Title of Project: Using Maackia amurensis seed lectin to target the podoplanin receptor as a functionally relevant biomarker to inhibit the growth of oral squamous cell carcinoma and precancerous lesions.

Principal Investigator: Mahnaz Fatahzadeh

Co-Investigators: Gary S. Goldberg, Soly Baredes, Evelyne Kalyoussef, Dylan Roden, Rabie Shanti, Ghayoour Mir, Eugenio Capitle, Htay Htay Kyi, David Suster, Alan J. Shienbaum

Locations: Recruiting, consenting, and specimen collection will take place at Rutgers.
Ex vivo analysis will take place at Rowan.

Name	Role on Project
Mahnaz Fatahzadeh, *DMD, MSD. Professor, Rutgers School of Dental Medicine.*	Sample precancerous oral lesions. Administer MASL. Consenting of Subjects. Obtains hematoxylin and eosin (H&E) stained and unstained specimens of previously diagnosed oral cancer/precancer patients who consent to participate in the study.
Gary S Goldberg, *PhD. Associate Professor, Rowan University School of Osteopathic Medicine.*	Study coordinator to manage regulatory paperwork, IRB approvals, and other documents. Evaluate PDPN expression and effects of MASL on oral cancerous and precancerous cells.
Soly Baredes	Sample oral cancers. Administer MASL. Consenting of Subjects. Obtains hematoxylin and eosin (H&E) stained and unstained specimens of previously diagnosed oral cancer/precancer patients who consent to participate in the study.
Evelyne Kalyoussef	Sample oral cancers. Administer MASL. Consenting of Subjects. Obtains hematoxylin and eosin (H&E) stained and unstained specimens of previously diagnosed oral cancer/precancer patients who consent to participate in the study.
Dylan Roden	Sample oral cancers. Administer MASL. Consenting of Subjects. Obtains hematoxylin and eosin (H&E) stained and unstained specimens of previously diagnosed oral cancer/precancer patients who consent to participate in the study.
Rabie Shanti	Sample oral cancers. Administer MASL. Consenting of Subjects. Obtains hematoxylin and eosin (H&E) stained and unstained specimens of previously diagnosed oral cancer/precancer patients who consent to participate in the study.
Ghayoour Mir	Sample oral cancers. Administer MASL. Consenting of Subjects. Obtains hematoxylin and eosin (H&E) stained and unstained specimens of previously diagnosed oral cancer/precancer patients who consent to participate in the study.
Eugenio Capitle	Evaluate patients before and after MASL or placebo administration and manage complications should they arise.
Htay Htay Kyi	Evaluate patients before and after MASL or placebo administration and manage complications should they arise.
David Suster	Surgical pathologist at the UH involved with the study. Evaluate tumor pathology, cell morphology, and PDPN expression in patient samples.
Alan J. Shienbaum *Keystone Medical Labs*.	Evaluate tumor pathology, cell morphology, and PDPN expression in patient samples.

Funding Source(s): Rowan University

1. Purpose/Specific Aims

This project will evaluate the expression of a receptor called podoplanin (PDPN) in cells from oral cancers and precancerous lesions. We will also determine how sensitive oral cancer cells are to a potential drug called Maackia amurensis seed lectin (MASL).

1.1 Objectives

Oral cancers will be biopsied by Dr. Soly Baredes, Dr. Roden, Dr Evelyne Kalyoussef and Dr.Ghayoour Mir and Dr. Rabie Shanti. Precancerous oral lesions will be biopsied by Dr. Mahnaz Fatahzadeh. For oral cancer/precancer patients biopsied at an outside institution and referred to Rutgers/University Hospital for care, outside H&E slides and unstained specimens for PDPN staining will be requested. Dr. Suster and Dr. Alan Shienbaum will evaluate tumor pathology, cell morphology, and PDPN expression in these patient samples. Dr. Gary Goldberg will evaluate PDPN expression and MASL sensitivity from primary cultures of these cells. Study clinicians will evaluate oral cavity and health of their respective patients before and after MASL treatment. Dr. Fatahzadeh, Dr. Baredes, Dr. Kalyoussef, Dr. Shanti, Dr. Mir or Dr. Roden will administer MASL dosage, evaluate any effects on oral structure and epithelium, and perform clinically indicated resection or biopsy to obtain specimen after treatment. Dr. Fatahzadeh, Dr. Baredes, Dr. Roden, Dr. Shanti, Dr. Mir or Dr. Kalyoussef along with Dr. Capitle and Dr. Kyi will measure overall health before and after administration, and evaluate potential allergic reaction to MASL and respond to any unlikely scenarios. Dr. Suster, Dr. Shienbaum and Dr. Goldberg will evaluate pathology, cell morphology, and PDPN expression in these samples. We will determine if MASL treatment can decrease PDPN expression and normalize cell morphology in oral cancers and precancerous lesions.

Thus, primary, secondary, and tertiary objectives are as follows:

a. Primary Objective: To evaluate and compare the morphology and podoplanin (PDPN) expression of cells included in the oral lesion initial biopsy to normal oral squamous epithelial cells (OSCCs).
b. Secondary Objective: To evaluate and compare the PDPN expression of cells included in the resected oral lesion to normal oral squamous epithelial cells.
c. Tertiary Objective: To evaluate and compare the morphology of cells included in the resected oral lesion to normal oral squamous epithelial cells.

1.2 Hypotheses

We hypothesize that the majority of oral cancers express PDPN, and that MASL inhibits the growth, migration, and transformed morphology of these cells. Our hypothesis predicts that more PDPN will be expressed in aggressive oral cancer cells (OSCCs) than normal oral epithelium (OSCs), and that MASL will inhibit the growth and migration of these OSCCs in a manner that correlates with PDPN expression *in vitro*, as well as normalize the morphology of these cells *in vivo*.

1.3 Primary, Secondary, and Tertiary Endpoints

Endpoints will be determined by cell morphology and PDPN expression examined by IHC. This work will be performed as an integral part of the study. The protocols rely on standard staining protocols to visual cells and PDPN expression with an approved antibody (D2-40). We have experience with this approach as previously described [56].

Our primary endpoint will be measured from patient biopsy material. We will compare the morphology and PDPN expression of cells included in the oral lesion to normal oral squamous epithelial cells. Patients eligible for the study will provide samples with lesion cells that are determined to be OSCC and express more PDPN than normal cells from the same sample specimen. In addition, these OSCC cells will express PDPN levels that are equivalent or greater than PDPN levels expressed by lymphatic endothelial cells (LECs) from the same specimen. Although this may be considered inclusion criteria, it is also an endpoint since these data will confirm that PDPN expression is increased in OSCC cells in our patient population. PDPN expression will be assessed by IHC for this endpoint since it can be used to qualitatively and quantitatively asses PDPN expression and be accomplished along with standard evaluations used for best care practice.

Our secondary endpoint will be measured from patient resection or second biopsy material. We will compare the PDPN expression of cells included in the oral lesion to normal oral squamous epithelial cells. Our secondary endpoint will be met if MASL treated OSCC cells express less PDPN than normal cells from the same sample specimen. In addition, these OSCC cells will express PDPN levels that are lower than PDPN levels expressed by lymphatic endothelial cells (LECs) from the same specimen. Our secondary endpoint will also require that the degree of PDPN expression decrease occurring after MASL administration is greater than any PDPN expression decrease observed after placebo administration. This comparison will be analyzed by t-test to achieve a p value less than 0.05.

Our tertiary endpoint will be measured from the same patient resection or second biopsy material used to measure our secondary endpoint. We will compare the morphology of cells included in the oral lesion to normal oral squamous epithelial cells. Our tertiary endpoint will be met if MASL treated OSCC cells appear less dysplastic than they appeared before treatment. Dysplasia will be graded on a scale of 1 to 5, with 5 being the most dysplastic. Our hypothesis predicts that MASL treated OSCC cells will appear more cuboidal and flatter than they were before treatment.

2. Background and Significance

Approximately 300,000 new cases of oral cancer are diagnosed each year, causing over 120,000 deaths worldwide [30,32]. Greater than 90% of these cancers are oral squamous cell carcinomas (OSCC) that proceed from hyperplasia to dysplasia, carcinoma in situ, and invasive carcinoma [55,27]. The potential for malignant progression of lesions including leukoplakia, erythroplakia, and leukoerythroplakia is primarily determined by the presence and degree of dysplasia [41,72,78,27].

Patients with early (stage I or II) OSCC are generally treated with surgery and radiation therapy, yielding 5-year survival rates between 70% and 95% [20,12,22,11,29,50,70,36]. However, patients with more advanced OSCC (stage III or IV) have much lower 5 year survival rates, ranging between 26% and 53% [54,66,69,36]. Metastasis to cervical lymph nodes is considered an extremely adverse prognostic factor for patients with OSCC [85,37,36].OSCCs and premalignant lesions often exhibit polymorphisms in cyclin D1 and inactivation of tumor suppressors including p53, p16 and p14 [77,4,48,64,35,67,15,18,17,21,58,71]. Increased expression of tumor promoters including EGFR, c-myc, cyclin D1, TGF-α, and Pdpn are also often seen in such lesions [14,35,68,15,71,75,82]. Indeed, EGF signaling can induce PDPN expression in a number of OSCC cell lines [28].

PDPN expression is clearly associated with OSCC aggression, invasion, metastasis, postoperative recurrence, and poor survival. Taken together, reports indicate that PDPN

expression is notably increased in over 30% or precancerous oral lesions and in over 60% of oral cancers. Moreover, PDPN expression appears to correlate with oral cancer stage. About 50% of stage T1/T2 oral cancers display elevated PDPN expression levels, and this number increases to about 75% for stage T3/T4 oral cancers. In addition, over 70% of primary OSCC tumors that metastasize to lymph nodes express elevated PDPN levels. Clinical studies also indicate that 5 year overall survival rates continuously decrease from 93% for patients with weak podoplanin expression, to 47% for patients with moderate expression, to 23% for patients with high levels of podoplanin expression. OSCC recurrence also correlate very well, with undetectable, weak, moderate, and high PDPN expression resulting in 100%, 93%, 71%, and 51% disease free survival rates, respectively [49,85,8,37,16,23,36,27].

The bulk of the PDPN protein, about 150 amino acids, lies outside of the cell and could serve as an ideal target to combat cancer growth and progression [40,80]. For example, antibodies against PDPN and a PDPN interacting partner (tetraspanin CD9) can inhibit lung metastasis of CHO cells transfected with PDPN [34,53]. However, while antibodies may offer significant targeting specificity, they cannot be administered orally [3,9,31] and may not possess intrinsic pharmaceutical effects on melanoma cell growth and migration seen with orally available lectins.

We have shown that *Maackia amurensis* seed lectin (MASL) targets PDPN to inhibit tumor cell growth and motility [1,38,57]. The extracellular domain of PDPN is *O*-glycosylated with sialic acid, α2,3 linked to galactose [80]. PDPN is activated by endogenous lectins that bind to these extracellular carbohydrate moieties [73,5,7] to induce tumor cell motility and metastasis [81,83,84,73]. Thus, blocking this interaction should inhibit malignant progression. For instance, compounds blocking the action of galectins, which activate mucin receptors, can inhibit tumor cell metastasis [26,19].

Although some lectins may nonspecifically bind to many glycoproteins, MASL can precisely target specific glycoproteins expressed by human cells [47,46]. We have shown that MASL, which has a high affinity for *O*-linked carbohydrate chains containing sialic acid [76,25], binds to PDPN in order to inhibit tumor cell growth and motility at nanomolar concentrations. As we have recently reported, MASL can be taken orally to inhibit tumor growth in mice. Moreover, no side effects were evident from MASL ingestion based on animal weight, behavior, or organ morphology.

Lectins such as MASL offer significant medicinal value. Toxic lectins (e.g. ricin and viscumin) are extremely rare. In fact, lectins are found in virtually all foods [51,52]. In addition to carbohydrate modifications, lectin interactions are guided by amino acid residues of their target receptor proteins. Lectins can bind to their receptors with dissociation constants (K_d) that rival the specificity of kinase inhibitors (e.g. lapatinib or imatinib) [33] and antibodies used against cancer (e.g. trastuzumab) [10,61,6,62]. For example, C-type lectin-like receptor 2 (CLEC-2) targets PDPN with an average dissociation constant (K_d) of less than 4 nM [74]. In addition, unlike antibodies, lectins are resistant to gastrointestinal proteolysis [63,60,62] and can be taken orally to treat cancer [61,19,62]. Indeed, lectins can block the action of endogenous pro-metastatic lectins (such as galectins or selectins) to inhibit tumor cell growth [59-61], and can be used medicinally to treat cancer in people [62]. However, most anti-cancer lectins that have been examined thus far have intrinsically toxic ribosome inhibitory protein (RIP) activity similar to that of ricin, and target receptors that have not been identified [62]. Unlike these other lectins, MASL is not toxic to normal cells and targets a known receptor on cancer cells - PDPN.

As mentioned above, antisera may offer greater targeting specificity but lectins offer the advantage of oral administration. Dietary legumes (lentils, peas, or beans) have been shown to significantly lower the incidence of skin cancer [39]. Digestion of 200 grams of peanuts results in

concentrations of up to 200 nM of intact peanut lectin (PNA) in circulating blood [79]. Our Preliminary Studies and recently published data [57] indicate that similar concentrations of our model leguminous lectin, MASL, survive digestion, enter the circulatory system, and can effectively inhibit OSCC cell growth and migration.

Human safety for MASL has already been demonstrated as a "coincidental" component in traditional medicines used to treat ailments including cancer. *Maackia amurensis* has been used as a medicinal plant in Asia (called "Huai Huai" or "怀槐" in China) for several centuries [43,45,42,44,13]. It should also be noted that, although its targets and mechanisms are not yet defined, Mistletoe lectin (viscumin) binds proteins containing α2,3-sialic acid, has undergone clinical trials, and is widely used to treat melanoma in Europe [2,10,24]. However, human safety studies for MASL have not been reported.

We plan to utilize PDPN as a functionally relevant biomarker and chemotherapeutic target to combat oral cancer. For this project, oral cancers and precancerous lesions will be removed and examined for PDPN expression. Sensitivity to MASL, which targets PDPN on transformed cells, will be evaluated in fixed samples and primary cell cultures established from these specimens. MASL will then be orally administered to patients with lesions that contain cells that express PDPN and are sensitive to MASL. Samples from subsequent biopsies or resections, as determined by best care practices, will then be analyzed for PDPN expression and cell morphology. Overall oral and physical health will also be examined to evaluate the effects of MASL on each patient.

Thus, disease, investigation products, and rationale are as follows:

a. Disease: oral cancer, specifically oral squamous cell carcinoma (OSCC).
b. Investigational products: Maackia amurensis seed lectin (MASL).
c. Rationale: MASL can be used as a nontoxic oral agent that targets the podoplanin (PDPN) receptor on OSCC cells topically and systemically to their growth, motility, and tumor progression.

3. Research Design and Methods

This trial will conducted in compliance with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirements. Up to 50 patients with visible oral lesions fitting the clinical criteria for cancerous or precancerous lesions undergoing surgery or biopsy (as part of clinical care) at the NJMS or RSDM oral medicine clinic/Rutgers Dental Associates will be targeted for consideration as participants in this study. This includes patients with oral cancer, proliferative verrucous leukoplakia (PVL), conventional oral leukoplakia (OL), and erythroplakia, plus suspect oral papillomas and oral lichen planus. When the identified oral lesion seems appropriate, patients will be asked to sign an informed consent allowing a portion of the biopsy sample or excised tumor to be utilized for research purposes. These specimens will be transferred directly to Dr. Goldberg at Rowan University-SOM for preparation of cell cultures and other research-related testing.

Oral cancers will be sampled surgically by Dr. Soly Baredes, Dr. Roden, Dr. Evelyne Kalyoussef, Dr. Ghayoour Mir or Dr. Rabie Shanti. Precancerous lesions will be biopsied surgically by Dr. Mahnaz Fatahzadeh. This work will be performed at the NJMS/RSDM/Rutgers Dental associates and University Hospital in Newark, NJ. Biopsy will be performed by original surgeon as determined by best care practice. These procedures will have been planned as a matter of course for the patient. Therefore, the patients' participation in this study should not affect their treatment. The operating surgeon will place a sample of the tissue on wet ice. Dr. Goldberg will receive the tissue from the pathology department after they agree that this tissue is not needed for further

analysis. Dr. Goldberg will be waiting in a room as instructed by the surgeon and pathologist and will package the specimens on wet ice and bring them to his lab according to IATA protocols.

Dr. Suster and Dr. Alan Shienbaum will evaluate PDPN expression and cell morphology by IHC from formaldehyde fixed section of these samples. Surgical specimens will be fixed in 10% formalin in PBS, paraffin embedded, sectioned (4 microns), and processed for hematoxylin/eosin staining and immunohistochemistry D2-40 monoclonal antibody (Dako) to detect PDPN as described [56]. Paraffin-fixed specimens will be prepared by the local oral pathology laboratories at the University Hospital in Newark, NJ as part of the diagnostic histopathology. Routine pathology reports generated during patient care will also be examined to verify the tumor grade and nature of these specimens. Data will be examined by Dr. Suster and Dr. Shienbaum in a blinded manner with coded labels such that they will not know whether the specimens are from MASL or placebo treated patients.

In addition to IHC analysis, Dr. Goldberg will establish primary cell cultures from these samples. He will evaluate cell morphology, assay PDPN expression by Western blotting, and evaluate the effects of MASL on the growth and migration of these cells by standard growth and motility assays [57]. This work will be performed at the Science Center in Stratford, NJ. Results from these studies will be used to elucidate understand how OSCC cells respond to MASL. However, these cell culture data will not be used as eligibility criteria since these assays may not be accomplished in a time frame consistent with treatment plans. The IHC analysis can be completed within 4 days. Therefore, this testing will not delay any subsequent treatment plans or options.

Patients with OSCC cells that express robust levels of PDPN will be considered for treatment. Robust levels of PDPN will be defined as greater than normal cells and equivalent or greater than PDPN levels expressed by lymphatic endothelial cells (LECs) from the same sample specimen. Patients that meet the eligibility criteria will be enrolled in the treatment phase of this study. Study clinicians (Dr. Baredes, Dr. Roden, Dr. Kalyoussef, Dr. Shanti, Dr. Mir or Dr. Fatahzadeh) will administer MASL or placebo to enrolled subject. Patients will be given a lozenge (troche)with or without MASL added to achieve a final dosage of 100 mg per adult. Clinicians administering the lozenge and Dr. Goldberg are not blinded to the type of the lozenge; however, this information is not available to the pathologist who evaluates tumor pathology, cell morphology, and PDPN expression in the patient samples.

In addition to the process described above, patients already diagnosed with oral cancer/precancer who present to one of the study clinicians for definitive care may also be enrolled in the study if they consent to participation. In these situations, it is routine to request H&E-stained slides from the original surgical pathology department for review as part of the surgical planning for clinical care. For patients who consent, the study clinician or PI would also request 1-3 unstained specimens for D2-40 staining to determine PDPN expression for research purposes. Patients with OSCC cells that express robust levels of PDPN and meet the eligibility criteria will be considered for treatment phase. The remainder of the protocol remains the same.

MASL and placebo lozenges will be prepared by an approved GMP certified facility by chromatography methods established by Dr. Goldberg as described [57]. Lozenges will be stored at the University Hospital Pharmacy and be hand delivered to the administering doctor by study investigators listed on page 1 in a sealed plastic container labeled with the patients name and ID. This work will be performed at the NJMS/RSDM/Rutgers Dental associates and University Hospital in Newark, NJ. Dr. Baredes, Dr. Kalyoussef, Dr. Roden, Dr. Shanti, Dr. Mir or Dr. Fatahzadeh along with Dr. Capitle and Kyi will perform a general physical evaluation of their respective patients before and after MASL or placebo administration. Dr. Capitle or Dr. Kyi will

evaluate patient vital signs including temperature, pulse, blood pressure, and overall health after administration. Dr. Capitle or Dr. Kyi will also assist with management of any complication should it arises.

In this approach, MASL will find administration through topical exposure to oral lesions in the mouth as the lozenge dissolves. Patients will be asked to dissolve the lozenge in their mouth for at least 5 minutes before being chewed, and refrain from spitting, eating, or drinking for at least 20 minutes after lozenge administration. Subjects will be asked to finish chewing the lozenge within 20 minutes. Systemic exposure through the blood stream may also be accomplished. Data from university studies on cell lines and animal models indicate that MASL can be applied topically or eaten to target PDPN in order to inhibit tumor cell growth and migration. However, pharmacokinetics of this compound have not been established.

3.1. Duration of Study

This study should be completed within 24 months of the start date. Each patient will participate in the study for about 2 to 6 months.

3.2 Study Sites

Keystone Medical Laboratories
781 Keystone Industrial Park
Throop, PA 18512

Science Center
Rowan University SOM
Stratford, NJ 08084

Rutgers School of Dental Medicine
Oral Medicine Clinic
110 Bergen Street
Newark, NJ 07103

Rutgers Dental Associates
90 Bergen Street (Suite 7700)
Newark, NJ 07103

Doctors Office Center
Allergy, Immunology and Rheumatology Clinic
90 Bergen Street (Suite 4500)
Newark, NJ 07103

Ambulatory Care Center
Otolaryngology Clinic
140 Bergen Street
Newark, NJ 07103

Doctors Office Center
Otolaryngology Clinic
90 Bergen Street (Suite 8100)
Newark, NJ 07103

University Hospital (Unit 1, Pathology, OR)
150 Bergen St
Newark, NJ 07103

3.3 Sample Size Justification

We will recruit up to 10 patients for MASL treatment and 10 patients for placebo treatment to attempt a p value of less than 0.05 based on a difference of 40% with respect to Podoplanin expression and MASL sensitivity. Patients will be selected for treatment with MASL versus placebo by 1:1 randomization.

3.4 Subject Selection and Enrollment Considerations

Subjects will be enrolled to fulfill a sample size in light of power considerations. These subjects will be enrolled from a well-defined population of patients presenting oral cancer or suspected lesions determined and treated by best care practice, and methods assuring human subjects' protections, including their voluntary & informed consent, privacy of person and confidentiality of data, and equitable access to research.

3.5.1 Inclusion Criteria

1.) Males and females of at least 18 years of age who are able to give consent.
2.) Smokers and non-smokers.
3.) Persons with white or red spots and/or lesions suspected or found to be oral cancer or precancer on the inner surface of the mouth.
4.) Oral lesions will be classified as OSCC or leukoplakia including, proliferative verrucous leukoplakia, conventional erythroplakia, suspect oral papillomas, or oral lichen planus. Only patients with such histologically confirmed diagnoses will be considered for inclusion.
5.) patients will be considered for inclusion at any stage of disease progression.
6.) Patients will be considered for inclusion if a subsequent biopsy or surgical resection are planned as part of their best care treatment.
7.) Patients with previously diagnosed oral cancer/precancer who present to the study clinicians for definitive surgical care, consent to participate, and meet the criteria for enrolment.
8.) Patients will have an Eastern Cooperative Oncology Group performance status of 0 or 1.
9.) Patients will display normal organ function as evidenced by standard laboratory blood tests including liver enzymes and creatine.
10.) Patients will not present evidence of comorbidities including ongoing or active infection, unstable illness, or medical conditions.

3.5.2 Exclusion Criteria

1.) Patients with cognitive impairments and cannot consent for themselves.
2.) Patients with language/hearing impairments.
3.) Use of a topical steroid product within the last 2 weeks.
4.) Pregnant women (to avoid any potential risk to the fetus) to be confirmed by standard blood or urine tests according to best care practice.
5.) Patients who are breastfeeding.
6.) Abstinence or use of adequate contraception will be required for women of childbearing potential and men of reproductive potential.

3.5.3 Subject Recruitment

Subjects will be recruited directly by Dr. Baredes, Dr. Kalyoussef, Dr. Roden, Dr. Shanti, Dr. Mir and Dr. Fatahzadeh. Patients will be selected for treatment with MASL versus placebo by 1:1 randomization.

3.5.4 Consent Procedures

The consent process will take place before the initiation of the study procedures. The physicians will discuss this process during the first office visit prior to surgery, or earlier. Patient will be approached with the forms and have the protocol described to them. Participants will discuss their participation with the surgeons before signing the consent form. A full description of the project will be given to the prospective participants in lay language in sufficient time for them to consider whether or not to participate in the study.

The entire process will be described to each subject in lay language in order for them to fully understand the nature of their involvement in the research. Consent will not be sought from subject with cognitive and/or language/hearing impairments as described in the experimental protocols.

3.5.5 Subject Costs and Compensation

There will be no costs or compensation for participants. This work will utilize samples to be taken during the course of standard care.

3.6 Chart Review Selection

All study documents such as consent forms, biopsy reports, and patient's identifiers (name, gender, birth date, etc.) are maintained in a locked cabinet in the PI's office (Dr. Mahnaz Fatahzadeh) in Newark. A copy of the relevant data (name, gender, DOB,..) including biopsy reports will also be kept in Dr. Gary Goldberg's office in the science center at SOM. This information will not be transferred to any files used for tissue analysis or other procedures. These identifiers will only be accessible to cross referencing a coded sample number that is used for all research purposes. Patient identifiers will only be used to verify results with the physicians that are involved in this study.

4. Study Variables

4.1 Independent Variables or Interventions

4.1.1 Drug or Device Interventions

This study involves the potential drug MASL (FDA IND#: 118210). MASL will be extracted, purified, and compounded into lozenges by the University of California GMP facility by protocols that result in MASL that is essentially free of salts and other contaminants that can be successfully used in cell culture and animals [57,56]. Patients will be given a lozenge with or without MASL added to achieve a final dosage of 100 mg per adult. Patients will be given a lozenge with or without MASL added to achieve a final dosage of 100 mg per adult. MASL will be prepared by the University of California Davis GMP facility. Patients will be watched for 2 hours after administration and examined for any reactions to the treatment.

As described above (see Section 3) MASL targets PDPN to inhibit tumor cell growth and motility [1,38,57]. Although some lectins may nonspecifically bind to many glycoproteins, MASL can precisely target specific glycoproteins expressed by human cells [47,46]. MASL can be taken

orally to inhibit tumor growth in mice. Moreover, no side effects were evident from MASL ingestion based on animal weight, behavior, or organ morphology [57,65]. Human safety for MASL has already been demonstrated as a “coincidental” component in traditional medicines used to treat ailments including cancer. *Maackia amurensis* has been used as a medicinal plant in Asia for several centuries, with doses averaging 100 mg per adult administration [43,45,42,44,13]. However, human safety studies for MASL have not been reported.

4.2 Dependent Variables or Outcome Measures

Up to 1 gram of tumor tissue will be analyzed. The cells in this tissue will be adapted to cell culture. Protein will be examined by IHC and Western blotting. Effects of MASL on PDPN expression, cell growth, migration, and morphology will be analyzed by standard growth, motility, and IHC assays. Patient age and sex will be correlated to these results to determine if correlations exist between age and sex.

4.3 Risk of Harm

Some medical risks are associated with this study since patients will be treated according to standard care and with an experimental nutraceutical (MASL). This compound has been used as an anecdotal incidental component of traditional medicines for many centuries without reports of harmful side effects. Risks of information distribution would involve patient name, age, sex, and tumor grade. These risks will be minimized by keeping information related to patient identifiers encoded, locked in the PI’s office, password protected, and destroyed after the study. Patient specimens will not be labeled with patient names or other identifiers; they will be labeled with unique identifiers to maintain patient privacy and confidentiality.

Protocols are in place for assessment and treatment of potential reactions to MASL. Patients will be observed for 2 hours after every administration for any development of effect such as hypersensitivity reaction. Any individual who develops suspected allergic episode will be evaluated for manifestations such as urticaria, angioedema, flushing, pruritus, upper airway obstruction, gastrointestinal symptoms, syncope, hypotension, and lower airway obstruction. Mild allergic reactions will be treated with anti-histamines and/or epinephrine. If the patient has moderate to severe anaphylaxis, intramuscular epinephrine will be administered into the lateral thigh. The adult dose of 1:1,000 epinephrine is 0.2 to 0.5 mL. If there is no significant improvement in symptoms, then the dose will be repeated approximately every 5 to 15 minutes, as the physician deems to be necessary. Intravenous or oral corticosteroid 1 to 2 mg/kg per dose up to 125 mg of methylprednisolone will be considered to decrease the biphasic or prolonged reactions. If needed, oxygen up to 100% will be administered at a flow rate of 6 to 10 L/min through a facemask. Oxygen saturation will be monitored and kept at 94% to 96% by oximetry. For signs and symptoms of bronchospasm (eg, wheezing, coughing, and shortness of breath) that has not responded to intramuscular epinephrine, albuterol will be administered through a nebulizer and facemask. Hypotension will be treated with rapid fluid replacement using 1 to 2 L of 0.9% normal saline, infused rapidly. For the normotensive patient in anaphylaxis, starting normal saline at an appropriate maintenance rate for weight (125 mL/h for adults) to maintain venous access for medications and/or rapid fluid replacement will be considered if necessary.

If the patient has severe anaphylaxis or is showing signs and symptoms of impending cardiopulmonary arrest, EMS will be summoned immediately in addition to all available office medical staff. Cardiopulmonary resuscitation will be started immediately in the event of cardiopulmonary arrest, with emphasis on adequate chest compressions without interruption.

Ventilations can be given once there are 2 medical staff members at the patient's side. For imminent or established cardiopulmonary arrest, venous access will be rapidly established to administer an intravenous bolus dose of epinephrine. For adults, the dose is 1 mg intravenously as a 1: 10,000 dilution that can be repeated every 3 to 5 minutes. If epinephrine is ineffective in treating anaphylaxis in patients taking b-blockers, then glucagon administration might be necessary.

Patient's blood pressure, cardiac rate and function, respiratory status, and oxygenation will be monitored and recorded at frequent and regular intervals. The duration of direct observation and monitoring after an episode of anaphylaxis will be individualized and based on the severity and duration of the anaphylactic event and response to treatment. Patients with moderate to severe anaphylaxis will be observed for a minimum of 4 to 8 hours. Mild anaphylactic symptoms that rapidly resolve with treatment usually will require a relatively shorter period of observation. At the time of discharge from medical supervision, patients will be instructed to call the office or call 911 or go to the emergency room in case there is recurrence of symptoms of an allergic reaction. Prescription, written action plan and follow up visits deemed necessary will be discussed upon discharge.

4.4 Potential for Benefit

This project presents great potential for benefit. Forthcoming results should elucidate the role of a specific receptor in oral cancer progression, and methods to target this receptor to prevent and combat cancer.

5. Data Handling and Statistical Analysis

Data will be analyzed in Excel and Prism software to evaluate means, standard errors, t-test, and ANOVA. Only the PI (Dr Goldberg) will have access to these raw files. Versions of these files without patient identifiers will be made available to the other members of this study group. Patient identifiers will only be used to verify results with the physicians that are involved in this study. Records with identifiers will be destroyed 6 years after completion of the study. This time is needed to complete laboratory experiments on the collected samples and allows tracing and re-examination of the histological slides should any question regarding diagnosis arises.

6. Data and Safety Monitoring

The effects of MASL on overall patient health and oral health will be monitored by trained physicians and dentists before and after each dose. No other drugs or devices will be given to patients. Attending investigators will evaluate adverse effects in real time. Data will be collected and stored with security measures used to protect it from loss or inappropriate use by password protection, restricted access, and, database backup.

An independent safety monitoring group has been set up for this study. The name of the Data Safety Monitoring Board (DSMB) is "MASL data safety monitoring board". The group will meet bimonthly, and will generate a written report from each meeting. These meetings will be closed to the public, and the group will be supplied with records and descriptions of patient responses in the study and scientific data that is obtained from the study. These descriptions will be complete, but the study coordinator will remove patient names and any personal identifiers before they are given to the group for evaluation. The group will use these descriptions to generate reports that

will contain 3 sections divided into: (1) Assessment of progress, (2) Presentation and analysis of any problems that arise, and (3) Recommendations based on assessment of progress and problems. Examples of problems that arise may include reactions of patients to the study drug including rashes, changes in heart rate, temperature, or breathing. Written reports will be available to the IRB since the director of the DSMB will send them directly to the study coordinator who will upload them to the Study Profile on the eIRB website. The group will be made up of the following professionals which include basic scientists (PhD) and a physician (MD, PhD) with experience in molecular and cell biology, acute reactions and shock, nutrition and digestion, and biostatistics:

Name and degrees	Position	Affiliation	Expertise
Kingsley Yin, Ph.D	Assistant Professor	Rowan-SOM	Molecular and cell biology, physiology, acute reactions and shock (Dr. Yin will serve as DSMB director)
Raymond Birge, Ph.D.	Professor	Rutgers-NJMS	Signal transduction, tumor cell survival and migration, chemotherapy mechanisms
Robert Steer, Ph.D.	Professor	Rowan-SOM	Basid and clinical research analysis and biostatistics
Peter Stein, Ph.D.	Professor	Rowan-SOM	Nutrition, digestion, effect of compounds on disease including diabetes, Alzheimer's, and autism, and research ombudsman

7. Reporting Results

7.1 Individual Results

Participants will not receive results since they are relevant to basic science and molecular events that underlie fundamental aspects of OSCC cell growth, migration, and tumorigenesis.

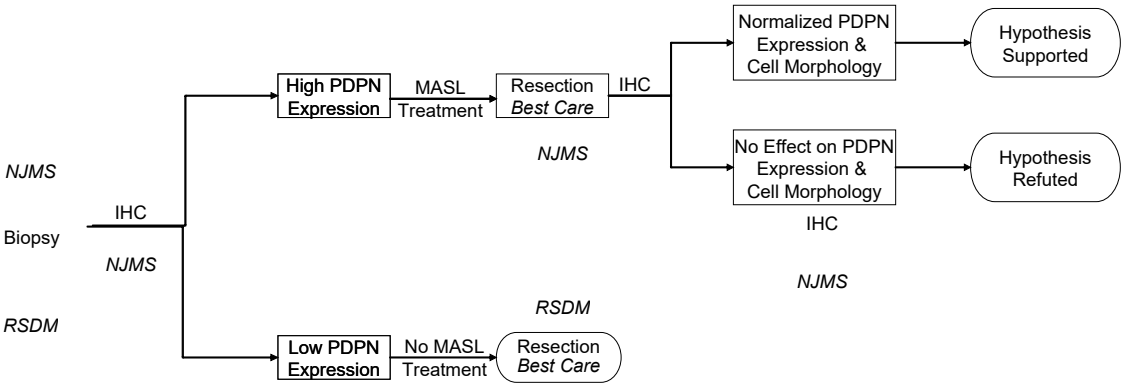
7.2 Aggregate Results

Participants will not receive results since they are relevant to basic science and molecular events that underlie fundamental aspects of OSCC cell growth, migration, and tumorigenesis.

7.3 Professional Reporting

Data will be collected and analyzed in a blind manner. Results will be published in peer reviewed journals to share results of this research with the scientific community.

Study Design Flowchart



Biopsies taken from patients will be examined by immunohistochemistry (IHC). Patients with lesions with high PDPN expression who meet the eligibility criteria for the trial phase will be offered MASL treatment before resection or second biopsy as determined by best care practice. Surgical specimens will then be examined by IHC to examine the effects of MASL on PDPN expression and cell morphology. Our hypothesis predicts that MASL will reduce PDPN expression and normalize the morphology of oral squamous carcinoma cells.

Schedule of Events

Subject Recruitment	
Identification of patients with premalignant or malignant lesions during the course of clinical care (this includes previously diagnosed oral cancer/precancer patients presenting for surgical resection as part of clinical care)	Dr. Fatahzadeh Dr. Baredes Dr. Roden Dr. Kalyoussef Dr. Shanti Dr. Mir
informed consent	
Clinical procedures as per best care practice	
Diagnostic tissue biopsy	Dr. Fatahzadeh Dr. Baredes Dr. Roden Dr. Kalyoussef Dr. Shanti Dr. Mir
Laboratory Procedures	
Diagnostic histopathology, Evaluation of cell morphology & PDPN expression	Dr. David Suster Dr. Alan Sheinbaum
Identification of patient samples with robust PDPN expression	
Clinical procedures as per best care practice	
Planning resection or second biopsy	Dr. Fatahzadeh Dr. Baredes Dr. Kalyoussef Dr. Roden Dr. Shanti Dr. Mir
Treatment Phase	
Evaluation of eligibility criteria for enrolment. Eligible patients: • Have tissue samples with robust PDPN expression • Have not used topical steroid products within the last 2 weeks • Have Eastern Cooperative Oncology Group performance status of 0 or 1. • Display normal organ function as evidenced by standard laboratory blood tests including liver enzymes and creatine. • Do not present evidence of comorbidities including ongoing or active infection, unstable illness, or medical conditions. • Not pregnant as per standard blood or urine tests according to best care practice • Not breast feeding • Consent to Abstinence or use of adequate contraception (women of childbearing potential and men of reproductive potential)	Dr. Fatahzadeh Dr. Baredes Dr. Kalyoussef Dr. Roden Dr. Shanti Dr. Mir
MASL administration within 1 week of planned biopsy or resection	Dr. Fatahzadeh Dr. Baredes Dr. Kalyoussef Dr. Capitle Dr. Kyi Dr. Roden Dr. Shanti Dr. Mir
Clinical procedures as per best care practice	
Planned resection or second biopsy	Dr. Fatahzadeh Dr. Baredes Dr. Kalyoussef Dr. Roden Dr. Shanti Dr. Mir
Laboratory Procedures	
Diagnostic histopathology, Evaluation of cell morphology & PDPN expression	Dr. David Suster Dr. Alan Sheinbaum

8. Bibliography

[1] J.A.O. Alvarez, C. George, H. Krishnan, X. Wu, and G.S. Goldberg, Contact Normalization: mechanisms and pathways to biomarkers and chemotherapeutic targets, in: J. Adams (Ed.), Extracellular and Intracellular Signaling, Royal Society of Chemistry, Cambridge, 2011, pp. 105-115.

[2] M. Augustin, P.R. Bock, J. Hanisch, M. Karasmann, and B. Schneider (2005) Safety and efficacy of the long-term adjuvant treatment of primary intermediate- to high-risk malignant melanoma (UICC/AJCC stage II and III) with a standardized fermented European mistletoe (Viscum album L.) extract. Results from a multicenter, comparative, epidemiological cohort study in Germany and Switzerland. *Arzneimittelforschung.* 55, 38-49.

[3] T. Bagnyukova, I.G. Serebriiskii, Y. Zhou, E.A. Hopper-Borge, E.A. Golemis, and I. Astsaturov (2010) Chemotherapy and signaling: How can targeted therapies supercharge cytotoxic agents? *Cancer Biol.Ther.* 10.

[4] J. Califano, R.P. van der, W. Westra, H. Nawroz, G. Clayman, S. Piantadosi, R. Corio, D. Lee, B. Greenberg, W. Koch, and D. Sidransky (1996) Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Research* 56, 2488-2492.

[5] C.M. Christou, A.C. Pearce, A.A. Watson, A.R. Mistry, A.Y. Pollitt, A.E. Fenton-May, L.A. Johnson, D.G. Jackson, S.P. Watson, and C.A. O'Callaghan (2008) Renal cells activate the platelet receptor CLEC-2 through podoplanin. *Biochem.J.* 411, 133-140.

[6] D.L. Costantini, C. Chan, Z. Cai, K.A. Vallis, and R.M. Reilly (2007) (111)In-labeled trastuzumab (Herceptin) modified with nuclear localization sequences (NLS): an Auger electron-emitting radiotherapeutic agent for HER2/neu-amplified breast cancer. *J.Nucl.Med.* 48, 1357-1368.

[7] L.N. Cueni, and M. Detmar (2009) Galectin-8 interacts with podoplanin and modulates lymphatic endothelial cell functions. *Experimental Cell Research* 315, 1715-1723.

[8] L.N. Cueni, I. Hegyi, J.W. Shin, A. Albinger-Hegyi, S. Gruber, R. Kunstfeld, H. Moch, and M. Detmar (2010) Tumor lymphangiogenesis and metastasis to lymph nodes induced by cancer cell expression of podoplanin. *Am.J.Pathol.* 177, 1004-1016.

[9] J.S. de Bono, and A. Ashworth (2010) Translating cancer research into targeted therapeutics. *Nature* 467, 543-549.

[10] E.G. De Mejia, and V.I. Prisecaru (2005) Lectins as bioactive plant proteins: a potential in cancer treatment. *Crit Rev.Food Sci.Nutr.* 45, 425-445.

[11] E.M. Diaz, Jr., F.C. Holsinger, E.R. Zuniga, D.B. Roberts, and D.M. Sorensen (2003) Squamous cell carcinoma of the buccal mucosa: one institution's experience with 119 previously untreated patients. *Head Neck* 25, 267-273.

[12] S. Dixit, R.K. Vyas, R.B. Toparani, H.A. Baboo, and D.D. Patel (1998) Surgery versus surgery and postoperative radiotherapy in squamous cell carcinoma of the buccal mucosa: a comparative study. *Ann.Surg.Oncol.* 5, 502-510.

[13] S.A. Fedoreev, N.I. Kulish, L.I. Glebko, T.V. Pokushalova, M.V. Veselova, A.S. Saratikov, A.I. Vengerovskii, and V.S. Chuchalin (2010) Maksar: A preparation based on amur maackia. *Pharmaceutical Chemistry Journal* 38, 605-610.

[14] J.K. Field, D.A. Spandidos, P.M. Stell, E.D. Vaughan, G.I. Evan, and J.P. Moore (1989) Elevated expression of the c-myc oncoprotein correlates with poor prognosis in head and neck squamous cell carcinoma. *Oncogene* 4, 1463-1468.

[15] A. Forastiere, W. Koch, A. Trotti, and D. Sidransky (2001) Head and neck cancer. *N.Engl.J.Med.* 345, 1890-1900.

[16] A. Funayama, J. Cheng, S. Maruyama, M. Yamazaki, T. Kobayashi, M. Syafriadi, S. Kundu, S. Shingaki, C. Saito, and T. Saku (2011) Enhanced expression of podoplanin in oral carcinomas in situ and squamous cell carcinomas. *Pathobiology* 78, 171-180.
[17] M. Gasco, and T. Crook (2003) The p53 network in head and neck cancer. *Oral Oncol.* 39, 222-231.
[18] S.A. Geisler, A.F. Olshan, M.C. Weissler, J. Cai, W.K. Funkhouser, J. Smith, and K. Vick (2002) p16 and p53 Protein expression as prognostic indicators of survival and disease recurrence from head and neck cancer. *Clin.Cancer Res.* 8, 3445-3453.
[19] S.S. Hasan, G.M. Ashraf, and N. Banu (2007) Galectins - potential targets for cancer therapy. *Cancer Lett.* 253, 25-33.
[20] W.L. Hicks, Jr., T.R. Loree, R.I. Garcia, S. Maamoun, D. Marshall, J.B. Orner, V.Y. Bakamjian, and D.P. Shedd (1997) Squamous cell carcinoma of the floor of mouth: a 20-year review. *Head Neck* 19, 400-405.
[21] S.L. Holley, C. Matthias, V. Jahnke, A.A. Fryer, R.C. Strange, and P.R. Hoban (2005) Association of cyclin D1 polymorphism with increased susceptibility to oral squamous cell carcinoma. *Oral Oncol.* 41, 156-160.
[22] C.J. Huang, K.S. Chao, J. Tsai, J.R. Simpson, B. Haughey, G.J. Spector, and D.G. Sessions (2001) Cancer of retromolar trigone: long-term radiation therapy outcome. *Head Neck* 23, 758-763.
[23] G.F. Huber, F.R. Fritzsche, L. Zullig, M. Storz, N. Graf, K. Haerle, W. Jochum, S.J. Stoeckli, and H. Moch (2011) Podoplanin expression correlates with sentinel lymph node metastasis in early squamous cell carcinomas of the oral cavity and oropharynx. *Int.J.Cancer* 129, 1404-1409.
[24] A. Icirsch (2007) Successful treatment of metastatic melanoma with Viscum album extract (Iscador (R) M). *J.Alternative and Complementary Medicine* 13, 443-445.
[25] A. Imberty, C. Gautier, J. Lescar, S. Perez, L. Wyns, and R. Loris (2000) An unusual carbohydrate binding site revealed by the structures of two Maackia amurensis lectins complexed with sialic acid-containing oligosaccharides. *J.Biol.Chem* 275, 17541-17548.
[26] L. Ingrassia, I. Camby, F. Lefranc, V. Mathieu, P. Nshimyumukiza, F. Darro, and R. Kiss (2006) Anti-galectin compounds as potential anti-cancer drugs. *Curr.Med.Chem* 13, 3513-3527.
[27] H. Inoue, Y. Miyazaki, K. Kikuchi, N. Yoshida, F. Ide, Y. Ohmori, A. Tomomura, H. Sakashita, and K. Kusama (2012) Podoplanin expression during dysplasia-carcinoma sequence in the oral cavity. *Tumour.Biol.* 33, 183-194.
[28] H. Inoue, Y. Miyazaki, K. Kikuchi, N. Yoshida, F. Ide, Y. Ohmori, A. Tomomura, H. Sakashita, and K. Kusama (2012) Podoplanin promotes cell migration via the EGF-Src-Cas pathway in oral squamous cell carcinoma cell lines. *J.Oral Sci.* 54, 241-250.
[29] S.G. Iyer, S.A. Pradhan, P.S. Pai, and S. Patil (2004) Surgical treatment outcomes of localized squamous carcinoma of buccal mucosa. *Head Neck* 26, 897-902.
[30] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, and D. Forman (2011) Global cancer statistics. *CA Cancer J.Clin.* 61, 69-90.
[31] K.A. Johnson, and P.H. Brown (2010) Drug development for cancer chemoprevention: focus on molecular targets. *Semin.Oncol.* 37, 345-358.
[32] N.W. Johnson, P. Jayasekara, and A.A. Amarasinghe (2011) Squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology. *Periodontol.2000.* 57, 19-37.
[33] M.W. Karaman, S. Herrgard, D.K. Treiber, P. Gallant, C.E. Atteridge, B.T. Campbell, K.W. Chan, P. Ciceri, M.I. Davis, P.T. Edeen, R. Faraoni, M. Floyd, J.P. Hunt, D.J. Lockhart, Z.V. Milanov, M.J. Morrison, G. Pallares, H.K. Patel, S. Pritchard, L.M. Wodicka, and P.P. Zarrinkar (2008) A quantitative analysis of kinase inhibitor selectivity. *Nat.Biotechnol.* 26, 127-132.
[34] Y. Kato, M.K. Kaneko, A. Kunita, H. Ito, A. Kameyama, S. Ogasawara, N. Matsuura, Y. Hasegawa, K. Suzuki-Inoue, O. Inoue, Y. Ozaki, and H. Narimatsu (2008) Molecular analysis of the

pathophysiological binding of the platelet aggregation-inducing factor podoplanin to the C-type lectin-like receptor CLEC-2. *Cancer Sci.* 99, 54-61.

[35] V.M. Kotelnikov, J.S. Coon, S. Mundle, S. Kelanic, S. LaFollette, S.I. Taylor, J. Hutchinson, W. Panje, D.D. Caldarelli, and H.D. Preisler (1997) Cyclin D1 expression in squamous cell carcinomas of the head and neck and in oral mucosa in relation to proliferation and apoptosis. *Clin.Cancer Res.* 3, 95-101.

[36] M. Kreppel, U. Drebber, I. Wedemeyer, H.T. Eich, T. Backhaus, J.E. Zoller, and M. Scheer (2011) Podoplanin expression predicts prognosis in patients with oral squamous cell carcinoma treated with neoadjuvant radiochemotherapy. *Oral Oncol.* 47, 873-878.

[37] M. Kreppel, M. Scheer, U. Drebber, L. Ritter, and J.E. Zoller (2010) Impact of podoplanin expression in oral squamous cell carcinoma: clinical and histopathologic correlations. *Virchows Arch.* 456, 473-482.

[38] H. Krishnan, W.T. Miller, and G.S. Goldberg (2012) SRC points the way to biomarkers and chemotherapeutic targets. *Genes Cancer* 3, 426-435.

[39] G.A. Kune, S. Bannerman, B. Field, L.F. Watson, H. Cleland, D. Merenstein, and L. Vitetta (1992) Diet, alcohol, smoking, serum beta-carotene, and vitamin A in male nonmelanocytic skin cancer patients and controls. *Nutr.Cancer* 18, 237-244.

[40] A. Kunita, T.G. Kashima, Y. Morishita, M. Fukayama, Y. Kato, T. Tsuruo, and N. Fujita (2007) The platelet aggregation-inducing factor aggrus/podoplanin promotes pulmonary metastasis. *Am.J.Pathol.* 170, 1337-1347.

[41] J.J. Lee, W.K. Hong, W.N. Hittelman, L. Mao, R. Lotan, D.M. Shin, S.E. Benner, X.C. Xu, J.S. Lee, V.M. Papadimitrakopoulou, C. Geyer, C. Perez, J.W. Martin, A.K. El-Naggar, and S.M. Lippman (2000) Predicting cancer development in oral leukoplakia: ten years of translational research. *Clinical cancer research : an official journal of the American Association for Cancer Research* 6, 1702-1710.

[42] J.F. Li, Z. Cui, and F.L. Zhang (2006) Research progress in the chemical constituents and pharmacological activities of Maackia. *Journal of Shenyang Pharmaceutical University* 23, 541-545.

[43] S. Li (1593) Bencao Gangmu (A Materia Medica, Arranged according to Drug Descriptions and Technical Aspects) Ming Dynasty, China.

[44] X. Li, D. Wang, M.Y. Xia, Z.H. Wang, W.N. Wang, and Z. Cui (2009) Cytotoxic prenylated flavonoids from the stem bark of Maackia amurensis. *Chem.Pharm.Bull.(Tokyo)* 57, 302-306.

[45] J. Luo, G. Liang, and S. Jiang (2003) Study on extraction and hepatoprotective function of isoflavones from callus cultures of Maackia Amurensis. *Food Science* 24, 139-142.

[46] K. Maenuma, M. Yim, K. Komatsu, M. Hoshino, A. Tachiki-Fujioka, K. Takahashi, Y. Hiki, N. Bovin, and T. Irimura (2009) A library of mutated Maackia amurensis hemagglutinin distinguishes putative glycoforms of immunoglobulin A1 from IgA nephropathy patients. *J.Proteome.Res.* 8, 3617-3624.

[47] K. Maenuma, M. Yim, K. Komatsu, M. Hoshino, Y. Takahashi, N. Bovin, and T. Irimura (2008) Use of a library of mutated Maackia amurensis hemagglutinin for profiling the cell lineage and differentiation. *Proteomics.* 8, 3274-3283.

[48] L. Mao, A.K. El-Naggar, Y.H. Fan, J.S. Lee, S.M. Lippman, S. Kayser, R. Lotan, and W.K. Hong (1996) Telomerase activity in head and neck squamous cell carcinoma and adjacent tissues. *Cancer Res* 56, 5600-5604.

[49] E. Martin-Villar, F.G. Scholl, C. Gamallo, M.M. Yurrita, M. Munoz-Guerra, J. Cruces, and M. Quintanilla (2005) Characterization of human PA2.26 antigen (T1alpha-2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. *Int.J.Cancer* 113, 899-910.

[50] W.M. Mendenhall, C.G. Morris, R.J. Amdur, J.W. Werning, and D.B. Villaret (2005) Retromolar trigone squamous cell carcinoma treated with radiotherapy alone or combined with surgery. *Cancer* 103, 2320-2325.
[51] M.S. Nachbar, and J.D. Oppenheim (1980) Lectins in the United States diet: a survey of lectins in commonly consumed foods and a review of the literature. *Am.J.Clin.Nutr.* 33, 2338-2345.
[52] A. Naeem, M. Saleemuddin, and R.H. Khan (2007) Glycoprotein targeting and other applications of lectins in biotechnology. *Curr.Protein Pept.Sci.* 8, 261-273.
[53] Y. Nakazawa, S. Sato, M. Naito, Y. Kato, K. Mishima, H. Arai, T. Tsuruo, and N. Fujita (2008) Tetraspanin family member CD9 inhibits Aggrus/podoplanin-induced platelet aggregation and suppresses pulmonary metastasis. *Blood* 112, 1730-1739.
[54] R.W. Nason, K. Sako, W.A. Beecroft, M.S. Razack, V.Y. Bakamjian, and D.P. Shedd (1989) Surgical management of squamous cell carcinoma of the floor of the mouth. *Am.J.Surg.* 158, 292-296.
[55] V.L. Noonan, and S. Kabani (2005) Diagnosis and management of suspicious lesions of the oral cavity. *Otolaryngol.Clin.North Am.* 38, 21-35, vii.
[56] J.A. Ochoa-Alvarez, H. Krishnan, J.G. Pastorino, E. Nevel, D. Kephart, J.J. Lee, E.P. Retzbach, Y. Shen, M. Fatahzadeh, S. Baredes, E. Kalyoussef, M. Honma, M.E. Adelson, M.K. Kaneko, Y. Kato, M.A. Young, L. Deluca-Rapone, A.J. Shienbaum, K. Yin, L.D. Jensen, and G.S. Goldberg (2015) Antibody and lectin target podoplanin to inhibit oral squamous carcinoma cell migration and viability by distinct mechanisms. *Oncotarget* 6, 9045-9060.
[57] J.A. Ochoa-Alvarez, H. Krishnan, Y. Shen, N.K. Acharya, M. Han, D.E. McNulty, H. Hasegawa, T. Hyodo, T. Senga, J.G. Geng, M. Kosciuk, S.S. Shin, J.S. Goydos, D. Temiakov, R.G. Nagele, and G.S. Goldberg (2012) Plant lectin can target receptors containing sialic Acid, exemplified by podoplanin, to inhibit transformed cell growth and migration. *PLoS.ONE.* 7, e41845.
[58] M.L. Poeta, J. Manola, M.A. Goldwasser, A. Forastiere, N. Benoit, J.A. Califano, J.A. Ridge, J. Goodwin, D. Kenady, J. Saunders, W. Westra, D. Sidransky, and W.M. Koch (2007) TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N.Engl.J.Med.* 357, 2552-2561.
[59] I.F. Pryme, S. Bardocz, A. Pusztai, and S.W. Ewen (1999) The growth of an established murine non-Hodgkin lymphoma tumour is limited by switching to a phytohaemagglutinin-containing diet. *Cancer Lett.* 146, 87-91.
[60] I.F. Pryme, S. Bardocz, A. Pusztai, and S.W. Ewen (2002) Dietary mistletoe lectin supplementation and reduced growth of a murine non-Hodgkin lymphoma. *Histol.Histopathol.* 17, 261-271.
[61] I.F. Pryme, S. Bardocz, A. Pusztai, and S.W. Ewen (2006) Suppression of growth of tumour cell lines in vitro and tumours in vivo by mistletoe lectins. *Histol.Histopathol.* 21, 285-299.
[62] A. Pusztai, S. Bardocz, and S.W. Ewen (2008) Uses of plant lectins in bioscience and biomedicine. *Front Biosci.* 13, 1130-1140.
[63] A. Pusztai, S.W. Ewen, G. Grant, W.J. Peumans, E.J. Van Damme, L. Rubio, and S. Bardocz (1990) Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. *Digestion* 46 Suppl 2, 308-316.
[64] A.L. Reed, J. Califano, P. Cairns, W.H. Westra, R.M. Jones, W. Koch, S. Ahrendt, Y. Eby, D. Sewell, H. Nawroz, J. Bartek, and D. Sidransky (1996) High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. *Cancer Research* 56, 3630-3633.
[65] E.P. Retzbach, S.A. Sheehan, E.M. Nevel, A. Batra, T. Phi, A.T.P. Nguyen, Y. Kato, S. Baredes, M. Fatahzadeh, A.J. Shienbaum, and G.S. Goldberg (2018) Podoplanin emerges as a functionally relevant oral cancer biomarker and therapeutic target. *Oral oncology* 78, 126-136.
[66] L.W. Rodgers, Jr., S.P. Stringer, W.M. Mendenhall, J.T. Parsons, N.J. Cassisi, and R.R. Million (1993) Management of squamous cell carcinoma of the floor of mouth. *Head Neck* 15, 16-19.

[67] M.P. Rosin, X. Cheng, C. Poh, W.L. Lam, Y. Huang, J. Lovas, K. Berean, J.B. Epstein, R. Priddy, N.D. Le, and L. Zhang (2000) Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin.Cancer Res.* 6, 357-362.

[68] G.J. Rubin, D.J. Tweardy, and M.F. Melhem (1998) Asynchronous modulation of transforming growth factor alpha and epidermal growth factor receptor protein expression in progression of premalignant lesions to head and neck squamous cell carcinoma. *Clin.Cancer Res.* 4, 13-20.

[69] D.G. Sessions, G.J. Spector, J. Lenox, B. Haughey, C. Chao, and J. Marks (2002) Analysis of treatment results for oral tongue cancer. *Laryngoscope* 112, 616-625.

[70] J.P. Shah, and Z. Gil (2009) Current concepts in management of oral cancer--surgery. *Oral Oncol.* 45, 394-401.

[71] N.G. Shah, T.I. Trivedi, R.A. Tankshali, J.A. Goswami, J.S. Shah, D.H. Jetly, T.P. Kobawala, K.C. Patel, S.N. Shukla, P.M. Shah, and R.J. Verma (2007) Molecular alterations in oral carcinogenesis: significant risk predictors in malignant transformation and tumor progression. *Int.J.Biol.Markers* 22, 132-143.

[72] M.N. Shiu, T.H. Chen, S.H. Chang, and L.J. Hahn (2000) Risk factors for leukoplakia and malignant transformation to oral carcinoma: a leukoplakia cohort in Taiwan. *Br.J.Cancer* 82, 1871-1874.

[73] K. Suzuki-Inoue, Y. Kato, O. Inoue, M.K. Kaneko, K. Mishima, Y. Yatomi, Y. Yamazaki, H. Narimatsu, and Y. Ozaki (2007) Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. *J.Biol.Chem* 282, 25993-26001.

[74] D. Systems (2009) Recombinant human podoplanin Fc chimera, 2009.

[75] S. Temam, H. Kawaguchi, A.K. El Naggar, J. Jelinek, H. Tang, D.D. Liu, W. Lang, J.P. Issa, J.J. Lee, and L. Mao (2007) Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. *J.Clin.Oncol.* 25, 2164-2170.

[76] E.J. Van Damme, F. Van Leuven, and W.J. Peumans (1997) Isolation, characterization and molecular cloning of the bark lectins from Maackia amurensis. *Glycoconj.J.* 14, 449-456.

[77] R.P. van der, H. Nawroz, R.H. Hruban, R. Corio, K. Tokino, W. Koch, and D. Sidransky (1994) Frequent loss of chromosome 9p21-22 early in head and neck cancer progression. *Cancer Research* 54, 1156-1158.

[78] d.W.I. van, K.P. Schepman, and E.H. van der Meij (2000) A modified classification and staging system for oral leukoplakia. *Oral Oncol.* 36, 264-266.

[79] Q. Wang, L.G. Yu, B.J. Campbell, J.D. Milton, and J.M. Rhodes (1998) Identification of intact peanut lectin in peripheral venous blood. *Lancet* 352, 1831-1832.

[80] A. Wicki, and G. Christofori (2007) The potential role of podoplanin in tumour invasion. *Br.J.Cancer* 96, 1-5.

[81] A. Wicki, F. Lehembre, N. Wick, B. Hantusch, D. Kerjaschki, and G. Christofori (2006) Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell* 9, 261-272.

[82] W.N. William, Jr. (2012) Oral premalignant lesions: any progress with systemic therapies? *Curr.Opin.Oncol.*

[83] I.P. Witz (2006) The involvement of selectins and their ligands in tumor-progression. *Immunol.Lett.* 104, 89-93.

[84] I.P. Witz (2006) Tumor-microenvironment interactions: the selectin-selectin ligand axis in tumor-endothelium cross talk. *Cancer Treat.Res.* 130, 125-140.

[85] P. Yuan, S. Temam, A. El-Naggar, X. Zhou, D.D. Liu, J.J. Lee, and L. Mao (2006) Overexpression of podoplanin in oral cancer and its association with poor clinical outcome. *Cancer* 107, 563-569.