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Detection and Delineation of Oral Cancer With a PARP1-Targeted Optical Imaging Agent

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Abstract

More sensitive and specific methods for early detection are imperative to improve survival rates in oral cancer. However, oral cancer detection is still largely based on visual examination and histopathology of biopsy material, offering no molecular selectivity or spatial resolution. Intuitively, the addition of optical contrast could improve oral cancer detection and delineation, but so far no molecularly targeted approach has been translated. Our fluorescently labeled small-molecule inhibitor PARPi-FL binds to the DNA repair enzyme poly(ADP-ribose)polymerase 1 (PARP1) and is a potential diagnostic aid for oral cancer delineation. Based on our preclinical work, a clinical phase I/II trial opened in March 2017 to evaluate PARPi-FL as a contrast agent for oral cancer imaging. In this commentary, we discuss why we chose PARP1 as a biomarker for tumor detection and which particular characteristics make PARPi-FL an excellent candidate to image PARP1 in optically guided applications. We also comment on the potential benefits of our molecularly targeted PARPi-FL-guided imaging approach in comparison to existing oral cancer screening adjuncts and mention the adaptability of PARPi-FL imaging to other environments and tumor types.

Keywords

poly(ADP-ribose)polymerase 1, PARP1, oral cancer, fluorescence, optical imaging, screening, clinical translation

Over the last 4 years, our laboratory has worked on developing fluorescent and radiolabeled poly(ADP-ribose)polymerase 1 (PARP1)-targeted inhibitors and has explored their tumor imaging capabilities for different applications in the preclinical setting. Recently, our most advanced optically active PARP imaging probe, PARPi-FL, has advanced to a phase I/II clinical trial and will be evaluated as a contrast agent for oral cancer imaging (NCT03085147). Implementation of the clinical trial was based on the major findings presented in the study by Kossatz et al,[1] where we showed that (1) PARP1 was highly overexpressed in human oral cancer biospecimen, (2) PARPi-FL accumulated with high specificity in PARP1-expressing oral cancer xenografts, and (3) oral cancer imaging was also feasible when PARPi-FL was applied topically instead of intravenously.

Although details on our research methodology, animal models, and validation techniques can be found in the above-mentioned manuscript, we would like to use this platform to expound why we consider PARPi-FL to be an exceptional candidate for translation as an optical imaging agent for early detection and delineation of oral and other cancers.

Optical molecular imaging probes are designed to enhance the visibility of tumor tissue against normal tissue by adding fluorescence contrast. They either rely on nonspecific mechanisms of tumor accumulation (eg, aberrant metabolism or physiological changes) or they are targeted against a particular biomarker. Design options for such probes are plentiful, ranging from nanoparticles to antibodies to peptides to small molecules.[2] One of the challenges for optical imaging agent design is to find the ideal target that is highly and consistently expressed in many different tumor types over all tumor stages, but not in the surrounding healthy tissues. Over the last few years, PARP has been identified as such a target and a series of optical and nuclear PARP1 imaging agents have been developed.[3] The first and most validated of the fluorescently labeled PARP-targeted imaging agents is PARPi-FL, a small-molecule

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inhibitor of the DNA repair enzyme PARP1 that has been conjugated to the fluorophore BODIPY-FL.[4] Since PARP1 regulates a process as fundamental as single-strand DNA repair,[5] it is highly conserved and its expression is much more universal and abundant than most membrane receptors. Remarkable overexpression of PARP1 has been shown in many tumor types, driven by genomic instability and proliferation rate, and has been linked to overall survival, making it a particularly attractive target for imaging (refer to study by Kossatz et al[1] for a list of references). Three PARP inhibitors (PARPi) have been recently approved for the treatment of ovarian cancer (olaparib, rucaparib and niraparib) and others are in late-stage clinical trials for a large variety of tumor types, including breast cancer, pancreatic cancer, prostate cancer, glioblastoma, small-cell lung cancer, and melanoma. Hence, our PARP1 targeting agent, PARPi-FL, is rooted in an already established, validated therapeutic platform, opening avenues for its use not only as a companion diagnostic but also as a stand-alone imaging agent for tumor delineation.

The main challenge in exploiting PARP overexpression for optical molecular imaging is reaching a target that is hidden away in the cell nucleus. Despite their often great potential as biomarkers, imaging of intranuclear targets is a rarity in molecular imaging and particularly in optical imaging. To access an intracellular target, a tracer does not only have to be delivered to the tumor itself, but it also needs to enter the tumor cells. Some small molecules can passively enter cells and bind intracellular targets without an active internalization mechanism, but this strongly depends on their size, charge, and polarity. Small-molecule PARP inhibitors are attractive in this respect since they typically have a size below 500 kDa and are uncharged and lipophilic. Importantly, therefore, the biggest hurdle in creating an efficient fluorescent PARP inhibitor is not related to maintaining affinity, but instead to maintaining permeability, since conjugation of a dye will affect size, charge, and polarity.[6] We have shown in vitro and in vivo that PARPi-FL is able to penetrate cell membranes and bind to nuclear PARP1 with high affinity, while unbound compound is rapidly cleared.[7] The conjugation of the green fluorescent BODIPY-FL as a fluorescent dye is crucial in this regard. While its wavelength in the visible range could be seen as a disadvantage, it is indeed a necessary trade-off to maintain functionality. Specifically, its high lipophilicity, neutral charge, and small size are imperative to preserve nuclear penetration and rapid clearance. There have been efforts to label PARP inhibitors with near-infrared dyes for improved tissue penetration of the excitation/emission light and better tumor to background ratios.[3] However, data on their in vivo performance have not been published and in vitro data suggest effects on affinity, nuclear penetration, and clearance that will perturb in vivo imaging characteristics.

After demonstrating that PARPi-FL targets a highly relevant imaging biomarker and has suitable photophysical and pharmacokinetic characteristics for fluorescence-guided applications, we initiated efforts for clinical translation. Our phase I/II clinical trial NCT03085147 was opened in March 2017. Currently, fluorescent agents that are standardly used in clinical applications are the dyes Fluorescein Isothiocyanate (FITC), 5-aminolevulinic acid, and Indocyanine green (ICG). And while ICG is continuously evaluated for new applications in intraoperative imaging and has proven useful in sentinel lymph node mapping, imaging of tumors, vital structures, and vascularization,[8] nonspecificity generally complicates tumor delineation and quantitative diagnostics. The development of targeted optical imaging probes, especially for fluorescence-guided surgery, has been actively pursued for several decades. However, it was not until 2011 that the first targeted optical imaging study in humans was published using an FITC-labeled folate conjugate for intraoperative detection of ovarian cancer.[9] Currently, a small number of early-stage clinical trials are ongoing that evaluate targeted fluorescent probes for intraoperative imaging, including approved therapeutic antibodies (bevacizumab, cetuximab, panitumumab) labeled with the near-infrared dye IRDye 800CW, as well as fluorescently labeled nanoparticles and folate analogs.[10,11]

Typically, fluorescently labeled probes are injected intravenously. While this is also possible for PARPi-FL, our tracer can also be applied topically and provides a promising alternative for generating optical contrast for tumors developing at the tissue surface. A major advantage of topical application is the ability to directly deliver imaging agents to the area of interest, thus requiring lower overall doses compared to systemic delivery. This lowers the translational regulatory burdens due to reduced risk of systemic toxicity. For oral cancer detection, the topical application of dyes has been explored for decades to improve the delineation of malignant lesions, and it was clear to biomedical researchers and physicians early on that a possible avenue toward improved detection and delineation of oral cancer could involve adding optical contrast to visual clues and palpation.

Preceding the development of new molecularly targeted contrast agents for oral cancers, several competing imaging systems and oral cancer screening technologies have been introduced to the market, all of which claim to enhance the detection of lesions. Underlying imaging technologies include the use of adjunctive aids such as diffused white light (Microlux DL), chemiluminescence (ViziLite), loss of tissue autofluorescence (VELScope), or toluidine blue contrast.[12,13] Collectively, however, there is insufficient evidence to justify existing technologies as screening adjuncts due to lack of specificity, selectivity, and the general ability to discover clinically undetected lesions, change of the provisional diagnosis, or alteration of the biopsy site.[14] Accordingly, the gold standard in diagnosis of oral cancer is still incisional biopsy and histopathological assessment, although this procedure itself is burdened by errors in both sampling and interpretation and lacks sensitivity to determine lesion progression.[15] PARP1–targeted optical imaging could fill this gap because it has the potential to provide highly accurate detection of cancerous lesions combined with spatial resolution.

In the study by Kossatz et al,[1] we were able to show that PARPi-FL can help delineate oral cancer xenografts after a 1

minute topical application and that the tracer penetrated up to 250 μm (~10 cell diameters) deep into the tissue. Encouraged by these results, we designed the clinical phase I/II study investigating PARPi-FL in patients with oral cancer that is currently recruiting patients (NCT03085147). In this study, PARPi-FL will be administered locally for 1 to 2 minutes, followed by rinsing with a clearing solution. Patients will then undergo fluorescence imaging of the oral cavity. The location of the tumor will be documented by photographic imaging, and the intensity of fluorescence in the tumor region relative to adjacent normal mucosa will be quantified. Successful results of this first study will warrant further studies to evaluate the accuracy of PARPi-FL in comparison with the gold standard.

Using PARPi-FL as a contrast agent also has the advantage of being not only cost-effective but also a point-of-care diagnostic platform, which decreases logistical burden. Unlike many other clinical imaging modalities, optical imaging is readily adaptable to settings outside of highly specialized and infrastructure-heavy hospitals. In combination with the simple "swish and spit" topical application of PARPi-FL, oral cancer screening could be performed remotely, for example during routine dental checkups, and without the presence of a trained oral cancer specialist. Using methodology developed in our laboratory, and information soon to be obtained from clinical cases, we are excited about the prospects of expanding our imaging approach to include gastrointestinal cancer, pharyngeal cancer, esophageal cancer, and colorectal cancer.

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Declaration of Conflicting Interests

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