PennState

HRP-592 - Protocol for Human Subject Research with Use of Test Article(s)

Protocol Title:
Effect of oral black raspberry administration on oral cell DNA adducts in smokers

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University Park and other campuses: Office for Research Protections Human Research Protection Program The 330 Building, Suite 205 University Park, PA 16802-7014 Phone: 814-865-1775 Fax: 814-863-8699 Email: irb-orp@psu.edu	**College of Medicine and Penn State Health:** Human Subjects Protection Office 90 Hope Drive, Mail Code A115, P.O. Box 855 Hershey, PA 17033 (Physical Office Location: Academic Support Building Room 1140) Phone: 717-531-5687 Email: irb-hspo@psu.edu

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1.0 Objectives

1.1 Study Objectives

In this Black Raspberry Oral Cancer Prevention (BROCAP) study we will determine for the first time the effects of local black raspberry (BRB) delivery on formation of 1) covalent carcinogen-derived DNA adducts and 2) oxidative DNA damage (8-OXO-dG) in exfoliated oral keratinocytes and 3) systemic oxidative DNA damage (8-OXO-dG) in urine in current smokers. Based on preclinical data, we hypothesize that daily black raspberry administration will reduce the levels of DNA damage due to tobacco smoke exposure.

1.2 Primary Study Endpoints

Exfoliated buccal mucosal cell levels of the tobacco specific nitrosamine (N'-nitrosonornicotine , NNN)-derived DNA adduct 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB)-releasing adduct.

1.3 Secondary Study Endpoints

Exfoliated buccal mucosal cell levels of the benzo[a]pyrene (B[a]P)-derived DNA adduct.

Exfoliated buccal mucosal cell levels of the oxidative DNA damage biomarker 8-OXO-dG.

Systemic oxidative DNA damage urinary biomarker 8-OXO-dG.

Tobacco smoke exposure urinary biomarker cotinine.

Systemic lipid peroxidation biomarker urinary 8-isoprostane (8-IsoP).

Urinary anthocyanin.

2.0 Background

2.1 Scientific Background and Gaps

Treating oral squamous cell carcinoma (OSCC) at late stages, even with recent advances in targeting therapies, continues to be a major challenge [1], and thus prevention should be the most effective strategy to manage and control this disease. Our prevention approach focuses on elucidating the molecular mechanisms responsible for the induction of OSCC by environmental carcinogens, followed by development of targeted intervention/chemoprevention strategies. Tobacco use remains a high OSCC risk factor resulting from exposure to potent tobacco carcinogens including polycyclic aromatic hydrocarbons (PAHs) such as dibenzo[a,l]pyrene (DB[a,l]P), benzo[a]pyrene (B[a]P) and tobacco-specific nitrosamines (TSNA) such as N'-nitrosonornicotine (NNN). To assess effects of these carcinogens on oral mucosa, we developed a novel OSCC mouse model using DB[a,l]P and its fjord region diol epoxide (DB[a,l]PDE) [2, 3]. Our preliminary results and literature data demonstrated the presence of covalent B[a]P and NNN derived adducts and 8-OXO-dG in buccal cells of smokers [4-6]. Importantly, we showed that dietary intervention with freeze-dried black raspberries (BRB) powder inhibited carcinogen-induced DNA damage, mutagenesis and carcinogenesis in the mouse oral cavity [3, 7]. Clinical trial data demonstrated topical application of a BRB mucoadhesive gel significantly reduced loss of heterozygosity (LOH) events at putative tumor suppressor gene loci [8]. These LOH data, which show BRB-enabled removal of cells with allelic imbalances or reduced additional LOH events, further support BRB inhibitory role of DNA damage [3, 7]. Altogether, these data suggest that BRB may be effective in reducing the

levels of DNA damage in the oral cavity resulting from tobacco smoke exposure in smokers. However, there have been no clinical studies of the effects of BRB administration in this regard.

2.2 Previous Data

Based on our published preclinical findings [7, 9, 10] and preliminary data, we hypothesize that BRB administration will reduce the levels of DNA damage (B[a]P & NNN-derived adducts & 8-OXO-dG) in buccal keratinocytes from healthy smokers. Previous clinical trials by members of our team and others have identified the efficacy of BRB at reducing the histologic grade, loss of heterozygosity events and clinical size of pre-malignant oral intraepithelial neoplasia (OIN) [8]. BRB also suppressed the expression of antiapoptotic and proinflammatory genes in tumor and at-risk mucosal tissues from oral squamous cell carcinoma patients [11] supporting a preventive/therapeutic role for BRB. Based on these results, we will conduct a phase 0 clinical trial to examine, for the first time, the efficacy of BRB at reducing smoking related DNA damage in healthy smokers. The previous BRB OIN clinical studies precluded tobacco use. As tobacco use remains a key OSCC risk factor, our study is essential to assess the protective efficacy of BRB on DNA damage induced by tobacco carcinogens in current smokers. Additionally, Mallery et al [12] demonstrated that human oral epithelium contains the requisite hydrolytic, phase II and efflux transporting enzymes necessary for local enteric recycling and sustained exposure to active BRB metabolites. We have had extensive experience with conducting similar nutrient-based intervention trials in healthy adult smokers and non-smokers with several of the same measured outcomes (e.g. biomarkers of oxidative stress) including interventions with selenium [13, 14], glutathione [15], liposomal glutathione [16], and omega-3 fatty acids [17, 18]. In most cases these previous trials were similarly designed in regard to number and frequency of visits or were even longer in duration and were highlighted by high levels of subject compliance.

2.3 Study Rationale

On the basis of our published results, preliminary data, and results reported in the literature, there is a strong premise underlying this research. This includes data suggesting that BRB can impact specific DNA repair mechanisms in oral cells [9, 10]. Major carcinogenic agents in tobacco smoke include polycyclic aromatic hydrocarbons such as benzo[a]pyrene (B[a]P) and tobacco specific nitrosamines such as N-nitrosonornicotine (NNN), as well as a variety of powerful oxidants including reactive free radicals. These agents can induce cancer development through formation of DNA adducts including B[a]P adducts, pyridyloxobutyl adducts from NNN, also known as “HPB-releasing adducts” since their measurement is based on the release of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), , and 8-oxo-2'-deoxyguanosine (8-OXO-dG) from free radicals.

Our previous studies confirmed that human surface epithelium and human OSCC keratinocytes possess the Phase 1 enzymes necessary for PAH activation [19] and BRB constituents can alter Phase I and II in a manner consistent with cancer prevention [3]. Furthermore, based on the abundant phenolic constituents in BRB (e.g. anthocyanins), it is logical to propose that BRB helps preserve the cellular redox poise, enhance redox scavenging and therefore function to prevent oxidative damage. In fact, this proposition is in line with our findings demonstrating that BRB inhibits oxidative stress in human oral cells [9]. In addition to a facilitating cytoprotection, cellular reducing equivalents are involved in many biosynthetic reactions including deoxyribonucleotides (dNTPs) that are essential components for DNA repair [20]. Furthermore, BRB contains appreciable levels of minerals such as Mg+2 that are key cofactors necessary for optimal DNA BER enzyme function [21-23]. In addition, our previous study showed that human oral mucosa possesses the requisite hydrolytic, Phase II and efflux transporting enzymes necessary for local enteric recycling which would increase BRB-target tissue contact [12].

Collection of oral mucosa cells is relatively simple and such cells are an excellent source of material for evaluating DNA adducts derived from structurally varied carcinogens identified in tobacco smoke [24] and molecular alterations potentially related to cancer [6, 25-27]. In fact, Stepanov et al.

quantified NNN-induced HPB-releasing DNA adducts in oral cells (44.7 pmol/mg DNA) collected by buccal brushing and by mouth wash of smokers [28]. In nonsmokers HPB releasing DNA adducts were not detected. A recent report demonstrated that NNN-induced DNA damage quantified in buccal cells was an independent risk factor for head and neck cancer [4]. Our preliminary results unequivocally demonstrate the presence of DNA adducts derived from B[a]P in buccal cells of smokers. Such damage can provide a novel target for cancer prevention using agents such as BRB, which enhance repair of bulky DNA lesions [9, 10]. It has been reported that 8-OXO-dG levels were relatively stable over a one week period [6]. To our knowledge, only the HPB-releasing DNA adducts, acrolein-DNA adducts, and 8-OXO-dG have been identified and quantified in human oral tissues. Therefore, we will examine the effect of BRB on levels of DNA lesions derived from B[a]P, NNN; and also on 8-OXO-dG in buccal cells of smokers. Due to the extensive heterogeneity in human Phase 1/Phase II, oral microflora population, antioxidants and DNA repair activity, we expect that levels of DNA lesions in the oral cavity are likely to vary appreciably among current smokers. Thus, we will compare the inhibitory effects of BRB on the levels of tobacco carcinogen-induced DNA damage in buccal cells in smokers at baseline and at the conclusion of our intervention on every participant which will enable intra- and inter-participant analysis.

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

1. Age 21-75
2. Smoke 5 cigarettes per day or more for at least the past 12 months
3. Have an expired air carbon monoxide measurement of greater than 6 parts per million
4. Made no serious cigarette smoking quit attempt or have used any FDA-approved smoking cessation medication in the prior 30 days
5. Not planning to quit in the next 4 months
6. Able to read and write in English
7. Able to understand and provide consent to the study procedures
8. Willingness and ability to attend regular visits over a 13-week and to respond to research contacts between visits

3.2 Exclusion Criteria

1. Unstable or significant medical conditions that affect participant safety or biomarker data in the past 3 months (e.g. recent heart attack, asthma, COPD, cancer (except basal cell carcinoma of the skin), tumors (except if benign and excised participation), , neurodegenerative diseases, kidney failure, stroke, heart failure, uncontrolled angina, , uncontrolled high blood pressure, uncontrolled lung or respiratory disease, diabetes (except well-controlled type 2 diabetes), liver disease (except Hepatitis A), infectious disease, autoimmune disorder)
2. Abnormalities found during oral cancer screening exam, including neoplastic and preneoplastic lesions
3. Women currently pregnant or nursing
4. Use of any non-cigarette nicotine delivery product in the past 7 days (e.g. e-cigarettes, pipe or cigar)
5. Uncontrolled mental illness or substance abuse or inpatient treatment in the past 6 months
6. Use of marijuana or other illegal drugs daily or weekly in the past 3 months
7. Any known allergy to raspberries
8. Use of high dose antioxidant supplements in the past month

9. Current use of antibiotics
10. Heavy drinking (>4 drinks/day, 5 days/week)
11. Currently in the process of reducing daily cigarette consumption or are planning to reduce the amount smoked in the next 3 months
12. Any other condition that in the opinion of the investigator would make it unlikely that the participants could comply with the study protocol

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

Subjects will be informed that they may voluntarily drop out at any time.

Participants will only be withdrawn from the study for the following reasons:

1. New pregnancy: Participants who report a new pregnancy at any point during the study will be withdrawn.
2. Suicide attempt: If at any time during the study it is discovered that a participant has made a suicide attempt, they will be withdrawn from the study.
3. New diagnosis of unstable or significant medical condition: Participants who are diagnosed with any of the medical exclusion conditions listed in section 3.2 during their participation in the study will be withdrawn due to the effect the condition may have on outcomes.
4. Increase of alcohol use: Participant's alcohol consumption increases to >4 drinks/day, 5 days or more per week will be withdrawn from the study due to the effect it may have on biomarkers.
5. Participant choice: Participants may choose to remove themselves from the study by informing the research team verbally or in writing at any point during the study
6. Noncompliance: On a case to case basis, participants who repeatedly are non-compliant with the intervention (not using the BRB lozenge as intended on a daily basis) will be withdrawn from the study, as this will impact study outcomes.
7. Significant change in smoking rate from baseline: A participant will be withdrawn from the study if they either increase their weekly average cigarette use to 150% of baseline or decrease their weekly average cigarette use to 50% of baseline.

The PI will evaluate the participant for possible withdrawal from the study at any point for the following:

1. Any hospitalization or debilitation in which participation in the study could be detrimental to the recovery process. This will be self-reported by the participant and will be reviewed by the PI and medical professional to determine whether continued participation in the study is appropriate.
2. Participant behavior: If a participant is behaving in an inappropriate or threatening manner, admits to lying about eligibility criteria, is participating in other smoking research studies that could affect the primary outcome measures, continually using other non-cigarette tobacco products, etc., then the PI can withdraw him/her from the study at the PI's discretion.
3. Study visit window: If a participant cannot attend their study visit within the study visit window then the PI can withdraw him/her from the study at the PI's discretion.

3.3.2 Follow-up for withdrawn subjects

There will be no planned follow-up of withdrawn subjects.

4.0 Recruitment Methods

4.1 Identification of subjects

For subject recruitment, we will make use of our existing tobacco research recruitment system (STUDY00002213) and large database of tobacco research volunteers developed as part of our Penn State Tobacco Center of Regulatory Science (TCORS). This database includes over 3,500 individual smokers who have provided basic details on their tobacco use history and demographics as well as contact details. We plan to use this database to select and recruit eligible cigarette smokers to the proposed study. These subjects are primarily from the Hershey/Harrisburg area. Additional recruitment will occur using our previously developed methods, including advertisements placed in local media (radio, newspapers, etc.), and through flyers, posters, and bulletin boards situated in close proximity to the Penn State Hershey Medical Center as outlined/uploaded in the IRB protocol STUDY00002213. Study flyers will be posted and distributed at the Bethesda Mission (Harrisburg, Pa).

This study will also identify subjects through the Penn State Health electronic medical record. Penn State EIM will release a data report of patients who have been diagnosed with nicotine dependence but without diabetes and those who have had a patient visit in the past 3 years. The report will include patient contact information (i.e. phone number, email address, and address). Researchers will reach out to potential participants through these avenues to let them know about the study opportunity.

This study will utilize BuildClinical to help identify potential participants. BuildClinical is a data-driven platform that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. They have worked with IRBs in the US to ensure they adhere to all the appropriate guidelines and procedures. They utilize already IRB approved study-specific advertisements to engage participants on digital platforms such as Facebook, Google, WebMD, etc. and redirect them to a study-specific landing page should they click it. On the landing page, the person can complete an online pre-screen questionnaire that gets routed into BuildClinical's platform. BuildClinical's Secure Socket Layer (SSL) software encrypts all inputted information and keeps information private and HIPAA compliant.

4.2 Recruitment process

4.2.1 How potential subjects will be recruited.

STUDY00002213 will serve as an initial recruitment point of contact for this study. Interested volunteers calling the study center number (located on all recruitment flyers) will go through an initial screening using STUDY00002213's eligibility script. Questions will assess basic eligibility, i.e. age, smoking status, access to transportation, etc. All volunteers calling this line must give verbal consent prior to answering any questions. If the participant's responses match our study's specified inclusion criteria, they will be forwarded to us, and will be screened a second time (eligibility script uploaded under recruitment materials) for certainty of eligibility. All subjects calling in to either line will complete the initial screener under IRB#2213 first, and then complete the second screener under this study's protocol.

Recruitment will include posting flyers (flyers have previously been approved under IRB STUDY00002213; they include images of people with the header "Do you smoke" and the study center's number listed below) around the Harrisburg, Lancaster and York area, radio advertisements and media use. All materials used in our recruitment process have been approved by the IRB under STUDY00002213. ResearchMatch.org will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University.

The ResearchMatch specific message is uploaded under Recruitment Materials. Researchers will also identify potential participants from the EIM data report generated from the EMR.

Digital ads run by BuildClinical will include a link to a BuildClinical landing page that provides information about the study, eligibility, etc. Once there, interested participants will click a link to go to a BuildClinical screening form that asks for contact information and basic study eligibility questions (a PDF of an example of a landing page and screening form have been uploaded for review).

4.2.2 Where potential subjects will be recruited.

Details on where participants will be recruited are in STUDY00002213. Generally, subjects will be recruited from the surrounding counties, with a focus of identifying participants in the following counties: Dauphin, Lebanon, Cumberland, Lancaster and York. Potential participants will also be recruited through ResearchMatch.org and through BuildClinical's services.

4.2.3 When potential subjects will be recruited.

Details of when participants will be recruited are in IRB STUDY00002213.

4.2.4 Describe the eligibility screening process and indicate whether the screening process will occur before or after obtaining informed consent. Screening begins when the investigator obtains information about or from a prospective participant in order to determine their eligibility. In some studies, these procedures may not take place unless HIPAA Authorization is obtained OR a waiver of HIPAA Authorization when applicable for the screening procedures is approved by the IRB. [*For FDA regulated studies, consent for any screening activities would need to be obtained prior to screening unless specifically waived by the IRB.]*

1. Call Routing Screener in IRB STUDY00002213 (Phone or online survey): We will consider the screening process and eligibility questions in IRB STUDY00002213 as Screening #1. This process includes a brief phone or online survey screening to determine basic eligibility for any of our study center protocols. Based on their answers to the questions participants will be routed to this study. Then, participants will complete the screening for this study in two additional steps.

2. Project Screener (Phone, email, or text): Participants will be contacted by researchers via phone, email, or text to complete the eligibility questions for the study. Those contacted via phone will complete the screening questions over the phone with the researcher, while those contacted by email or text will be provided a REDCap link to complete the Eligibility survey online on their own. A full script and screening questions specific to this study are in the "Consent Forms and Recruitment Materials" section of the IRB application. If a participant has met basic eligibility criteria, they will be scheduled to come into the study center for their first visit.

3. Project Screener (In person at Visit 1): At Visit 1 they will be re-screened using the Project Screener (Phone). If the participant's answers have changed from the phone screener and they are no longer eligible, they will be informed that they cannot participate. If they remain eligible, they will be consented to the study and further screened for eligibility. A full script and screening questions are uploaded in the "Consent Forms and Recruitment Materials" section of this IRB application.

5.0 Consent Process and Documentation

5.1 Consent Process:

Check all applicable boxes below:

☒ **Informed consent will be sought and documented with a written consent form *[Complete Sections 5.2 and 5.6]***

☒ **Implied or verbal consent will be obtained – subjects will not sign a consent form (waiver of written documentation of consent) *[Complete Sections 5.2, 5.3 and 5.6]***

☐ **Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception). *[Complete section 5.2, 5.4 and 5.6]***

☐ **Informed consent will not be obtained – request to completely waive the informed consent requirement. *[Complete Section 5.5]***

5.2 Obtaining Informed Consent

5.2.1 Timing and Location of Consent

When participants attend their first in-person visit, they will have the study explained to them in detail, have the opportunity to ask questions and then be asked to sign the consent form. Participants will be given a signed copy of the form. This will take place in a private clinic room at the Penn State Hershey Clinical Research Center (CRC).

Consenting may also take place in a private space at the Bethesda Mission.

5.2.2 Coercion or Undue Influence during Consent

The researchers obtaining consent will clearly indicate that the participant's enrollment in the study is purely voluntary; the researchers will not offer comments about whether they believe the participant should enroll in the study or not. Given the number of contacts and visits involved in the study protocol, the compensation provided to the participant is modest.

5.3 Waiver of Written Documentation of Consent

5.3.1 Indicate which of the following conditions applies to this research:

☒ The research presents no more that minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

OR

☐ The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern. (*Note: This condition is not applicable for FDA-regulated research. If this category is chosen, include copies of a consent form and /or parental permission form for participants who want written documentation linking them to the research.)*

OR

☐ If the subjects or legally authorized representatives are members of a distinct cultural group or community in which signing forms is not the norm, that the research presents no more than minimal risk of harm to subjects and provided there is an appropriate alternative

mechanism for documenting that informed consent was obtained. (*Note: This condition is not applicable for FDA-regulated research.)*

Describe the alternative mechanism for documenting that informed consent was obtained:

For participants determined to be eligible, a study visit will be made where they will complete a written consent form.

5.3.2 Indicate what materials, if any, will be used to inform potential subjects about the research (e.g., a letter accompanying a questionnaire, verbal script, implied consent form, or summary explanation of the research)

A verbal phone script will be used to inform potential subjects about the research and is uploaded with the screener under the Consent Form and Recruitment Materials section.

5.4 Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception).

5.4.1 Indicate the elements of informed consent to be omitted or altered

Not applicable

5.4.2 Indicate why the research could not practicably be carried out without the omission or alteration of consent elements

Not applicable

5.4.3 Describe why the research involves no more than minimal risk to subjects.

Not applicable

5.4.4 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.

Not applicable

5.4.5 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.

Not applicable

5.4.6 Debriefing

Not applicable

5.5 Informed consent will not be obtained – request to completely waive the informed consent requirement

5.5.1 Indicate why the research could not practicably be carried out without the waiver of consent

N/A

5.5.2 Describe why the research involves no more than minimal risk to subjects.

N/A

5.5.3 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.

N/A

5.5.4 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.

N/A

5.5.5 Additional pertinent information after participation

N/A

5.6 Consent – Other Considerations

5.6.1 Non-English-Speaking Subjects

Non-English speaking subjects will not be eligible for this study.

5.6.2 Cognitively Impaired Adults

5.6.2.1 Capability of Providing Consent

N/A. Cognitively impaired adults will not be eligible for this study.

5.6.2.2 Adults Unable to Consent

N/A

5.6.2.3 Assent of Adults Unable to Consent

N/A

5.6.3 Subjects who are not yet adults (infants, children, teenagers)

5.6.3.1 Parental Permission

Only adults are eligible for this study.

5.6.3.2 Assent of subjects who are not yet adults

Not applicable

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☐ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

☒ **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☐ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☒ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2.1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2.1.1 Plan to protect PHI from improper use or disclosure

Information is included in the "Confidentiality, Privacy and Data Management" section of this protocol.

6.2.1.2 Plan to destroy identifiers or a justification for retaining identifiers

All study data will be retained indefinitely for any future analyses on the study population.

6.2.2 Explanation for why the research could not practicably be conducted without access to and use of PHI

PHI must be collected to determine the participant's eligibility in to the study. Some medical conditions listed in section 3.2, would exclude the participant from being eligible to participate. Contact information (name, phone number, address) will be used to follow-up about scheduling and for appointment reminders.

6.2.3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

In order to screen the participants prior to inviting them into the study center, the study coordinator will conduct a phone screen to determine if the participant is eligible to participate in the study.

6.3 Waiver or alteration of authorization statements of agreement

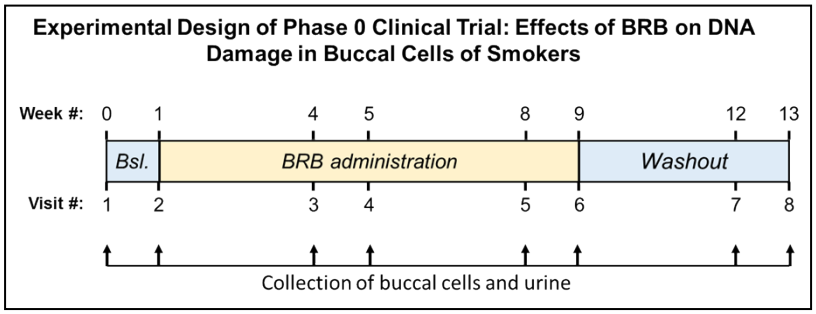
Protected health information obtained as part of this research will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other permitted uses and disclosures according to federal regulations.
The research team will collect only information essential to the study and in accord with the 'Minimum Necessary' standard (information reasonably necessary to accomplish the objectives of the research) per federal regulations.

Access to the information will be limited, to the greatest extent possible, within the research team. All disclosures or releases of identifiable information granted under this waiver will be accounted for and documented.

7.0 Study Design and Procedures

7.1 Study Design

This Phase 0 trial will consist of a single arm, where participants, after a 1-wk baseline period, will be placed on daily BRB (oral) lozenges for 8 wks followed by a 4-wk washout. Biological samples will be collected at 0, 1, 4, 5, 8, 9, 12 and 13 wks (See figure). An 8-wk BRB administration period was selected based on previous clinical and preclinical data (1) and a 4-wk washout period is included to examine the effects of BRB withdrawal on major outcomes.



7.2 Study Procedures

Based on COVID-19 restrictions (Participant mask removal must PAUSE for all observational studies at the College of Medicine), the study team will mail or drop off buccal cell collection kits and exhaled CO monitoring devices to participants homes. Participants will complete these study procedures before they come in for their scheduled study visit. The participants will also be provided with instructional handouts for completing the study procedures. If needed participants will be provided additional instruction through Zoom or phone. With these adjustments in study procedures there will be no need for participants to take off their mask during study visits. Once the restrictions are lifted, the study team will go back to collecting these samples during the study visit.

All clinical work will be conducted at the Smoking Research Laboratory in the Clinical Research Center and within the Dental Clinic Suite/PSH Medical Group (University Physician's Center, Entrance 4) at Penn State College of Medicine in Hershey, PA. Eligible subjects, after phone screening, will receive a study packet in the mail with directions to the clinic, a copy of the consent form, a Participant Medical History form, and a Participant Current Medications form to review and complete at home. Participants will visit the clinic (Visit 1) for additional screening after obtaining informed consent, including measurement of exhaled carbon monoxide (CO), oral cancer examination (performed by Dr. Susan Mallery, DDS from the Ohio State University collaborative site, or a trained study team member and pregnancy test (females). If the physician is not available at Visit 1, the oral cancer screening can be performed at Visit 2. Study team members who are trained to do the oral health exam can also complete the exam at Visit 1 or 2. Eligible subjects will be entered into the protocol where questionnaire data on demographics, medical history, lifestyle, tobacco and alcohol consumption, and usual dietary intake, biological samples (exfoliated buccal cells & urine) and anthropometric data will be collected. Subjects will return after 1 wk (Visit 2, 2nd baseline) where biological samples will be collected and the test agent (BRB lozenge) will be supplied (includes a usage diary and instructions on the proper application). At Visits 3 & 4 (Mid-test Phase, weeks 4 & 5) subjects will return unused product, complete a brief questionnaire on compliance and provide biological samples. At Visit 4, subjects will receive a new batch (4-week supply) of BRB lozenges. At Visits 5 & 6 (End-test Phase, Weeks 8 & 9) subjects will return unused product, complete a brief questionnaire on compliance and provide biological samples. At Visit 6, subjects will return study diaries and enter the Washout Phase where no test agent will be provided. At Visits 7 & 8 (End-washout Phase, weeks 12 & 13), subjects will provide biological samples. The timing for collection of biological samples is based, in part, on the need for collecting ample quantities of DNA for analyses of carcinogen-DNA adducts and 8-OXO-dG. Based on previous results and preliminary findings, DNA requirements are as follows: NNN-derived (HPB-releasing) adducts, 10 µg (5); B[a]P-derived adducts, 10 µg (preliminary data); 8-OXO-dG, 2 µg (104). Typically, 20 µg of DNA can be isolated from exfoliated buccal mucosal cells collected according to the protocol outlined below, a value consistent with others (9, 105). Thus, to assure that adequate levels of DNA are obtained, 2 sample collections (1 week apart) for each Phase of the study will be obtained as follows: Baseline Phase (weeks 1 & 2); Mid-test Phase (weeks 4 & 5); End-test Phase (weeks 8 & 9); End-washout Phase (weeks 12 & 13). The 1-week waiting period between sampling is consistent with the 5-7 day turnover rate for the buccal epithelium (106) allowing for adequate recovery of cells and avoids potential bias towards collection of differentially affected cell populations (cell surface vs. underlying cells) (9, 106). All subjects will be remunerated for their participation ($300 in total; $25/visit and an additional $100 at the final visit).

	Phone Screening	Enrollment/ Baseline	BRB Intervention							BRB Washout		
Study Visit #		1	2	Phone Call 1	3	4	Phone Call 2	5	6	Phone Call 3	7	8
Study Week #	-1	0	1	2.5	4	5	6.5	8	9	10.5	12	13

Measures/Questionnaires												
Eligibility Questions Screener	X	X										
Name Registry	X											
Informed consent		X										
Medical history		X										
Concomitant medications		X										
Oral Cancer Screening		X										
Demographics		X										
Cigarette Details		X										
Cigarette Dependence		X										
Menstrual Cycle		X	X		X	X		X	X		X	X
Product Evaluation									X			
Dietary intake interviews (between visits)		X						X				
BRB Dispensation			X			X						
Daily Study Log			X		X	X		X	X		X	X
Adverse Event Trigger questions/ Adverse Event Log			X		X	X		X	X		X	X
Biomeasures												
Height		X										
Weight		X	X		X	X		X	X		X	X
Exhaled CO		X	X		X	X		X	X		X	X
Blood pressure/pulse		X	X		X	X		X	X		X	X
Pregnancy test		X				X			X			X
Buccal cells collection		X	X		X	X		X	X		X	X
Urine collection			X			X			X			X

7.2.1 Visits 1 & 2.

Baseline Phase: (Visits 1 & 2, Weeks 0 & 1). Recruited subjects will visit the clinic twice during this phase at week 0 and week 1. At visit 1, eligible subjects will come to the Dental Clinic Suite/PSH Medical Group (University Physician's Center, Entrance 4) or the Clinical Research Center. The research coordinator will complete the screener again to ensure answers did not change. The coordinator will review the informed consent with the participant and go over any inquiries the participant may have. After consent, medical history and current concomitant medications will be reviewed (participants will be prompted when scheduling their first study visit to either bring in a list or the bottles of their current medication/s). Next, an oral cancer screening (performed by Dr. Susan Mallery, DDS from the Ohio State University, collaborative site, or a trained study team member) will occur. Study team members who are trained to do the oral health exam can also complete the exam at Visit 1 or 2. If, upon screening, a subject is found to have a suspicious oral lesion or abnormality, they will be removed from the study and referred to their personal physician for follow-up. The details of the suspicious oral lesion or abnormality will be provided to the subject using the Medical Professional letter. If needed, a

list of low cost dental and health clinics in the area will also be provided to the subject. The oral cancer screening can be done at Visit 2 if the physician is not available on the day of Visit 1.Participants will complete a urine sample (for pregnancy determination for women of child-bearing potential, i.e. those who have had a period in the past 12 months and who have not had a hysterectomy), exhaled CO (by a piCO+ Smokerlyzer), blood pressure, heart rate, height and weight during the visit. Participants will provide an exfoliated buccal mucosal cell sample obtained by using a procedure we have previously used and optimized for maximum DNA yield (72, 121). Subjects will rinse their mouth with distilled water before collection of the buccal cells and then gently brush the inside of their cheeks (both sides) with a soft bristle toothbrush. The mouth is then rinsed with saline for 2-3 minutes and participants will expectorate the solution into a collection tube. The toothbrush will be agitated in the collection tube. The participant may be taken to a private patient room in the IO Silver Heart and Vascular Practice Clinic in the University Physicians Center (UPC, Suite 600) to complete part of the study visit. Female participants will be asked on a continual monthly basis to report dates of their menstrual cycle for biomarker purposes. Participants will be sent their baseline questionnaires via a survey link to fill out at home after the visit. These include Demographics, Cigarette Dependence, and Cigarette Details. Participants will be encouraged to fill them out before their next visit. All participants will be provided a daily study log to complete independently to tally the number of cigarettes smoked per day and number of alcoholic drinks. During the baseline phase, 2 unannounced 24-food recall assessments will be conducted by trained dieticians through the BioNutritional Service of the Penn State Clinical Research Center to obtain information regarding dietary intake of phytochemicals.

At Visit 2, participants will have measurements and biosamples taken, including a urine sample, exhaled CO, heart rate, blood pressure, and weight. Additionally, the participant will provide an exfoliated buccal mucosal cell sample as described above. Participants will be asked for their daily study log from the week prior. Participants will be asked about adverse events using the Adverse Event Trigger questions. At this time, all participants will receive a four week supply of BRB lozenges and be instructed to consume 1 lozenge, 5 times/day by placing the lozenge in their mouth and slowly sucking on it until fully dissolved (~10-12 min). Participants will be asked to consume their first lozenge while at the clinic to ensure proper usage. Participants will be instructed to not eat or drink 15 minutes following lozenge use. Along with the lozenges, participants asked to complete independently a daily study log to tally all lozenges, alcoholic drinks, and cigarettes consumed per day.

7.2.2 Visits 3 & 4

Mid-test (Mid-BRB administration) Phase: (Visits 3 & 4, Weeks 4 & 5). At Visits 3 & 4, participants will return unused product and complete a brief questionnaire on compliance and adverse events, including possible changes in medication and health status. Females will be asked to report the date of their last menstrual cycle as changes can affect biomarkers. The coordinator will collect the BRB lozenges and daily study log for review. The coordinator will review any uncaptured cigarette or lozenge usage data not recorded on the daily log. Then, measurements and biosamples will be taken, including a urine sample (Visit 4 only), exhaled CO, blood pressure, heart rate, and weight. A urine pregnancy test will be completed at Visit 4. Additionally, the participant will provide an exfoliated buccal mucosal cell sample at Visits 3 and 4. At Visit 4, subjects will receive a new batch (4-week supply) of BRB lozenges along with new log (if needed) for cigarette, alcohol, and lozenge usage.

7.2.3 Visits 5 & 6.

End-test (End BRB administration) Phase: (Visits 5 & 6, Weeks 8 & 9). At Visits 5 & 6, participants will return unused product and complete a brief questionnaire on compliance and adverse events, including possible changes in medication and health status. Females will be asked to report the date of their last menstrual cycle as changes can affect biomarkers. A urine pregnancy test will be completed at Visit 6. The coordinator will collect the BRB lozenges and daily study log. The coordinator will review any uncaptured cigarette or lozenge usage data not recorded on the daily log. Then, measurements and biosamples will be taken, including a urine sample (Visit 6 only), exhaled CO, blood pressure, heart rate, and weight. Additionally, the participant will provide an exfoliated buccal mucosal cell sample at Visits 5 and 6. At Visit 6, subjects will enter the washout period where no more BRB lozenges will be provided. Participants will receive a new daily study log (if needed) to record cigarette and alcohol use in the next phase. During the last week of the BRB administration period, 2 unannounced 24-food recall assessments will be conducted by trained dieticians through the BioNutritional Service of the Penn State Clinical Research Center to obtain information regarding dietary intake of phytochemicals.

7.2.4 Visits 7 & 8.

End-washout Phase: (Visits 7 & 8, Weeks 12 & 13). At Visits 7 & 8, participants will complete a brief questionnaire (BRB AE Trigger Questions) on adverse events, and possible changes in medication and health status. Females will be asked to report the date of their last menstrual cycle. A urine pregnancy test will be completed at Visit 8. The coordinator will collect the daily study log. The coordinator will review any uncaptured cigarette usage data not recorded on the daily study log. Then, measurements and biosamples will be taken, including a urine sample (Visit 8 only), exhaled CO, blood pressure, heart rate, and weight. Additionally, the participant will provide an exfoliated buccal mucosal cell sample at Visits 7 and 8.

7.2.5 Phone Interviews

Since there is a 3-week gap between Visits 2 & 3, and Visits 4 & 5, and 6 & 7, the coordinator will contact the participant midway through the off clinic weeks to determine if the participants are having any issues with compliance and to remind them of their next scheduled visit. The coordinator will document in Redcap whether or not they are able to get a hold of the subject.

7.3 Duration of Participation

Subjects will be in the study for a total of 13 weeks. This will include 8 clinic visits which should typically last 30-45 mins each.

7.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

7.4.1 Description

The test agent will be freeze-dried black raspberry in the form of a dissolvable slow-release lozenge. This product is available over-the-counter without prescription (https://www.berrihealth.com/collections/frontpage/products/authentic-1000-mg-black-raspberry-lozenges-bottle-of-30). See attachment (BRB lozenge label) for layout of the bottle label. Each lozenge contains 1 gram (g) BRB freeze-dried powder equivalent to ~5 black raspberries and will be purchased from BerriProducts LLC, Corvallis, OR. A similar dosage regimen has been used in a previous trial.[11] All lozenges will be prepared from a single

harvest/year lot of black raspberries obtained from Corbett and Sandy Oregon (see BRB Certificate of Analysis and BRB COA Letter). The plants are Rubus occidentalis of the Munger cultivar. This form of BRB administration was selected over other forms previously used, including consumption in diet or application in gels, based on ease of use, the ability to more accurately control dose to target tissue, and a prolonged oral mucosa contact time facilitating bioactive component delivery. Lozenges will be packaged in Polyethylene terephthalate (PETE) tamper proof bottles with oxygen absorbers and desiccants. The shelf life is more than 3 yrs when unopened and 3 months after opening at normal room conditions. Immediately after we receive the lozenges, they will be analyzed for composition and stability of their constituents. No toxicity has been associated with freeze-dried black raspberry powder. In a 9 month study in rats, freeze-dried black raspberry powder in the diet at levels equivalent to 1.8 oz per day (representing approximately 1.2 lb of raspberries per day in humans) was not associated with any signs of toxicity. [29]

7.4.2 Intervention Regimen

A dose of 5 g freeze-dried BRB per day will be provided to each participant in the form of dissolvable slow-release BRB lozenges (each containing 1 g BRB freeze-dried powder) (BerriProducts LLC, Corvallis, OR). Each subject will consume 1 lozenge, 5 times/day and instructed to not eat or drink 15 minutes following lozenge use.

7.4.3 Method for Assigning Subject to Intervention Groups

In this Phase 0 trial, all subjects will receive the same intervention.

7.4.4 Subject Compliance Monitoring

Adherence to the study protocol will be assessed by analysis of diary entries and comparison of returned BRB lozenges.

7.4.5 Blinding of the Test Article

This will not be a blinded study.

7.4.6 Receiving, Storage, Dispensing and Return

7.4.6.1 Receipt of Test Article

BRB lozenges will be obtained from BerriProducts LLC, Corvallis, OR. They will be shipped in boxes to Penn State Hershey directly. Lozenges will be packaged in PETE tamper proof bottles containing 30 lozenges with oxygen absorbers and desiccants.

7.4.6.2 Storage

The test agents will be stored in a refrigerator in the PSU Tobacco Center pharmacy. We will monitor and record the temperature of the refrigerator.

7.4.6.3 Preparation and Dispensing

The research coordinator will be responsible for preparing and distributing the lozenges to participants. Lozenges will be prepared for distribution in the PSU Tobacco Center pharmacy. Research coordinator will prepare a package with the appropriate allotment of BRB lozenges the participant will need until the next study visit.

7.4.6.4 Return or Destruction of the Test Article

Any unused lozenges that are returned from the participant will be counted and destroyed on site.

7.4.6.5 Prior and Concomitant Therapy

Concomitant medications will be surveyed at all study visits to serve as covariates during analysis and to monitor participant health conditions. Medications related to certain medical conditions that are exclusions to the study will serve to alert the study staff of the presence of these conditions during screening.

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

75 subjects will be enrolled to account for participants who drop out of the study. A total of 47 will be needed to complete the research procedures.

8.2 Sample size determination

We are unware of any published data about the intervention effect on the primary endpoint. But the huge difference between smokers and non-smokers in Stepanov et al (63) suggests a huge potential for intervention effect. For the sample size calculation, we assume that 70% of subjects will have lower HPB-releasing adduct values from beginning to end of a considered period and 30% will have a higher values. We will test the hypothesis that 50% of subjects will have a lower values and 50% will have higher values. It then follows that 47 subjects provides 80% power at 5% type I error. In the case of 75% of subjects having a lower value and 25% have a higher value, 30 subjects are required. For this study, we will use 47 subjects to ensure sufficient power and will recruit 75 subjects (an additional 28 to account for potential drop-outs).

8.3 Statistical methods

The main endpoints of this trial are the carcinogen-induced HPB-releasing adducts and secondarily, B[a]P-DNA adducts and 8-OXO-dG in buccal cells. The statistical analysis will focus on the difference between measurements at baseline (wks 1 & 2) and after BRB intervention (wks 8 & 9). Based upon the lack of preliminary data on inter-individual variation of B[a]P-DNA adducts and the known importance of HPB-releasing adducts on carcinogenesis (4), we have focused on this latter outcome as the primary endpoint. Stepanov et al (63) provides data on HPB-releasing adducts which show a drastic difference

between smokers (mean [SD]=12.0 [35.1], n=30) and non-smokers (0.23 [0.43], n=15). The distribution of these adducts for smokers severely deviated from normality, with the majority of values at lower end (<5), but a few at high end (>15). For this reason, the Wilcoxon rank test will be used to analyze the change pre- and post-BRB intervention. In addition, boxplots of HPB-releasing adducts at beginning of and end of BRB intervention and of resultant changes will be used to summarize the information. The other endpoints are expected to show similar distributions, so the same analysis strategy will be used. Paired t-test will be used for endpoints that are approximately normally distributed. Additionally, outcome measurements at different time points (baseline, 4-5 wk, 8-9 wk, and at the end (12-13 wk after BRB initiation, 4 wk after withdrawal of BRB), after appropriate transformation, will be modeled using linear mixed effects to study their change over time after BRB administration where appropriate.

9.0 Data and Safety Monitoring Plan

9.1 Periodic evaluation of data

The PI of the project will be responsible for monitoring the overall safety and integrity of the research project with the assistance of the study coordinator. Participants will meet during regularly scheduled visits with the coordinator who will assess adverse events throughout the study. If there are any concerns on participant safety, they will be brought to the PI's attention. Dr. El-Bayoumy will meet monthly and as needed with the study staff to review data, including any adverse events.

The Penn State Cancer Institute Data and Safety Monitoring Committee (PSCI DSMC) will be the Data and Safety Monitoring Board of record. The PSCI DSMC will review adverse event reporting and data and safety monitoring through annual study progress reports submitted by the study team.

9.2 Data that are reviewed

Data that will be included in the study progress reports and reviewed by the PSCI DSMC include:

- Accrual and retention
- Subject enrollment status
- Participant demographic information
- Protocol deviations/violations
- Adverse events summary

9.3 Method of collection of safety information

All safety data will be directly coded into REDCap during study visits. At each visit after visit 1, participants will be asked a series of non-directive questions that would trigger an assessment for an adverse event to be documented. An adverse event will also be documented if a participant volunteers information about changes in their health at a study visit. An adverse event log (uploaded in the Supporting Documents section) will be used to document the description of the adverse event, including start/stop dates, type, grade, relationship to the study and action taken.

9.4 Frequency of data collection

Questions assessing adverse events will be collected at each visit after visit 1 and may also be collected when the participant volunteers information between visits (e.g. compliance reminder phone calls).

9.5 Individuals reviewing the data

The PI of the project will be responsible for monitoring the overall safety and integrity of the research project with the assistance of the study coordinator. The study coordinator will be responsible for the daily oversight of the study and recording all AEs. Dr. Susan Mallery, PhD, DDS (Professor and Chair, Division of Pathology and Radiology, The Ohio State University College of Dentistry, collaborative site) will review AEs with the study coordinator as needed.

The PSCI DSMC and the Committee's appointed Medical Monitor, Dr. Shin Mineishi, will review the annual study progress reports. If a serious AE occurs the study team will notify the PSCI DSMC as soon as possible. The serious AE will be reviewed by the Medical Monitor and PSCI DSMC.

9.6 Frequency of review of cumulative data

The PSCI DSMC and the Committee's appointed Medical Monitor, Dr. Shin Mineishi, will review the study progress reports annually. If a serious AE occurs the study team will notify the PSCI DSMC as soon as possible.

9.7 Statistical tests

Basic summary data will be compiled to report the study accrual, dropout rate, completion rate, and the proportions of adverse events.

9.8 Suspension of research

Due to the low risk of the study intervention, it is unlikely that there will be a need to suspend the research. However, should the PSCI DSMC identify any issues after reviewing the data, these recommendations will be followed.

10.0 Risks

A potential risk with using the black raspberry lozenges is stomach discomfort (i.e. nausea/vomiting), especially for people with certain stomach sensitives to berries. Other potential risks to subject volunteers in this study will be minimal as all sampling is non-invasive. There is the potential for minor irritation from the collection of exfoliated buccal mucosal cells which involves brushing the inside of the mouth with a soft toothbrush. There is minimal risk of breach of confidentiality in this study. Precautions will be taken to prevent this, including direct coding of data in REDCap. It is possible that some of the questions in the questionnaires may make participants uncomfortable. They will be instructed that they are free to skip any questions that make them uncomfortable.

11.0 Potential Benefits to Subjects and Others

11.1 Potential Benefits to Subjects

None

11.2 Potential Benefits to Others

Society as a whole will benefit from the research because it is expected to provide important information on the effectiveness of BRB to reduce DNA damage resulting from cigarette smoking. Cigarette smoke is the leading preventable cause of disease and current evidence-based interventions are ineffective for many current smokers. The study should provide empirical data on whether BRB intervention may be a valid disease prevention approach for addicted smokers.

12.0 Sharing Results with Subjects

The study is not designed to diagnose or treat any disease or condition. If during the course of conducting clinical procedures (e.g., blood pressure) a participant is found to have a result outside of clinical norms, the result will be discussed with the participant at the visit where the result is identified. If during the oral exam any abnormalities or issues are found, the observation will be shared with the participant and they will be advised to follow up with their dentist or doctor. Pregnancy tests will be given to female participants at the initial screening visit and throughout the study. If the test is positive, the results will be shared with the participant and they will be advised to follow up with their doctor. They will not be allowed to participate in the study. No results will be shared with others, such as the participant's Primary Care Provider.

13.0 Subject Payment and/or Travel Reimbursements

Subjects will receive a nominal payment of $35 each visit completed for visits 1 - 8, and an additional $100 at the final visit. Total possible payment is $380 and covers traveling to site, buccal cell and urine samples and for their time and participation. Payments will be issued on the Greenphire ClinCard.

14.0 Economic Burden to Subjects

14.1 Costs

The only cost associated with participation in the research is travel to and from the clinic on clinic visit days. Reimbursement for these costs is included in the participant's stipend.

14.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

15.0 Resources Available

15.1 Facilities and locations

All clinical work will be conducted at the Dental Clinic Suite/PSH Medical Group (University Physician's Center, Entrance 4) and the Smoking Research Laboratory in the Clinical Research Center at Penn State College of Medicine in Hershey, PA.

The Bethesda Mission will be used as a site to conduct the study.

15.2 Feasibility of recruiting the required number of subjects

Recruitment is open to all smokers meeting inclusion and exclusion criteria. Favorable word of mouth from smoking participants to their family/friends/coworkers has served as a significant source of recruitment in past research studies. Feasibility of acquiring participants is very good. Additionally, the estimated smoking prevalence in 2017 in Pennsylvania was 19% which is higher than the national average.

15.3 PI Time devoted to conducting the research

PI has committed 5% FTE in years 1-5 for the project.

15.4 Availability of medical or psychological resources

It is not anticipated that participants will need any medical or psychological resources as a result of being in this study. Safety of the participants will be monitored throughout the study.

15.5 Process for informing Study Team

All members of the study team have completed IRB/CITI/GCP training; documentation of such has been submitted to the IRB. The PI and study coordinator will train additional personnel when needed on protocol procedures.

16.0 Other Approvals

16.1 Other Approvals from External Entities

Dr. Richie's lab is approved under the Institutional Biosafety Committee JPR12-01-2 for work with human body fluids. Dr. El-Bayoumy's lab (co-investigator) is also approved under the Institutional Biosafety Committee (KEE20-01-2) for work with human body fluids. In addition, the study will be registered on clinicaltrials.gov.

16.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – **Penn State** Health **only** – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of "HRP-902 - Human Tissue For Research Form" in CATS IRB.

☐ Animal Care and Use – **All campuses** – Human research involves animals and humans or the use of human tissues in animals

☒ Biosafety – **All campuses** – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☐ Clinical Laboratories – **Penn State Health only** – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes but are no longer needed for clinical use. Upload a copy of "HRP-901 - Human Body Fluids for Research Form" in CATS IRB.

☒ Clinical Research Center (CRC) Advisory Committee – **All campuses** – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – **All campuses** – Research has one or more of study team members indicated as having a financial interest.

☐ Radiation Safety – **Penn State Health only** – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of "HRP-903 - Radiation Review Form" in CATS IRB.

☐ IND/IDE Audit – **All campuses** – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☐ Scientific Review – **Penn State Health only** – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Health Cancer Institute (PSCI) Protocol Review Committee or the PSCI Disease Team is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website.

17.0 Multi-Site Study

17.1 Other sites

Collaborative Site: The Ohio State University College of Dentistry, Columbus, OH.

Site PI: Susan Mallery, DDS, MS, Ph.D., Professor and Chair, Division of Oral and Maxillofacial Pathology and Radiology.

Contact Information: 2191-B Postle Hall, 305 W. 12th Ave, Columbus, OH 43210. Phone: 614-292-5892. Email: mallery.1@osu.edu.

IRB: Ohio State University Biomedical Sciences IRB

17.2 Communication Plans

There will be no subject recruitment occurring at the Ohio State University site as all subjects will be recruited at Penn State University. Dr. Mallery's role will be to provide support for the clinical trial by assisting with review of adverse events and to provide oral cancer screenings of participants. There will be direct lines of communication between the Penn State NIH-funded grant PI (Dr. El-Bayoumy) and the Ohio State University PI, Dr. Mallery. There will be no other Ohio State personnel involved in this study. Dr. Mallery will participate in bi-weekly study meetings attended by all study personnel where progress, issues and problems will be discussed. AE's will be communicated with Dr. Mallery in an immediate fashion as described in the study protocol. Dr. El-Bayoumy will be in charge of ensuring that IRB approval has been obtained from both institutes and for the prompt distribution of the most current protocols, consent forms, etc. through direct communication with Dr. Mallery.

17.3 Data Submission and Security Plan

All raw data from the study will be maintained at the Penn State University site. Identifiable information shared with Dr. Mallery will be kept to a minimum. If identifiable or health information needs to be shared with Dr. Mallery it will be password protected.

17.4 Subject Enrollment

There will be no subject enrollment at the Ohio State University site.

17.5 Reporting of Adverse Events and New Information

N/A

17.6 Audit and Monitoring Plans

N/A

18.0 Adverse Event Reporting

18.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility.* For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse event or Serious suspected adverse reaction	Serious adverse event or Serious suspected adverse reaction: An adverse event or suspected adverse reaction that in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or

	subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Life-threatening adverse event or life-threatening suspected adverse reaction	An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
Unexpected adverse event or Unexpected suspected adverse reaction.	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

For device studies, incorporate the following definitions into the below responses, as written:	
Unanticipated adverse device effect	Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

18.2 Recording of Adverse Events

Research subjects will be questioned on possible adverse events at study visits 2-8. An adverse event may also be collected if the participant volunteers information between visits, for example, during their phone call check-ins.

All adverse events (serious or non-serious) and abnormal test findings observed or reported to the study team believed to be associated with the study drug will be followed until the event, its sequelae, or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.
An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention, including significant additional concomitant drug treatment or other therapy. Note: Simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study.
- The test finding is considered an adverse event by the investigator.

18.3 Causality and Severity Assessments

The investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s) or device(s)", the adverse event will be classified as associated with the use of the study

drug(s) or device(s) for reporting purposes. If the investigator's final determination of causality is "unknown but not related to the study drug(s) or device(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

18.4 Reporting of Adverse Reactions and Unanticipated Problems to the FDA

18.4.1 Written IND/IDE Safety Reports

N/A

18.4.2 Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions

N/A

18.5 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

18.6 Unblinding Procedures

N/A

18.7 Stopping Rules

No formal a priori statistical stopping rules will be used for interim monitoring of the primary or secondary endpoints. Analyses will be performed and comprised of endpoints associated with safety and study integrity (i.e. recruitment rate, completion rate, rates of SAEs/AEs), and any other variables that are requested from the Penn State Cancer Institute Data and Safety Monitoring Committee. An annual summary report of this information will be prepared by the study team for the committee to review. The committee will use these reports as the primary basis assessing data quality and subject safety, and if necessary making recommendations of amendment to the protocol or stopping the trial.

19.0 Study Monitoring, Auditing and Inspecting

19.1 Study Monitoring Plan

19.1.1 Quality Assurance and Quality Control

Study data will be managed using REDCap (Research Electronic Data Capture). REDCap is a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium which includes The Pennsylvania State University and was initiated at Vanderbilt University. The database is hosted at the Penn State Health Medical Center and College of Medicine data center, which will be used as a central location for data processing and management. REDCap data collection projects rely on a thorough study-specific data dictionary

defined in an iterative self-documenting process by all members of the research team. This iterative development and testing process results in a well-planned data collection strategy for individual studies.

REDCap is HIPAA compliant. Data are stored on a secure server; data in REDCap are encrypted; access to the database requires authentication (a unique username and password); data are accessed based on the individual's role on the project; every interaction with the data is logged, creating an audit trail.

Data quality tools included in REDCap will be utilized to identify incorrect data types, out of range data and outliers in real-time.

19.1.2 Safety Monitoring

The **Principal Investigator** (Dr. El-Bayoumy) will confirm that AEs are entered into the AE case report forms by the coordinator; and will notify the IRB, sponsor and/or DSMC of all applicable AEs as appropriate.

The **Co-Investigator** (Dr. Susan Mallery) will be available to answer any questions that the coordinators may have concerning AEs; make final assessments of causality of AEs.

The **Research Coordinator** will complete appropriate report forms and adverse event logs; assist the PI to prepare reports and notify the IRB, sponsor and/or DSMC of all applicable AEs as appropriate.

The **Medical Monitor** appointed by the PSCI DSMC is Dr. Shin Mineishi. He will review annual reports submitted to the PSCI DSMC and will be contacted for any urgent safety matters during the study.

20.0 Future Undetermined Research: Data and Specimen Banking

20.1 Data and/or specimens being stored

Urine and exfoliated buccal mucosal cell samples will be banked for future undetermined research. Specimens will be stored with an ID code attached. All data associated with the ID code will be retained in REDCap.

20.2 Location of storage

Specimens will be stored in a locked freezer room in the research laboratory on the 3rd floor of the Cancer Institute. All data will be stored in REDCap.

20.3 Duration of storage

Specimens will be stored indefinitely with code number attached. Data will be stored indefinitely with identifiers attached in REDCap.

20.4 Access to data and/or specimens

The lab managers will have access to the locked freezer room where the specimens will be stored. The research coordinators will have access to the stored data in REDCap.

20.5 Procedures to release data or specimens

Investigators who are interested in obtaining samples from this project for ancillary studies will first be required to submit a detailed written proposal to Dr. El-Bayoumy. Dr. El-Bayoumy will then review the proposal and, if approved, the investigator will be required to obtain all other regulatory approvals (IRB, departmental scientific committees, etc.) prior to samples being released. Only de-identified data, as approved in the investigator's IRB application, will be released to the investigator.

20.6 Process for returning results

Investigators will be required to provide a written report on their study results to the PI.

21.0 References

See bibliography

22.0 Confidentiality, Privacy and Data Management

IMPORTANT: The following section is required for all locations EXCEPT Penn State Health and the College of Medicine. Penn State Health and College of Medicine should skip this section and complete "HRP-598 Research Data Plan Review Form." In order to avoid redundancy, for this section state "See the Research Data Plan Review Form" if you are conducting Penn State Health research. Delete all other sub-sections of section 22.

See the Research Data Plan Review Form

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