However when proto-oncogenes become oncogenes through successive mutations, this triggers a series of cells division creating a 'tumor' (3). The tumor microenvironment is a mixture of fluids, immune cells and blood vessels which surround the tumor. Interactions between tumor cells and the tumor microenvironment favor its development and choose evasion and escape from immune surveillance.(4)

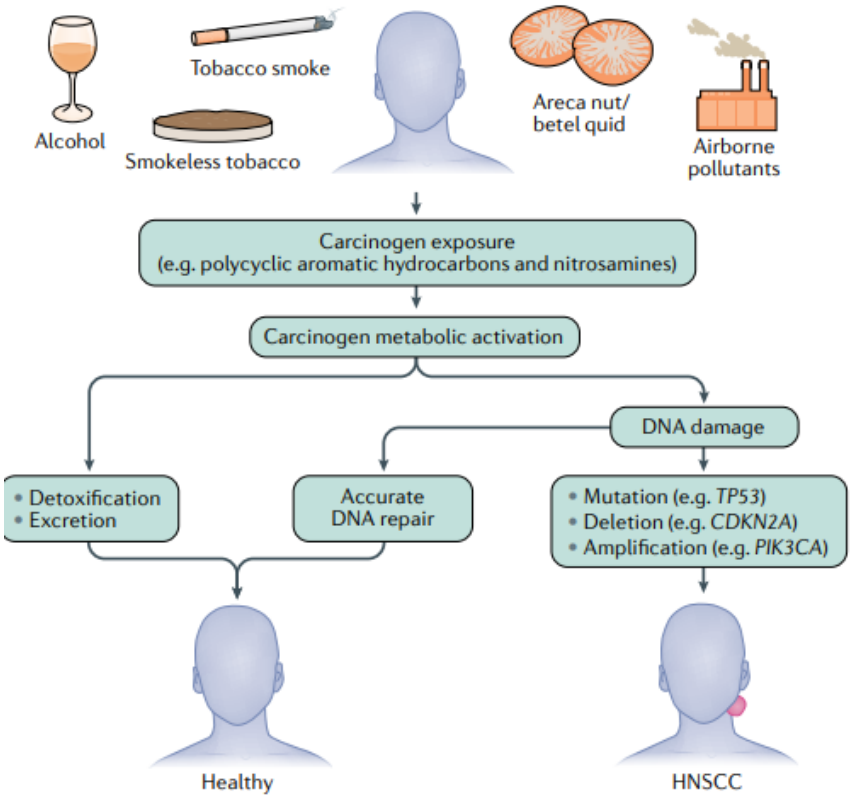
Squamous cell carcinoma (HNSCC) is known to be the type of head and neck cancer with the most prevalence. It has an average 5 year survival rate of 47%. (5)
Worldwide, *GLOBACON* have estimated an incidence level of head and neck cancer of approximately 830,000 new cases in 2020. These arose from the lip, oral cavity (377,713), larynx (184,615), nasopharynx (133,354), hypopharynx (84,254), and salivary glands (53,582) (5).
As it stands in 2020, head and neck cancer had a morbidity rate of 3.4% of global deaths related to cancer. This accounts for 420,000 patients according to *GLOBACON,* a vast phenomenon. (4) The majority of these patients presented with advanced stage III–IV disease and with loco-regional spread. (4)

1.2. Etiology

Alterations in tumor suppressor genes are a major oncogenic event in head and neck squamous cell cancer. Common somatic mutations are involved in the following genes: CDKN2A (22% of tumors), TP53 (72% of tumors), FAT1, NOTCH1, PIK3CA, KMT2D and NSD1. (6)

There are many etiological factors involved in head and neck cancer types, including environmental risk factors and even though they are highly preventable, they account for 75% of cases. These include tobacco, heavy alcohol consumption and prolonged sun exposure. Heavy consumption of alcohol and tobacco is known to have a >35 fold higher risk for developing HNSCC. (7)

Figure 1: *Development of carcinogen-associated, HPV negative HNSCC* (8)



1.2.1. HPV and the head and neck

Despite the increasing educational preventive programs for environmental risk factors such as alcohol and tobacco, human papilloma virus-type 16 is a significant genotype risk factor, culpable for the overall increasing number of cancer cases in the oropharyngeal region.

HNSCC patients with HPV are more commonly found in younger patients who are non-smokers. HPV related cancers has a unique risk factor profile, but has a more favorable prognosis than the other environmental risk factors discussed. (9) The virus is a known prognostic biomarker as it can be easily detected. The viral genome integrates itself into the host cellular genome by the expression of E6 and E7 viral oncoproteins, degradation of P53 (retinoblastoma protein, a tumor suppressor gene) and inactivation of the protein RB (proto-oncogenic tumor suppressor) which inhibits cell cycle progression. (4) Primary prevention of HPV related cancer includes the use of the new 'Gardasil-9' vaccine that is recommended for all individuals between the ages of 9-26 and has shown an 88-93% efficacy in reducing HPV type-16/18/31/33/52/55 related diseases. (7)

1.3. Traditional therapies

Traditional therapy of HNSCC involves surgery followed by radiation or chemotherapy. An additional latest first line treatment was also approved in 2009, known as 'trans-oral robotic surgery'. Following that trimodal therapy is recommended, for example the use of support teams that aid in speech and swallow therapy and physical therapy for rehabilitation. Accompanying, occupational therapy, smoking cessation and nutritional programs are highly advocated. (4)

Surgery, chemotherapy, or radiation are most effective when detected early before rapid growth, spread to lymph nodes and metastasis have occurred. These forms of therapy are most essential in HPV negative related carcinomas, due the unique immunophenotype of human papilloma virus. (10)

1.3.1. Surgery

The goal of surgery is to directly remove the tumor along with associated lymph nodes, restore structure, as well as preserving functions such as speaking, swallowing and expression. (11). Open surgery involves directly removing the tumor if it has a large presentation but generally results in significant scarring. Transoral surgery functions by accessing the mouth with the use of robotic devices and is typically used to treat smaller cancers in the mouth, tongue, throat, or tonsils. (12) In comparison to traditional surgery, transoral surgery may reduce the risks of side effects such as swallowing difficulties and result in quicker remission, less scarring and shorter hospitalization. It creates a more accurate incision and successful procedure. Overall surgery in general, is a very rigorous treatment and has not proven to be very efficacious in long term recuperation…locoregional recurrence remains the most common surgical failure for patients with HNSCC (12).

1.3.2. Chemotherapy

In comparison to surgery, concurrent cisplatin-based chemotherapy often provides a better prognosis, but the 5-year survival rate remains suboptimal, more specifically in HPV negative cancer patients. Cisplatin in general exacerbates acute toxicities of mucositis and dermatitis as well as nausea, vomiting, neutropenia, kidney damage, tinnitus, and peripheral neuropathy, thus significantly affecting the overall quality of life for the patient. (13) A significant number of patients cannot withstand the side effects related to cisplatin therapy every 3 weeks during radiation. (13)

1.3.3. Radiation

Research demonstrates severe toxic side effects associated to radiation therapy of the head and neck region. One in five patients who receive radiation are known to have mucositis, which is dose limiting and affects patients in different severity according to their unique oral microbiome. Radiation may cause partial muscle paralysis, dysgeusia,

xerostomia and reversible damage to salivary glands. (7) Osteonecrosis may be induced, causing patients to be vulnerable to fractures in the mandible, which is caused due to low perfusion to the osseous structures. Furthermore such destruction to the oral microbiome may result in opportunistic infections, such as the growth of bacteria *Porphyromonas gingivalis* and *Fusobacterium Nucleatum* which cause periodontal disease. Lastly, aspiration pneumonia is another infection that may arise due to impaired swallowing, xerostomia, thickened oral secretion and mucositis.(7)

Despite recent advances in treatments, cancer in the head and neck region remains to be a significant challenge particularly to patients but also to health organizations worldwide.(8) As the head and neck region is anatomically complex, not to mention highly vascular and innervated through nerves, treatment can often be invasive and often unsuccessful. Overall, single modality therapy may cause extreme side effects affecting quality of life of the patient and the potential need for cessation of therapy and hospitalization.

1.4. Introduction to immunotherapy

William B. Coley, now known as the Father of Immunotherapy, first attempted to use the immune system for the treatment of cancer in the late 19th century. Since then, it has constantly been developed and recognized for its success. He subsequently was awarded a Nobel prize due to his thriving research. (14).

Head and neck squamous cell carcinoma has a high tumoral mutational burden (TMB) and immune infiltration.(8) Immunotherapy has become one of the most current standards of care, due to its benefits in terms of tumor progression control. The mechanism involves counterbalancing the resistant mechanisms used by the tumor, which in turn helps the endogenous immune system to reject it.(15) In addition, it aims to reactivate anti-tumor immune cells and overcome immune escape mechanisms.

This form of therapy has proven to have a high success rate (more specifically in HPV-related cancers), in preventing long term regression of stubborn tumors which have not been successful with other treatments (15). It results in a relatively high survival and response rate 12-24 months after treatment for cases of recurrent or metastatic HNSCC, and this therapy has been approved as a neoadjuvant therapy option for previously untreated cases. (16)

1.4.1. What are biomarkers

The use of biomarkers in immunotherapy is a highly specialized method in medicine. A biomarker is "a biological molecule found in blood, other bodily fluids and tissue, secreted by a tumor or during a specific response, that signifies normal or abnormal process of a condition or disease" defined by the National Cancer Institute (4). They ideally are collected non-invasively through blood or serum. (1)

1.42 Uses of biomarkers

Excitingly, biomarkers are being explored at a genetic, soluble, and cellular level and have a wide variety of uses, such as risk assessment of a patient to detect if they have a certain predisposition to a particular cancer by studying mutations. Mutant proteins detected are the most specific example of biomarkers and are secreted directly by the existing tumor. (16,17) Examples of biomarkers include P-16 and P-14 which are tumor suppressor proteins that are used in the diagnosis and prognosis of HNSCC, and high levels of expression of these proteins indicate better prognosis and survival rate. RB is a gene which regulated cell growth and division and is often mutated in cancer cases. Low levels of RB1 expression are often associated with poor survival rate, therefore being a useful biomarker. (18)

Some other significant biomarkers aid in selecting certain patients who will best respond well to therapy. Examples in head and neck immunotherapy include the PD-L1 expression; PD-L1 is a protein expressed on the surface of tumor cells and it inhibits immune response against cancer. Tumors expressing high levels of PD-L1 have shown

promising results as it can be used in targeted immunotherapy.(19) Other biomarkers include tumor infiltrating lymphocytes, which are immune cells that infiltrate cancerous tissues and are involved in the immune response against tumors and cytokine expression patterns, such as interferon-gamma and interleukin-12. These can help to predict treatment response to immunotherapy and modulate immune responses against cancer. (1)

Additionally, biomarkers have proven to be useful in the diagnosis process to determine if a tumor originates from a primary or metastatic origin by screening chromosomes in both sites. Biomarkers are furthermore useful in the determination of the prognosis, and in the process of pharmacodynamics and pharmacokinetics to help with drug dosing in treatment of the cancer. (18)
They aid in the identification of a tumors and in evaluating the successful outcome of a treatment, as well as monitoring disease progression and the individual's risk of recurrence. This process in turn improves the quality of life and reduces the cost of patient care. (20)

Table 1: *The use of Biomarkers* (1) *Basheeth and Patil, 2019*

Table 1 has been made by summarizing the most relevant biomarkers in head and neck immunotherapy in the article 'Biomarkers in Head and Neck Cancer an Update'

Biomarkers	***Role of Biomarker***	***Significance***
AChE	Prognostic	Low AChE activity in HNSCC can be used to predict survival
P53	Prognostic	High p53 expression has a negative prognostic effect and is used in detection of recurrence In HNSCC
PI3K pathway mutations	Predictive for survival rate and efficacy of treatment	PI3K pathway mutations and inflammatory cytokine expression help identify OSR and patients that may benefit from therapy of drug Durvalumab
RB	Prognostic gene	Rb1 alterations have prognostic implications, particularly in high P16 expression
P16	Predictive for survival rate and efficacy of treatment	High expression of p16 predicts better response to chemoradiations in patients with stage 4 cancer.

Cyclin D1	Prognostic protein and efficacy of treatment	High levels of cyclin D1 may be associated with poorer survival high risk of recurrence and treatment resistance as well as lymph node metastasis.
CD44	Prognostic &Therapeutic target	Low CD44 is related to decreased survival as it can be related to the tendency of tumors to metastasize. Monoclonal antibodies can be used in therapy to block CD44
Epidermal growth factor receptor (EGFR)	Prognostic and efficacy	This overexpression corresponds to tumor growth and progression, resistance to therapy and poor outcome
PDL-1	Prognostic protein and therapeutic target	Protein that is expressed on cancer cells; High levels of PDL-1 expression is associated to better response to immunotherapy
TMB (tumoral mutational burden)	Efficacy of immunotherapy	TMB is the number of mutations in a tumors DNA. High TMB is associated with better response.

1.4.2. Examples of biomarkers in head and neck immunotherapy

Currently, more than 70 markers have been reported in the head and neck region and some of the most important have been represented in this table above. (1)
Identifying specific molecular changes in malignant tumors has proven to be beneficial when trying to understand the genetic and molecular basis of human malignancies. Amongst some of the key biomarkers, TP53, p16, epidermal growth factor receptor (EGFR), cyclin D1, HPV, PD-1/PDL-1 and TMB seem to be the most significant.

TP53 is one the most altered genes in HNSCC and in many cases its mutation can be caused by HPV infection. By detecting this gene, we can identify tumor progression and treatment response thus making it a useful biomarker. A high association has been found between tabaco and alcohol consumption and chromosomal loss at the site of the TP53 gene causing its mutation. (21). The main therapeutic mechanism involves restoring its tumor suppressor activity and by identifying this gene we can predict tumor resistance to radiotherapy. Clinically, this specific mutation is associated with a short survival time and tumor resistance, therefore it makes it a useful biomarker for patient risk stratification and as a predictor of clinical response. (1,22).

Another prognostic biomarker is the protein P-16 which slows cell division acting as a tumor suppressor. Approximately, one third of HNSCC express P-16 and it has a high correlation with HPV associated cancer. P-16 protein is detected by immunohistochemistry and a high expression of this protein is indicative of primary HNSCC with a higher overall survival rate. It is also a predictive value for specific treatments as patients positive for this protein have a high response to radiotherapy or EGFR targeted therapy. (18)

Additionally, another biomarker is epidermal growth factor receptor (EGFR). This family of receptors play an essential role in cancer cell proliferation, vessel angiogenesis and dissemination and it is overexpression in more than 90% of the cases in HNSCC. EGFR inhibition therapy uses antibodies such as cetuximab and

panitumumab that target extracellular ligands and blocks its function. It has been found that there was a significant improvement in survival rate of patients with recurrent or metastatic HNSCC, when combining cetuximab and platinum chemotherapy, when comparing those who received chemotherapy alone. It was also found that patients with P-16 positive tumors responded well to cetuximab based therapy whereas those who were P16 negative responded better to chemotherapy alone. (18)

Human papilloma virus is detected easily by DNA/MRNA PCR and the individual's status can be used as a biomarker for anti PD-1/PDL-1 targeted therapy. Multiple studies have concluded that there was a high response rate for HPV positive patient when treated with PD-1/PDL-1 targeted therapy. (23)

Overall, these biomarkers help identify patients who are most likely to benefit from immunotherapy, provide insights into the mechanism of response and resistance. They can also guide the development of new immunotherapeutic approaches for treatment.

1.5. Immunotherapeutic drugs

Table 2: *Current immunotherapy target drugs* (2) *Wen and Grandis 2015*

Table 2 has been made by summarizing the most current immunotherapy target drugs in head and neck immunotherapy in the article 'Emerging drugs for head and neck cancer' by Yihui Wen and Jennifer R Grandis.

Drug name	***Target***	***Mechanism***
Pembrolizumab	PD-1 inhibitor	Blocks Pd-1/PD-L1 pathway, allowing for increased T cell activity
Nivolumab	PD-1 inhibitor	Shown to improve survival rate in patients with advanced HNSCC.
Durvalumab	PD-L1 inhibitor	Currently investigated as a monotherapy and in combination with other drugs.
Atezolizumab	PD-L1 inhibitor	Shown promising results in combination with chemotherapy.

Cetuximab	Epidermal growth factor receptor inhibitor	In combination with chemotherapy has shown to improve overall survival when given as first-line treatment in patients with recurrent or metastatic cases.
Ipilimumab	CTLA-4 inhibitor	It can be used alone in monotherapy or can be combined with Nivolumab or chemotherapy.
AZD5069	CXCR2 Inhibitor	CXCR2 is a receptor for cytokines. Used in combination with Durvalumab.
AZD9150/ Danvatirsen	STAT3 Inhibitor	It is a transcription factor for immunosuppressive tumor microenvironment. Used in combination of Durvalumab.

Ficlatuzumab	Anti-HGF IgG1 mAb	Antibody that blocks growth signals and helps the immune system recognize and fight HNSCC. Can be used in combination with cetuximab
In combination with chemotherapy	Cisplatin-based CRT	In combination with pembrolizumab for HPV-positive patients.

Table 2 has listed the most current Immunomodulatory drugs, which functions by targets immunosuppressive pathways, that are involved between the interaction of tumor cells and T lymphocytes. Drugs that block checkpoint proteins are called *checkpoint inhibitors* and have proven to be beneficial in immunotherapy, by blocking proteins that stop the immune system from attacking the cancer cells (6).

1.5.1. PD-1/PDL-1

PD-1(programmed cell death 1) is a protein that is highly expressed by activated T lymphocyte cells, B cells, dendritic cells and natural killer cells and it suppresses T cell inflammatory activity, overall preventing the immune system from killing cancer cells. PDL-1 (programmed cell death-ligand 1) is another protein expressed on several types of tumor cells and are specific targets for immunotherapeutic drugs. By blocking the interaction between PD-1 and PDL-1, it can improve the T cell response and brings about an antitumor activity. (19). Targeting PD-1/PD-L1 is therefore able to restore anti-tumor immune response, which is mediated by CD8 + lymphocytes.(24).

The development of anti PD-1/ PDL-1 antibodies is a great development in targeted immunotherapy and has shown an improvement in treatment outcomes for multiple tumor types, as it brings clinical benefits with limited toxicity. (23)
Anti-PD-1/ PDL-1 drugs include Nivolumab, Pembrolizumab (both anti PD-1) and Durvalumab and Atezolizumab (both anti PDL-1). PD-1 therapy effect is mediated by binding with T lymphocytes resulting in a systemic effect, whereas PDL-1 is directed against the receptors expressed on the tumor cells, causing a local effect.
Nivolumab and pembrolizumab were the first immune checkpoint inhibitors that were approved for recurrent/metastasis head and neck carcinoma in first line and with cisplatin based chemotherapy in patients whose tumors show a PD-1/PDL1 combined positive score (19).

Research has shown that anti PD1 therapy seemed to be more successful for male and smoker patients. The response between patients who smoked tobacco and immunotherapeutic treatment in several studies has shown to have a positive correlation, and this could be due to mutation created in smoker patients on the DNA which increases the tumour mutational burden, overall impacting the immunogenicity. (25) Moreover, for metastatic patients, anti PD1 therapy was associated with a greater survival rate. The microenvironments of metastatic cancers is generally different from the primary tumour sites and presents greater expression of PD-1 and TILS. (25)

In general anti PDL-1 therapy seems to be more effective in female patients, recurrent cases and in HPV positive patients. The gender differences are an interesting aspect in oncology due to perhaps the presence of autoimmune diseases between male and female subgroups. Furthermore, the immunological difference between recurrent and metastatic cancers of the head and neck could be used to conclude the great efficacy of Durvalumab with Tremelimumab, both being anti PDL-1 agents, in recurrent cases. Moreover, there was a slight increase in efficacy of patients who were HPV positive for anti PDL-1 agents. This could be due to the specific tumor antigens and immune microenvironment in the HPV positive patient who has a unique, non self, antigenic

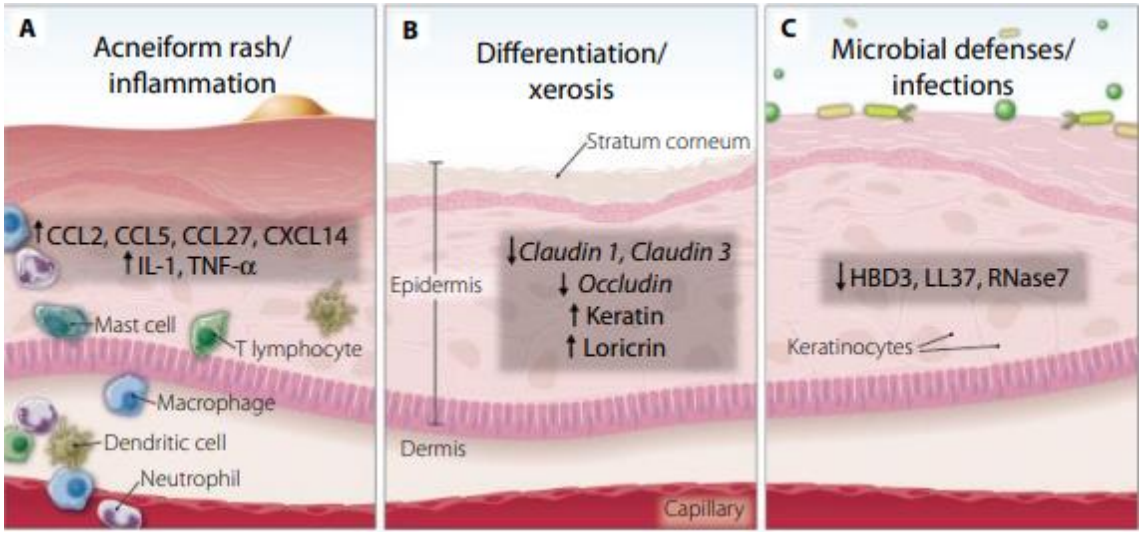
target. (25). For HPV positive agents; their unique microenvironment includes P-16 overexpression as well increased CD8 T cells activation, greater tregs infiltration and additional markers of immune filtration in comparison to HPV negative cases of HNSCC Furthermore there is an increased expression of PD-L1 compared to that of HPV negative cases and with all of these increased factors it induces the immune system to detect tumour cells much easier, therefore making targeted treatment more specific. (23) Anti PD-1/ PDL-1 antibodies can be used alone in immunotherapy as first line treatments or in combination therapy.

1.5.2. Combination therapy

Combination therapy is a treatment modality that combines two or more therapeutical agents improving the overall efficacy. Using a combination of therapies and drugs in turn potentially reduces resistance and tumour growth as well as metastatic potential, and has been proven to be more effective in HPV positive HNSCC patients.(26).

In many cases of head and neck cancers, there is 80-90% probability that the cells overexpress epidermal growth factor (EGFR). EGFR overexpression is associated with poor prognosis, making it a significant predictive biomarker in HNSCC. (27) Cetuximab was developed as an IgG1 antibody that inhibits EGFR activity and stimulated cytotoxicity of the cancer cells. It is most successful in recurrent or metastatic cases of HNSCC and it often used in first line therapy when in combination with platinum, fluorouracil chemotherapy, as it enhances its activity. There is ongoing current research into the prediction of clinical outcomes after anti-EGFR therapy for HNSCC. It has been found that there was a positive correlation between an increased survival rate for recurrent and/or metastatic HNSCC and skin toxicity for patients undergoing EGFR therapy. Patients who benefited from the therapy , often presented grade 3 dermatological toxicities such as rashes and inflammation by chemokine expression, changed in epidermal differentiation and a reduction in microbial defences in keratinocytes causing infections, as shown in figure 2. (27,28)

Figure 2: *EGFR inhibitors go skin deep* (28)



Several trials have been carried out in the development of immunotherapeutic agents such as the use of pembrolizumab alone or in combination with 5-fluorouracil used in chemotherapy. This has been compared to the use of chemotherapeutic agents with cetuximab. It was found that in the cases of patients with a high expression of the PDL-1 1 biomarker, a combination of chemotherapy with pembrolizumab improved the overall survival rate, compared with chemotherapy and cetuximab, when comparing the response to treatment and toxicity results.(8) The combination of chemotherapy with pembrolizumab was found to be more effective in patients with a higher disease burden who were more symptomatic, whereas the use of pembrolizumab monotherapy was greater for those patients who had a smaller tumour volume and greater PDL-1 expression. (8)

CTLA-4 is expressed in T cells and produces the immunosuppressive molecule transforming growth factor (TGF-β) when activated with CD28. Both CTLA-4 and CD28 act as transmembrane receptors. CTLA-4 can bind the B7 protein to induce T cell dysfunction and participate in negative regulation of the immune response. Blocking CTLA-4 can abolish the inhibition of T cells, leading to an antitumor immune response

in the host.(29) Ipilimumab is an anti-CTLA antibody and can be used alone in monotherapy or can be combined with nivolumab or chemotherapy. Ipilimumab may be promising for HNSCC in improving survival rates for those with metastatic cases. However, certain side effects have been found such as diarrhea, fatigue, skin rash and inflammation of the liver and colon, therefore it is not normally chosen as a first line treatment but a last resource.(30)

CXCR2 is a chemokine receptor expressed on many immune cells and is responsible to attracting neutrophils to tumor cells, in turn promoting tumor growth, invasion and metastasis. Inhibiting the signaling of this chemokine, has shown to enhance anti-tumor activity in head and neck cancer. They function by recruiting immunosuppressive myeloid cells into the tumor microenvironment and enhancing the activity of tumor infiltrating lymphocytes (TILS). There are many ongoing clinical trials which are investigating the combination of CXCR2 inhibitors with anti PDL-1 immunotherapy to evaluate their safety and efficacy, and if approved it could significant improve the treatment outcome for patients with HNSCC. (30)

The development of STAT3 pathway inhibitor, leads to growth inhibition and increases apoptosis of cancer. STAT3 pathway plays a vital role in the regulation of cell growth and differentiation and its hyperactivation is associated with poor clinical prognosis. By blocking this signaling pathway, it aids in the inhibition of growth of cancer cells. The drug can be used in combination with other immunotherapeutic agents and can enhance the activity of T cells. Several STAT3 inhibitors are being studied in clinical trials for head and neck cancers and can contribute to a great advance in immunotherapy. (31)

Further current research has found that the combination of chemotherapy with cetuximab (EGF inhibitor) and Ficlatuzumab is efficacious in recurrent or metastatic HNSCC, increasing the overall survival rate.(26) Ficlatuzumab is a monoclonal antibody that targets hepatocyte growth factors (HGF) and its 'Met' receptor. The signalling

pathway between HGF and Met plays a crucial role in growth of cancer cells and its inhibition, blocks growth factors produced by cells surrounding the tumour and therefore inhibits cancer cells to grow. Results from clinical trials demonstrated that only HPV negative cancers responded well to the combination of these three treatments, perhaps due to the presence of the Met receptor, making it a significant biomarker. Further clinical trials are needed to evaluate the safety and side effects of this immunotherapeutic agent. (26)

Overall, therapy for head and neck squamous cell carcinoma is rapidly evolving due to the development of new immunotherapeutic agents and has shown to improve survival outcomes for patients with recurrent or metastatic cases, as well as in combination with other immune checkpoint inhibitors. However only a small percentage of around 20-30% of patients have benefited from this type of treatment therefore stratification has a vital role to determine which kind of patients will benefit from immunotherapy. (30)

1.5.3. Adverse effects of immunotherapy

As with every line of therapy, adverse effects do occur and some of the most common include fatigue, nausea, skin reactions and join or muscle pain. More serious side effects can occur such as severe allergic reactions, pneumonitis, hepatitis, colitis, hormone imbalances due to affectation of the thyroid gland or even neurological problems such as seizures. In these cases, treatment interruption and medication such as systemic corticosteroids are indicated. Additionally, hyper-progression, a process of accelerated disease progression, was found to occur most likely in patients who were HPV negative with large local or regional recurrence, treated with immune checkpoint inhibition without chemotherapy. (8) There must be a high attention to symptom management and functional rehabilitation which are key in improving the quality of life of these patients.

2. OBJECTIVES.

1) **The main objective is to identify genetic biomarkers of immunotherapy in patients with head and neck cancer deposited in CBioPortal.**

2) **To identify genetic biomarkers in patients with Oropharynx Squamous Cell Carcinoma and treated with combo versus PD-1/PDL-1.**

3) **To analyze the genetic profile of metastatic versus primary tumor and the response to immunotherapy treatment**.

4) **To identify epidemiological parameters (smoking, drinking…) related with Head and Neck Squamous Cell Carcinoma**..

3. MATERIAL AND METHORDS.

As the use of biomarkers immunotherapy is such a vast and recent advance in oncologic medicine, I was able to utilize a wide range of articles to support my review study.

The bibliography was compiled using a variety of English-language publications.

To carry out this review, scientific articles and formally conducted studies were used. Different websites were also used to find information and studies relevant to the subject. Electronic databases were searched:

- PubMed,
- Medline,
- CraiLibrary (UEM),
- Google academics,
- Research gate

Specific keywords were used to find articles related to immunotherapy for head and neck and its new advances and treatments. Searches were made using the following words: ' epidemiology', 'etiology', 'HPV and the head and neck', 'surgery', 'chemotherapy', 'radiation', 'immunotherapy', 'biomarkers', 'uses of biomarkers', 'biomarkers head and neck', 'immunotherapeutic drugs', 'PD-1/PDL-1 head and neck', 'CTLA-4', 'EGFR', 'cetuximab', 'STAT3', 'adverse effects of immunotherapy'

The final search equation was as follows; 'Immunotherapy' AND/OR 'head and neck cancer' AND/OR 'genetic biomarkers AND/OR 'oropharynx squamous cell carcinoma AND/OR 'PD-1/PDL-1' AND/OR 'combination treatment' were used. Furthermore sci-hub was used as an additional database for articles.

Articles were filtered using exclusion and inclusion criteria to ensure only significant information was used.

Inclusion Criteria:

- Academic publications.
- Articles published from 2010 to the present date.
- Articles available in complete PDF text.
- Articles of free public access.
- Literature review articles.
- Clinical studies.
- English language articles.

Exclusion Criteria:

- Irrelevant to the subject of head and neck immunotherapy.
- Articles related to immunotherapy of cancers other than in the head and Neck region.
- Articles published before 2010.
- Studies with low sample numbers.

- Articles that had to be paid to access.
- Studies based on non-randomised clinical trials.
- Articles published in languages other than English.

In the results section of this study CBioPortal (www.cbioportal.org) was used which is an analysis tool and provides visualization, analysis and download of cancer genomic data sets.

Using this platform, a sample of 37 patients with oropharynx squamous cell were studied and we used TMB (tumor mutational burden) as a predictive biomarker in immunotherapy as their first line of therapy.
Using this sample of patients, we were able to identify the gender discrepancy as well as the mutated genes in primary tumor type vs metastatic type. We also were able to identify different etiological factors associated and success of the treatments with anti PDL/PDL-1 verses combo.

4. RESULTS.

We used CBioPortal to conduce the results for head and neck cancer to evaluate statistical cases. (MSK, Nat Genet 2019).

Figure 3: Table showing cancer types: From this database we used a sample of 37 patients who had oropharynx squamous cell carcinoma.

Cancer Type Detailed	#	Freq
Head and Neck Squamous Cell ...	37	26.6%
Oropharynx Squamous Cell Car...	37	26.6%
Oral Cavity Squamous Cell Car...	25	18.0%
Larynx Squamous Cell Carcinoma	9	6.5%
Nasopharyngeal Carcinoma	9	6.5%
Sinonasal Squamous Cell Carci...	6	4.3%
Hypopharynx Squamous Cell C...	5	3.6%
Head and Neck Carcinoma, Other	3	2.2%
Head and Neck Neuroendocrin...	3	2.2%
Head and Neck Squamous Cell ...	3	2.2%
Clear Cell Odontogenic Carcino...	1	0.7%

Figure 4: Pie chart to showing gender differences.

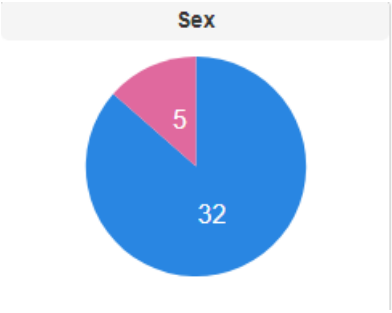


Figure 5: Pie chart showing age groups at diagnosis in years.

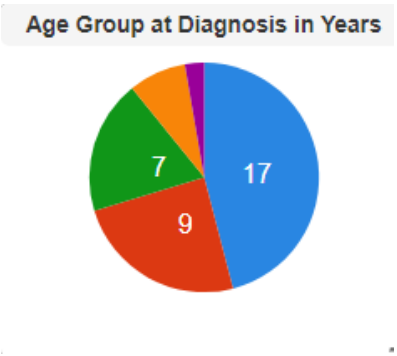
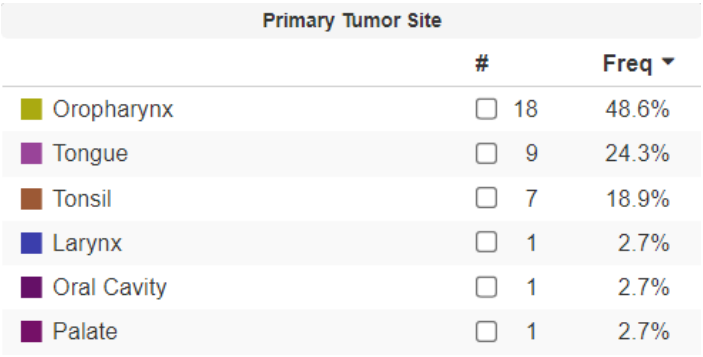
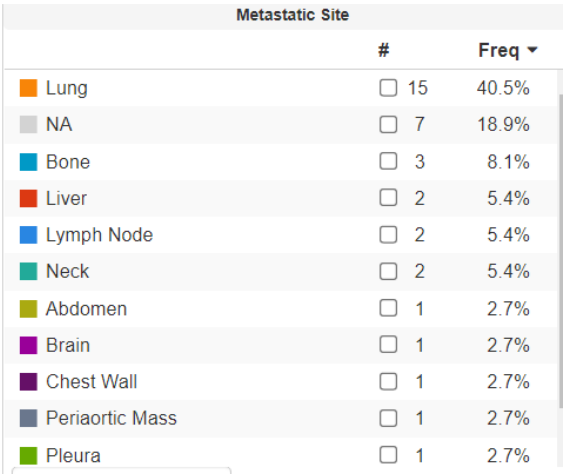


Figure 6: Primary tumor site locations.



Primary Tumor Site	#	Freq
Oropharynx	18	48.6%
Tongue	9	24.3%
Tonsil	7	18.9%
Larynx	1	2.7%
Oral Cavity	1	2.7%
Palate	1	2.7%

Figure 7: Metastatic tumor site location.



Metastatic Site	#	Freq
Lung	15	40.5%
NA	7	18.9%
Bone	3	8.1%
Liver	2	5.4%
Lymph Node	2	5.4%
Neck	2	5.4%
Abdomen	1	2.7%
Brain	1	2.7%
Chest Wall	1	2.7%
Periaortic Mass	1	2.7%
Pleura	1	2.7%

Figure 8: Pie chart representing the drug type. In Blue represents those who received PD-1/PDL-1 and in orange for those who received combination treatment.

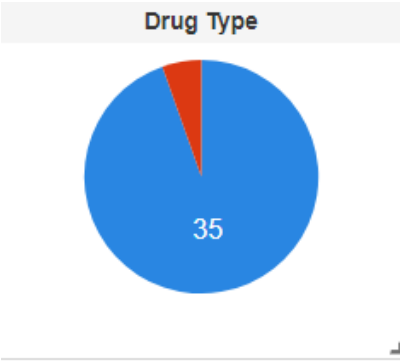


Figure 9: Pie chart representing patients who survived vs those who were deceased post treatment PD-1/PDL-1.

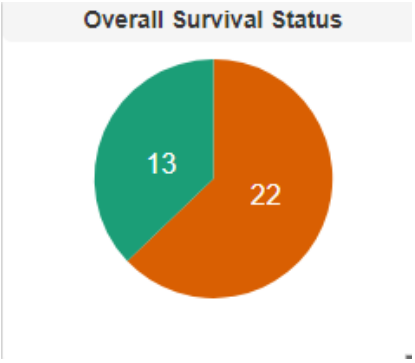


Figure 10: Sex differences between patients who survived PD1/PDL-1.

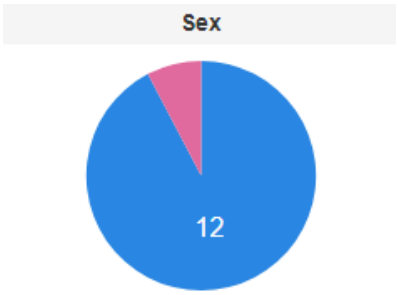


Figure 11: Overall survival rate of patients who received PD1/PDL1 who presented with metastasis.

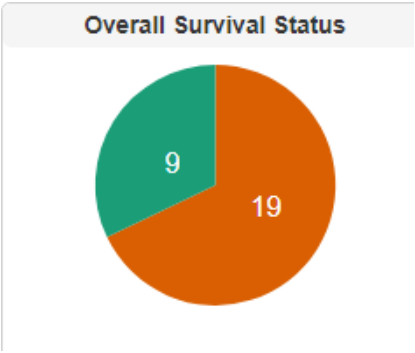


Figure 12: Overall survival rate of patients who received PD1/PDL1 who presented with a primary tumor.

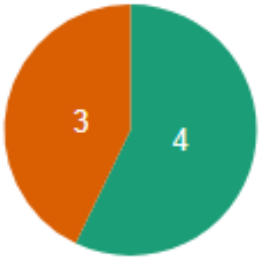


Figure 13: Overall survival rate of the two patients who received combo treatment presented with metastasis.

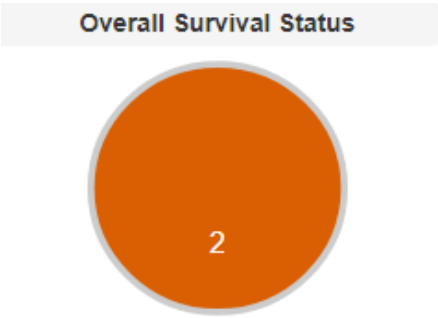
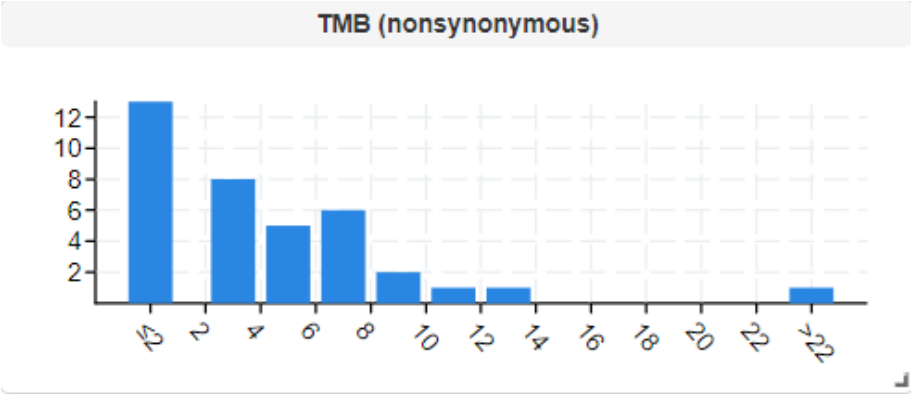


Figure 14: Graph to demonstrate the TMB (tumoral mutational burden) within the sample.



Mutated Genes (37 profiled samples)			
Gene	# Mut	#	Freq
PIK3CA	12	10	27.0%
TERT	8	7	18.9%
EP300	6	5	13.5%
NOTCH1	5	5	13.5%
KMT2C	6	5	13.5%
TP63	6	5	13.5%
ALK	5	4	10.8%
TP53	4	4	10.8%
EPAS1	1	1	8.3%
PREX2	1	1	8.3%
NSD2	1	1	8.3%

Figure 15: Table to demonstrate mutated genes within the sample. Genetic sampling was carried out to evaluate the most affected mutated genes which can act as significant biomarkers for the patients.

Mutated Genes (13 profiled samples)			
Gene	# Mut	#	Freq
PIK3CA	7	5	38.5%
TERT	4	4	30.8%
TP63	5	4	30.8%
EPHA7	3	2	16.7%
GPS2	2	2	16.7%
EP300	3	2	15.4%
BRCA1	3	2	15.4%
AXL	2	2	15.4%
NFE2L2	2	2	15.4%
SPEN	2	2	15.4%

Figure 16 :

Mutated genes of patients who survived PD1/PDL-1 therapy.

Mutated Genes (22 profiled samples)			
Gene	# Mut	#	Freq
UPF1	1	1	33.3%
NOTCH1	4	4	18.2%
PIK3CA	4	4	18.2%
EP300	3	3	13.6%
ALK	3	3	13.6%
TP53	3	3	13.6%
KMT2C	3	3	13.6%
ANKRD11	2	2	11.1%
STK11	2	2	9.1%
MAPK1	2	2	9.1%
TERT	3	2	9.1%

Figure 17 :

Mutated genes of patients who were deceased after PD1/PDL-1 therapy.

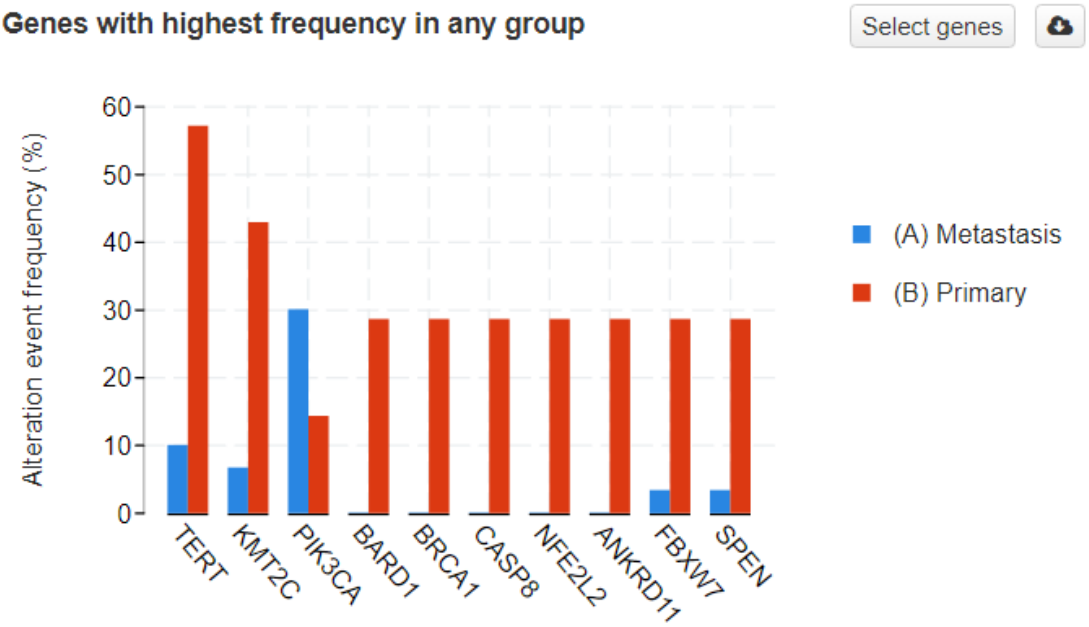
Mutated Genes (2 profiled samples)			
Gene	# Mut	#	Freq
H3C11	1	1	50.0%
TERT	1	1	50.0%
SOX17	1	1	50.0%
NSD1	1	1	50.0%
PMS1	1	1	50.0%
ERBB3	2	1	50.0%
PIK3CA	1	1	50.0%
RBM10	1	1	50.0%
FBXW7	1	1	50.0%
KMT2D	1	1	50.0%
PTEN	1	1	50.0%

Figure 18 :

Mutated genes of patients who received combination treatment and were deceased after.

Figure 19:

The bar chart corresponding compares the genes mutated in metastasis vs the genes mutated in the primary tumor sites.



Mutated Genes (9 profiled samples)			
Gene	# Mut	#	Freq
PIK3CA	4	4	44.4%
AXL	2	2	22.2%
TP63	3	2	22.2%
TERT	2	2	22.2%
EPAS1	1	1	20.0%
KMT2B	1	1	20.0%
SLX4	1	1	20.0%
H3C1	1	1	12.5%
EIF4A2	1	1	12.5%
EPHA7	1	1	12.5%

Figure 20: Genes of patients who had metastasis, who received PD1/PDL-1 therapy and survived.

Mutated Genes (19 profiled samples)			
Gene	# Mut	#	Freq
UPF1	1	1	33.3%
PIK3CA	4	4	21.1%
EP300	3	3	15.8%
ALK	3	3	15.8%
NOTCH1	3	3	15.8%
MAPK1	2	2	10.5%
TP53	2	2	10.5%
ATR	3	2	10.5%
KMT2C	2	2	10.5%
NFKBIA	1	1	6.7%

Figure 21: Genes of patients who had metastasis, who received PD1/PDL-1 therapy and died.

Mutated Genes (4 profiled samples)			
Gene	# Mut	#	Freq
BRCA1	3	2	50.0%
NFE2L2	2	2	50.0%
TERT	2	2	50.0%
FBXW7	3	2	50.0%
TP63	2	2	50.0%
BARD1	2	2	50.0%
KMT2C	3	2	50.0%
DICER1	1	1	25.0%
CDK6	1	1	25.0%
EP300	2	1	25.0%

Figure 22: Genes of patients who had primary tumor, who received PD1/PDL-1 therapy and survived.

Figure 23:

Genes of patients who had primary tumor, who received PD1/PDL-1 therapy and died.

Mutated Genes (3 profiled samples)			
Gene	# Mut	#	Freq
TERT	3	2	66.7%
ANKRD11	2	2	66.7%
PTPN11	1	1	33.3%
STK11	1	1	33.3%
TP53	1	1	33.3%
AXIN2	1	1	33.3%
SPEN	1	1	33.3%
FAT1	1	1	33.3%
KMT2D	1	1	33.3%

Figure 24: Genes of patients who received combination treatment who both had metastasis and died.

Mutated Genes (2 profiled samples)			
Gene	# Mut	#	Freq
H3C11	1	1	50.0%
TERT	1	1	50.0%
SOX17	1	1	50.0%
NSD1	1	1	50.0%
PMS1	1	1	50.0%
ERBB3	2	1	50.0%
PIK3CA	1	1	50.0%
RBM10	1	1	50.0%
FBXW7	1	1	50.0%

5.DISCUSSION.

Using the CBioPortal, 37 patients were studied and sampled, each having squamous cell carcinoma in the oropharynx region. In figure 3, we can see that oropharynx affected 26.6% of the total head and neck cancer cases, when searching for TMB and immunotherapy on this database. The oral pharynx, larynx, nasopharynx, sinonasal and hypopharynx were also commonly affected regions of head and neck cancer but there was a significantly smaller number of samples, hence they were not included.

Of the 37 patients within this sample 32 were male and 5 were female, disproportionately affected the sexes as seen in figure 4. Men are at an increased risk of acquiring this cancer, perhaps because men are more likely to drink heavier than women and have a greater daily tobacco use. Figure 5 is a pie chart showing age groups at diagnosis. From the results we can evaluate that there was an increased proportion of older patients who were diagnosed with oropharynx squamous cell carcinoma. The most common ages were between 61-70 years of age, corresponding to 45.9% of the sample. Following that; 9 of the patients (24.3%) were detected at 50-60 years old, 7 patients (18.9%) at 31-50 years old, 3 patients (8.1%) at ages greater than 71 and finally only 1 patient (2.7%) were less than 30 years old.

7 of the patients had oropharynx squamous cell carcinoma as a primary tumor site and 30 of the patients had secondary metastasis of oropharynx squamous cell carcinoma. Within the primary tumor site, the most common location for the cancer was in the oropharynx for 18 patients corresponding to 48.6% of the cases. Squamous cell carcinoma in the tongue (9 patients, 24.3%) was second most common site, followed by the tonsils (18.9%), larynx (2.7%), oral cavity (2.7%) and palate (2.7%) (Figure 6).

Metastasis was very common in this sample group. Spread to the lungs was the most common site and occurred in 15 patients. Spread to the bone was the second highest site in 3 patients. Following this metastasis in the liver, lymph node and neck occurred

in 2 patients each. The abdomen, brain, chest wall, periaortic mass and pleura were the least occurring sites of metastasis affecting 1 patient each. (Figure 7)

Figure 8 represents the number of patients who received PD-1/PDL-1 therapy and those who received combination treatment. 35 of 37 of the patients received PD1/PDL-1 therapy and only 2 of the patients received combo treatment. Of the 35 patients that received PD1/PDL1, the survival rate remained at 37.1 percent as only 13 patients survived and 62.9% of the patients (22 of the patients) were deceased post treatment, at a median of 7 months post treatment.(Figure 9) As only 2 patients received combination treatment, the results cannot be conclusive of whether combination treatment affects the overall survival rate of these patients.

The survival rate is shown In figure 10 for male and female patients. Of the 35 patients who received PD1/PDL1, 30 of the patients were male and 5 were female. Of the 30 male patients, 12 survived and 18 died, making the overall survival rate for male patients 40%. 1 out of the 5 female patients survived whom received PD1/PDL-1 making the female survival rate 20%. Therefore we are able to conclude that this kind of therapy had a greater efficacy amongst the male population. On the other hand, 2 of the patients who received combination treatment were both male and they both were deceased. Due to the low sample, we are not able to conclude that combination treatment was not effective amongst the male population.. As discussed in the introductive of the article, studies have shown that sex makes a significant difference between the choice of PD1 or PDL-1 treatment.

Another variable to take into consideration for the efficacy of treatment is the location of the tumor site. Of the 35 patients who received PD1/PDL-1, 28 of the patients presented with metastasis in this region and 7 of the patients had a primary tumor site. As seen in figure 11, 9 of the patients survived this treatment who presented with metastasis making the survival rate 32.1% within this sample, and 19 of the patients died (67.9%). Of the 7 patients who presented with a primary tumor (figure 12), 4 of

the patients survived (survival rate of 57.1%) and 3 of the patients died. We can therefore conclude that within this sample the survival rate for patients who received PD1/PDL-1 higher for those who presented with a primary tumor site rather than those who had metastasis. In figure 13 we can see that both of the patients who received combo treatment died and they both presented with metastasis, therefore for our sample combination treatment was not effective for metastatic cases. As no patients who received combination treatment presented with a primary tumor, we were not able to decipher whether combination treatment for a particular location of tumor.

As we have demonstrated within this article, biomarkers are an integral part of immunotherapy, in not only diagnosis of tumors but also prediction and efficacy of treatment. Within the study, we used tumoral mutation burden as the biomarker of choice, to predict how the sample would respond to immunotherapy. In the case of this study, TMB was used to compare the mutations of those who survived PD1/PDL-1 and those who died, as well as for those who received combination treatment. In figure 14, we were able to see the number of tumoral mutations burdens, therefore the number of mutations of DNA that were sequenced. 13 patients presented with less than 2 mutations sequenced, therefore it was the greater range within the sample. Only 1 patient had a range of more than 22 mutated genes present.

Genetic sampling was carried out within the sample to evaluate the most commonly mutated genes that could act as significant biomarkers for targeted immunotherapy. Demonstrated in figure 15, PIK3CA (phosphoinositide 3-kinase) was the most mutated gene affecting 27% of the overall sample. TERT (telomerase reverse transcriptase) was the second highest recurrent mutation found affecting 18.9% of this sample and its mutation in general represents a fundamental step in tumorigenesis.
The third most common mutation was in the gene EP300 (13.5%) which is responsible in producing the protein p300 which plays a role in controlling cell growth and division. This mutation is frequently associated with increased TMB. The fourth most common

mutation occurred within the gene NOTCH1 (13.5%) which is a tumor suppressor gene. Mutation of TP63 was another common mutated gene (13.5%) of the sample and it promotes HNSCC progression and metastasis. Following, there were alterations in the genes; ALK and TP53 (both 10.8%) and EPAS1, PREX2 and NSD2 (all 8.3%).

Figure 16 is a table of the genes most commonly affected amongst the patients who survived PD1/PDL-1 therapy. PIK3CA was the most commonly affected gene (38.5%) of the sample; following that TERT (30.8%), TP63 (30.8%), EPHA7 (16.7%), GPS2 (16.7%), EP300 (15.4%), BRCA1 (15.4%), AXL (15.4%), NFE2L2 (15.4%). Therefore we are able to conclude that the presence of these particular genes within the sample resulted in a greater efficacy of treatment to PD-1/PDL-1 immunotherapy. On the other hand, in figure 17 we are able to see the most commonly affected genes amongst those patients who were deceased after PD-1/PDL-1 therapy. UPF1 (33.3%) was the most commonly mutated gene, following; NOTCH1 (18.2%), PIK3CA (18.2%), EP300 (13.6%), ALK (13.6%), TP53 (13.6%), KMT2c (13.6%), ANKRD11 (11.1%), STK11 (9.1%) and MAPK1 (9.1%). Therefore within the sample, the presence of the mutated genes TERT, TP63, EPHA7, GP52, BRCA1, AXL and NFE2L2 favored PD1/PDL-1 therapy. However the presence of UPF1, NOTCH1, ALK, TP53, KMT2C, ANKRD11, STK11 and MAPK1 was disfavored for PD-1/PDL-1 therapy. However both the genes PIK3CA and EP300 were highly present in both sample types, therefore perhaps these genes have no specific response to this kind of therapy.

Figure 18 is another graph representing the genes of the two patients who were deceased after combo treatment. Both of the patients had the mutated genes; H311, TERT, SOX17, NSD1, PMS1, ERBB3, PIK3CA,RMB10, FBXW7,KMT2D and PTEN. When comparing the genes with those patients who survived PD-1/PDL-1 we can see that the presence of PIK3CA and TERT which were the most expressed, responded better to PD1/PDL-1 than to combination treatment. We can also evaluate the presence of the genes H3C11, SOX17, NSD1,PMS1, ERBB3, RBM10, FBXW7, KMT2D and PTEN did not favor combination treatment.

Further genetic sampling within this sample found that within the primary tumor site there was a greater frequency of the mutated genes; TERT, KMT2c, BARD1, BRCA1, CASP8, NFE2L2, ANKRD11, FBXW7 and SPGN, when comparing with genes found in the metastatic sites. However, there was a significantly greater presence of the gene PIK3CA in the metastatic sites. This can be represented in figure 19 and again genetic sampling can be useful in the diagnosis process, to determine if a tumor originates from a primary or metastatic origin by screening chromosomes in both site.
For the patients who had metastasis, we are able to clearly compare the genes present who those patients who survived and were deceased after PD-1/PDL-1 therapy, as shown in figure 20 and 21. For metastatic cases the genes that favored PD1/PDL-1 therapy were AXL, TP63, TERT, EPAS1, SLX4, H2CA1 and E1F4A2. However the genes that did not favor this therapy were UPF1, EP300, ALK, NOTCH1, MAPK1, TP53, ATR and NFKB1A. There was the presence of both the genes KMT2B and PIK3CA in metastatic cases in patients who survived and were deceased after this therapy.

In figure 22 and 23 were are able to compare the genes of the patients who presented with a primary tumor site in this region and who survived verses those who were deceased post PD1/PDL-1 therapy. Of the 7 patients who had primary tumor site, 4 survived and the most commonly mutated genes were BRCA1, NFE2L2, TERT, FBXW7, TP63, BARD1, KMT2C, DICER1, CPK6 and EP300. However for the 3 patients who were deceased after the treatment the most commonly affected genes were TERT, ANKRD11, PTPN11, STK11, TP53, AX1N2, SPEN, FAT1 and KMT2D. Therefore we can evaluate that the presence of the genes BRCA1, NFE2L2, FBXW7, TP63, BARD1, KMT2C, DICER1, CPK6 and EP300 favored PD1/PDL-1 therapy for those who had a primary tumor site.

Lastly in figure 24 we are able to evaluate the genes of the patients who both had metastasis and died after receiving combination treatment. The presence of the genes H3C11, TERT, SOX17, NSD1, PMS1, ERBB3, PIK3CA, RBM10 and FBXW7 did not favor

combination treatment in primary metastatic sites. Interestingly the presence of the TERT gene was found in all scenarios of survival and death of patients who had primary or metastatic sites.

Overall, genetic sequencing plays a very integrate role, as we are clearly able to see which genes respond well to different immunotherapies, potentially increasing the survival rate of cancer patients.

6. CONCLUSIONS

Based on this research the following conclusions can be made:

1. Squamous cell carcinoma is the main histologic type of head and neck cancer with alcohol, tabaco and HPV as well defined risk factors.

2. The identification and analysis of genetic biomarkers have shown promising results and we used a TMB as the potential biomarker of choice using the CBioPortal database. This may aid in personalized treatment options for patients. The use of genetic biomarkers has the potential to improve the treatment outcome for cancer patients and it should be a focus area for immunologists in the future.

3. When comparing treatments of combo versus PD-1/PDL-1 there may potentially be specific mutated genes that respond better or worse to each treatment

4. Genetic sequencing is useful in the diagnosis process for metastatic versus primary tumor and an analysis into genetic profiling can aid in their response to treatment for each cases.

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8. ANNEX REFERENCES

TABLES:

Table 1. Basheeth N, Patil N. Biomarkers in Head and Neck Cancer an Update. Indian J Otolaryngol Head Neck Surg. 2019 Oct;71(S1):1002–11.

Table 2. Wen Y, Grandis JR. Emerging drugs for head and neck cancer. Expert Opin Emerg Drugs. 2015 Apr 3;20(2):313–29.

FIGURES:

Figure 1: Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primer. 2020 Nov 26;6(1):92.

Figure 2: Lacouture ME, Rodeck U. Skinflammation and Drug Toxicity—A Delicate Balance. Sci Transl Med [Internet]. 2013 Aug 21 [cited 2023 Apr 11];5(199). Available from: https://www.science.org/doi/10.1126/scitranslmed.3006993

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