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RESEARCH

Effects of *Maackia amurensis* seed lectin (MASL) on OSCC cell morphology, PDPN expression, growth, and motility in a phase 1 clinical trial

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Abstract

Background Podoplanin (PDPN) has emerged as a functionally relevant biomarker and chemotherapeutic target expressed by OSCC cells. PDPN signaling can directly increase tumor cell invasion and metastasis, and also inhibit host lymphocyte activation and immune response. Accordingly, antibodies and *Maackia amurensis* seed lectin (MASL) can target the PDPN receptor to inhibit OSCC cell migration and viability. However, the effects of MASL on OSCC cells in oral cancer patients has not yet been reported.

Methods We conducted a Phase 1 human clinical trial to examine the effects of a single 100 mg oral dose of MASL on OSCC cell morphology, PDPN expression, and immune cell infiltration in lesions in oral cancer patients. We also examined the effects of MASL on the PDPN expression, motility, and viability of cells cultured from these patient lesions. In addition, we examined the ability of antibodies to target PDPN and kill OSCC cells by near-infrared photoimmunotherapy.

Results MASL administration was found to be safe and did not produce any adverse effects in any patients. While this single dose did not affect OSCC cell morphology in lesions in situ, it did appear to increase lymphocyte infiltration into tumor fields in one patient by over 5 fold ($p < 0.01$). In addition, MASL inhibited the growth and motility of all OSCC cells cultured from these patient lesions in a dose responsive manner in vitro ($p < 0.05$ in all cases) We also report that antibodies can target PDPN on OSCC cells obtained from these patients to destroy them by near-infrared photoimmunotherapy (NIR-PIT).

Conclusion These results suggest that protocols using MASL and photoimmunotherapies that target PDPN can be developed to effectively treat OSCC lesions in oral cancer patients.

Keywords Keywords: Maackia amurensis seed lectin · MASL · Oral cancer · Oral squamous cell carcinoma · OSCC · PDPN · Podoplanin · Near-infrared photoimmunotherapy · NIR-PIT

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Introduction

Over 400,000 new oral cancer cases are diagnosed each year, causing over 120,000 deaths worldwide (Bray et al. 2024). In addition to these fatalities, surgery and radiation treatments used to treat oral cancer can be disfiguring and cause acute patient discomfort and permanent sequelae. More effective treatments are clearly needed to treat oral cancer patients (D'Cruz et al. 2018; Mohamad et al. 2023; Parmar et al. 2021).

Over 90% of oral cancers are oral squamous cell carcinomas (OSCC) that proceed from hyperplasia to dysplasia, carcinoma in situ, and invasive carcinoma (D'Cruz et al. 2018; Parmar et al. 2021; Retzbach et al. 2018). The podoplanin (PDPN) receptor has emerged as a functionally relevant biomarker and chemotherapeutic target to identify and neutralize these OSCC cells (Hwang et al. 2021; Retzbach et al. 2018; Sayuddin et al. 2024). PDPN is a unique transmembrane receptor that promotes tumor cell motility, invasion, and metastasis (Krishnan et al. 2018; Quintanilla et al. 2019; Suzuki et al. 2022). PDPN also acts as an inhibitory receptor on lymphocytes to decrease immune and inflammatory activity (Chihara et al. 2018; Nylander et al. 2017; Peters et al. 2015).

PDPN is a transmembrane receptor with a sialic acid modified extracellular region that can serve as an enticing chemotherapeutic target (Krishnan et al. 2018; Quintanilla et al. 2019; Suzuki et al. 2022). Antibodies can target PDPN to inhibit cancer progression in animal models of cancer including OSCC (Kamoto et al. 2020; Shinada et al. 2023; Takemoto et al. 2022; Yoneda et al. 2024). Preclinical studies have also employed near-infrared photoimmunotherapy to dynamically target PDPN in order to destroy OSCC cells in mice (Kato et al. 2023).

In addition to antibodies, *Maackia amurensis* seed lectin (MASL) also targets the PDPN receptor with a KD value of ~10nM to inhibit OSCC cell growth and motility (Hamilton et al. 2021; Hellmig et al. 2023; Ochoa-Alvarez et al. 2015; Yin et al. 2024). MASL is a unique lectin that consists of a tetramer formed around central cysteine residues that bridge intramolecular dimers (Nayak et al. 2025). MASL also offers the advantage of oral administration since, like other lectins, MASL activity can survive digestion to enter the circulatory system and inhibit cancer progression and inflammation (Carpintero-Fernandez et al. 2020; Hamilton et al. 2022; Singh et al. 2024). Indeed, *Maackia* lectins are found as a component in traditional medicines used for centuries to treat cancer and inflammation in Asia (Hellmig et al. 2023). However, to the best of our knowledge, controlled human studies to investigate the effects of MASL on cancer progression have not been reported.

Here, we describe how MASL affected OSCC cells in human oral cancer patients in a Phase 1 clinical trial. This is the first human study to investigate the effects of MASL or any other agent that targets PDPN that we know of. Patients were treated with a 100 mg oral dose of MASL to administer topical oral and systemic exposure. This MASL dosage appeared safe as it did not cause any adverse effects. This single treatment did not significantly affect oral lesion OSCC cell morphology or PDPN expression in situ. However, MASL appeared to increase CD3 labeled lymphocyte tumor infiltration in one patient, suggesting that MASL might help to increase immune tumor surveillance. In addition, MASL significantly decreased the motility and viability of every OSCC cell line obtained from these patient lesions when adapted to cell culture. Interestingly, MASL exhibited different effects of PDPN expression in these cells, and significantly increased PDPN in some patient cell lines. We, therefore, also utilized NIR-PIT to demonstrate that antibodies conjugated to photoactivatable dye can target PDPN to kill human OSCC cells exposed to near-infrared light.

Materials and methods

Human tissues and cells

Human protocols and informed consent were approved by University Institutional Review Boards. These include an initial clinical study to examine PDPN expression on OSCC cells in oral lesion resections (Rutgers #CR00006596 and Rowan #Pro2012001544) and a Phase 1 clinical trial (USFDA IND #118210 and clinicaltrials.gov #NCT04188665) to study the effects of MASL on OSCC cell morphology, PDPN expression, growth, and motility in oral lesion biopsies and resections (Rutgers #CR00024686 and Rowan #Pro20140000009). Patients diagnosed with OSCC were randomized 1:1 by coin toss and treated with placebo or oral lozenges containing 100 mg of essentially pure MASL prepared and compounded at the University of California GMP facility (2315 Stockton Boulevard, Sacramento, CA 95817) in a manner consistent with best care practice. This 100 mg MASL dose was based on the concentration of *M. amurensis* lectins found in traditional concoctions used to treat cancer and inflammation (Hellmig et al. 2023). Cells obtained from surgical biopsies and resections were cultured from minced tissue washed in PBS, digested with trypsin (Hyclone SH30042.01 or Gibco 25200-056), and incubated in DMEM (Hyclone SH30021) supplemented with 25 mM HEPES (Hyclone SH30237), 1000/100 IU/ml penicillin/streptomycin (Corning 30-002-CI), 2.5 ug/ml amphotericin b (HyClone SV30078.01), and 10% FBS (Seradigm 1400–500) at 37 °C in 5% CO2 and 100%

humidity as described (Yin et al. 2024). Cell morphology and pan-cytokeratin expression were evaluated to confirm the malignant identity of these cells. Cells obtained from 11 out of the 19 patients (~58%) were successfully adapted to culture while 8 (~42%) failed to grow or succumbed to contamination.

Immunohistochemistry

Surgical specimens were fixed in 10% formalin in PBS, paraffin embedded, sectioned (4 µm), and processed for hematoxylin/eosin (H&E) staining and immunohistochemistry with antibodies specific for PDPN (D2-40 Agilent M361901-2), CD3 (2GV6 Roche/Ventana 790–4341), E-cadherin (26 Roche/Ventana 790–4497), and N-cadherin (SP90 Fisher PIMA516324) as described (Ochoa-Alvarez et al. 2015; Retzbach et al. 2018; Yin et al. 2024). Tissue morphology and PDPN expression was graded on a scale of 1 to 5, with five displaying the highest level of cellular dysplasia and PDPN expression, by two board certified pathologists in an independent and blind manner. Numbers of CD3 positive T cells were counted in 200 × 200 micron areas central to OSCC tissue to quantitate patient immune cell infiltration into tumors. Cells were visualized by phase contrast and bright field microscopy with a Zeiss Axiovert 5 microscope equipped with ZeissAxioCam Mrc cameras and Zen software as previously described (Nicoletto et al. 2024; Yin et al. 2024).

Western blot analysis

Western blotting was performed as previously described (Nicoletto et al. 2024; Sheehan et al. 2022; Yin et al. 2024). Briefly, cells were grown to confluence and lysed with 2% SDS, 10% glycerol, 10 mM EDTA, 50 nM DTT, 50 mM NaF, 1 mM Na3VO4, and 1 mM PMSF in 62.5 mM Tris pH 6.8. Protein concentration was measured by Coomassie (Fisher 1856209) with BSA standards (Fisher 23210). Equal amounts of protein (10ug/lane) were resolved by SDS-PAGE, transferred to Immobilon membranes (Millipore IPVH00010), and incubated with primary antiserum specific for PDPN (Agilent Dako M3619), pan-cytokeratin (Cell Signaling 4068 S), and GAPDH (Cell Signaling 2118 S). Primary antiserum was probed with appropriate secondary anti-rabbit (Cell signaling 7074 S) or anti-mouse (Invitrogen 31430) IgG antibodies and detected by chemiluminescence (Fisher 32106 or Fisher 34095). Signal intensity was quantitated by Image J (NIH version 1.54f). Transferred gels and membranes were stained with Coomassie dye and India ink, respectively, to verify equal loading and transfer.

Cell viability and migration assays

Effects of reagents on OSCC cell growth and motility were performed as described (Yin et al. 2024). Briefly, cells were grown to confluence on 6 well tissue culture cluster plates (Falcon 353224). For migration assays, cell monolayers were scratched and visualized at 0 and 24 h after treatment with 0 nM, 770 nM, 1540 nM, and 3080 nM MASL. Images were taken at 0 and 24 h after treatment, and migration was quantitated as the distance cells traveled from the edge to the center of the wound. Sister plates treated with MASL for 24 h were incubated with alamarBlue (BioRad #BUF012A) for 2 h and assayed (ex/em: 570/600 nm) to assess cell viability. Cell morphology was also examined on cells cultured on chambered culture slides (Falcon 354104) and stained with H&E (Abcam ab2458801). Cells were visualized by phase contrast microscopy with a Zeiss Axiovert 5 microscope equipped with ZeissAxioCam Mrc cameras and Zen software as previously described (Nicoletto et al. 2024; Yin et al. 2024). All assays were performed at least 3 times with similar results.

Cell surface PDPN expression analysis by flow cytometry

OSCC cells were grown to confluence, trypsinized, suspended in media (2×10^5 cells/ml), incubated with 1 ug/ml PE labeled anti-PDPN mAb clone NC-08 (Biolegend 337003) (Suzuki et al. 2022) or PE labeled isotype mAb control (Biolegend 402304) for one hour at 4 °C, washed with PBS, and analyzed with a flow cytometer (FACSLyric, BD Biosciences, San Jose, CA, USA) with FlowJo software (BD Biosciences). PDPN expression in each cell line was compared as relative fluorescent intensity (RFI) calculated as the median fluorescent intensity (MFI) of antibody staining divided by the MFI of isotype control.

IR700 antibody conjugation and NIR-PIT analysis

IR700 antibody conjugation and NIR-PIT analysis were performed as described (Furusawa et al. 2022; Kato et al. 2023). Briefly, 1 mg (6.8 nmol) NZ-1 mAb was incubated with 66.8 µg (34.2 nmol) IR700 NHS ester (LI-COR Biosciences, Lincoln, NE, USA) in phosphate buffer (pH 8.5) at room temperature for one hour. Conjugated antibodies were purified by Sephadex G25 column chromatography (PD-10; Cytiva, Piscataway, NJ, USA) and concentrations were determined by absorption at 689 nm using UV-Vis (8453 Value System; Agilent Technologies, Santa Clara, CA, USA). Effects of IR700 conjugated antibodies on OSCC cell viability after near-infrared exposure were evaluated by flow cytometry. Briefly, OSCC cells were seeded on 24

well plates (2×10^5 cells/well) and incubated overnight to form confluent monolayers. IR700 labeled antibody was then added to each well to a final concentration of 4 ug/ml and incubated for 1 h at 37 °C. NIR light (150 mW/cm^2) was applied to achieve 0, 5, 10, 20, or 50 Joules (J) before cells were immediately trypsinized, collected, stained with Fixable Viability Dye eFluor 506 (ThermoFisher Scientific 65–0866), and analyzed by flow cytometry (FACSLyric, BD Biosciences, San Jose, CA, USA) with FlowJo software (BD Biosciences).

Statistical analysis

Statistical analysis was performed with Prism software (GraphPad Prism version 10) as described in text and figure legends.

Results

Effects of a single MASL dose on OSCC lesion tissue morphology, PDPN expression, and immune cell infiltration in situ

This investigation was initiated with a clinical study to examine PDPN expression in surgical excisions obtained from patients with oral lesions. Four lesions were examined as shown in Fig. 1a. Three of these lesions were diagnosed as OSCC, while one of them (MF1) was diagnosed as epithelial dysplasia. Dysplastic oral squamous cells expressed robust PDPN levels in these lesions as expected from reports describing increased PDPN expression in oral cancers (Monteiro et al. 2024; Retzbach et al. 2018; Sayuddin et al. 2024). These results prompted initiation of a clinical trial designed to target PDPN in order to combat oral cancer.

Maackia amurensis seed lectin (MASL) targets the PDPN receptor with a KD value of 1.0×10^{-8} M to inhibit OSCC cell growth and motility (Hamilton et al. 2021; Ochoa-Alvarez et al. 2015; Yin et al. 2024). We, therefore, initiated a Phase 1 clinical trial to investigate how MASL affects OSCC cell morphology in oral cancer patients. Oral lesions were examined from biopsies obtained from 15 patients enrolled in this study as shown in Fig. 1b and c. A total of 19 biopsies were examined when combined with the 4 patients enrolled in the initial study. These patients ranged between 40 and 87 years old with a mean age of 57.3 years and were 79% male as shown in Fig. 1d.

Thirteen patients enrolled in the clinical trial were diagnosed with OSCC, while three (Sen2, Sen7, and Sen9) were diagnosed with other types of oral squamous lesions. All lesions in this study were taken from the tongue, buccal, lip, or gingival regions of the oral cavity. PDPN was detected in squamous cells at the borders of many lesions as shown in Fig. 1a, b, and c. PDPN expression and cellular dysplasia were graded in these lesions as shown in Fig. 1e. Consistent with previous reports (Monteiro et al. 2024; Retzbach et al. 2018; Sayuddin et al. 2024), PDPN expression correlated with the degree of cellular dysplasia as shown in Fig. 1f.

Eight patients enrolled in the clinical trial proceeded to the treatment phase of the study. Half (four) of these patients were treated with oral lozenges containing 100 mg of MASL, while the remaining four were treated with placebo lozenges. Surgical resections were then taken from these patients consistent with best care practice 18 to 65 h after lozenge administration. No adverse effects of any kind were evident from this oral MASL or placebo administration. PDPN and lesion morphology were assessed and compared with pretreated biopsy samples as shown in Fig. 1c. The single dose of MASL tested in this study did not appear to significantly affect OSCC cell morphology or PDPN expression in oral cancer patient lesions as shown in Fig. 1g.

PDPN has been identified as a co-inhibitory receptor induced by IL27 on CD4$^+$ T helper (Th17) cells, regulatory T (Treg) cells, and tumor infiltrating lymphocytes along with PD-1 and TIM3 (Chihara et al. 2018; Peters et al. 2015). We, therefore, examined if MASL might target PDPN to increase T cell infiltration into tumor tissue. We compared CD3 positive immune cells in OSCC tissue biopsies and resections in patients 18 to 24 h after MASL or placebo treatment shown in Fig. 2a. These results were variable with one patient (Sen12) showing reduced T cell infiltration after placebo treatment, another patient (Sen8) showing increase T cell infiltration after MASL treatment, and one patient (Sen15) showing decreased T cell infiltration after MASL treatment as shown in Fig. 2b.

Effects of MASL on patient derived oral lesion cell PDPN expression, motility, and viability in culture

Cells from patient lesions were adapted to culture in order to investigate the effects of MASL on their PDPN expression, motility, and viability. Effects of MASL on PDPN expression and behavior of HSC2 cells have been previously reported (Hamilton et al. 2021; Ochoa-Alvarez et al. 2015; Sheehan et al. 2021), and they are included here as well characterized human OSCC cells. Cells derived from lesions obtained from patients in this study exhibited a squamous morphology with varied degrees of differentiation as shown in Fig. 3a, and PDPN expression as shown in Fig. 3b and c. SB2 and Sen1 cells expressed the highest levels of PDPN at over 50% higher than HSC2 cells. MF1, SB3, and Sen9 cells expressed moderate PDPN levels within 25% of that seen in HSC2 cells. Sen6, Sen7, Sen12, and Sen15 cells expressed low PDPN levels at less than 50% of that seen in

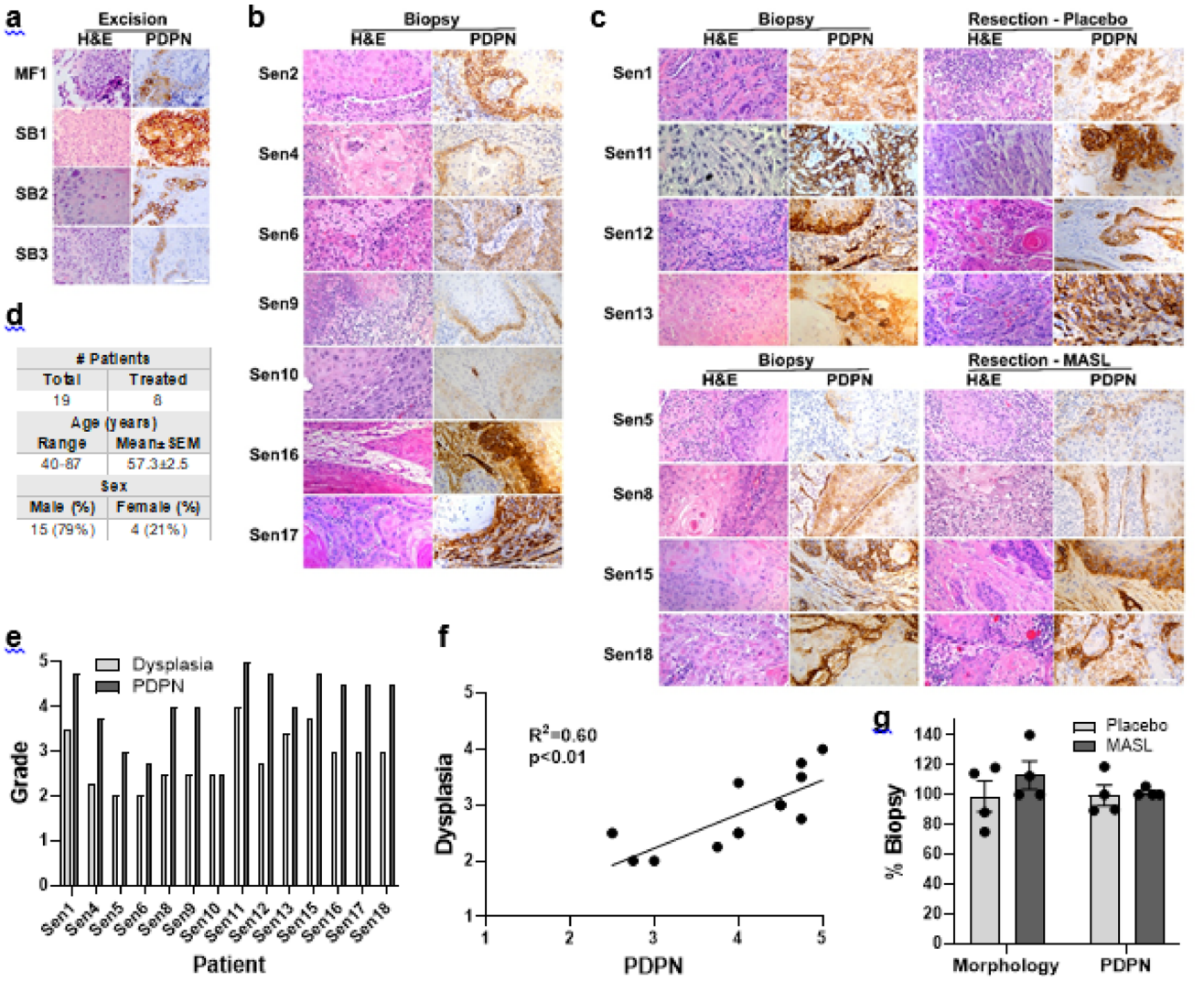

Fig. 1 Cell morphology and PDPN expression in oral lesions. Surgical samples from oral lesions in patients were examined by H&E staining and immunohistochemistry to detect PDPN expression. **a** Patient resections from initial clinical investigation (bar = 100 microns). **b** Biopsies from untreated patients enrolled in clinical trial (bar = 100 microns). **c** Biopsies and resections from placebo and MASL treated patients enrolled in clinical trial as indicated (bar = 100 microns). **d** Patient numbers, age, and sex. **e** Cellular dysplasia and PDPN expression in oral legions were quantified on a scale from 1 to 5 for each patient as indicated. **f** Linear regression analysis of cellular dysplasia and PDPN expression from patients with oral lesions. **g** Effects of placebo or MASL treatment on cellular dysplasia and PDPN expression in patient oral lesions were quantitated as percent of grading compared to biopsies as indicated (mean + SEM, $n = 4$)

HSC2 cells. Only two cell lines, Sen8 and Sen18, did not express detectable levels of PDPN.

MASL had variable effects on PDPN expression in cells derived from patient lesions grown in culture as shown in Fig. 4. Overall, MASL significantly inhibited PDPN expression in two cell lines (HSC2 and Sen7), did not significantly affect PDPN expression in cell from 5 patients (SB2, Sen1, Sen6, Sen9, and Sen12), and significantly increased PDPN expression in cells from 3 patients (MF1, SB3, and Sen15). However, it should be noted that MASL appeared to increase PDPN expression in Sen1, Sen6, and Sen12 cells, although these effects were not found to be significant with $p > 0.05$ by ANOVA. Two patient cell lines (Sen8 and Sen18) did not express detectable PDPN levels regardless of MASL exposure in culture.

In contrast to variable effects on PDPN expression, MASL inhibited migration and viability of every patient derived cell line in culture. In general, MASL inhibited OSCC cell migration in a dose responsive manner from the high nanomolar to low micromolar range shown in Fig. 5. MASL required higher concentrations in the 1–3 micromolar range to inhibit OSCC cell viability than was required to inhibit migration as shown in Fig. 6. These data confirm that MASL inhibits OSCC cell motility before inhibiting viability as previously reported (Hamilton et al. 2021; Ochoa-Alvarez et al. 2015; Yin et al. 2024). Interestingly,

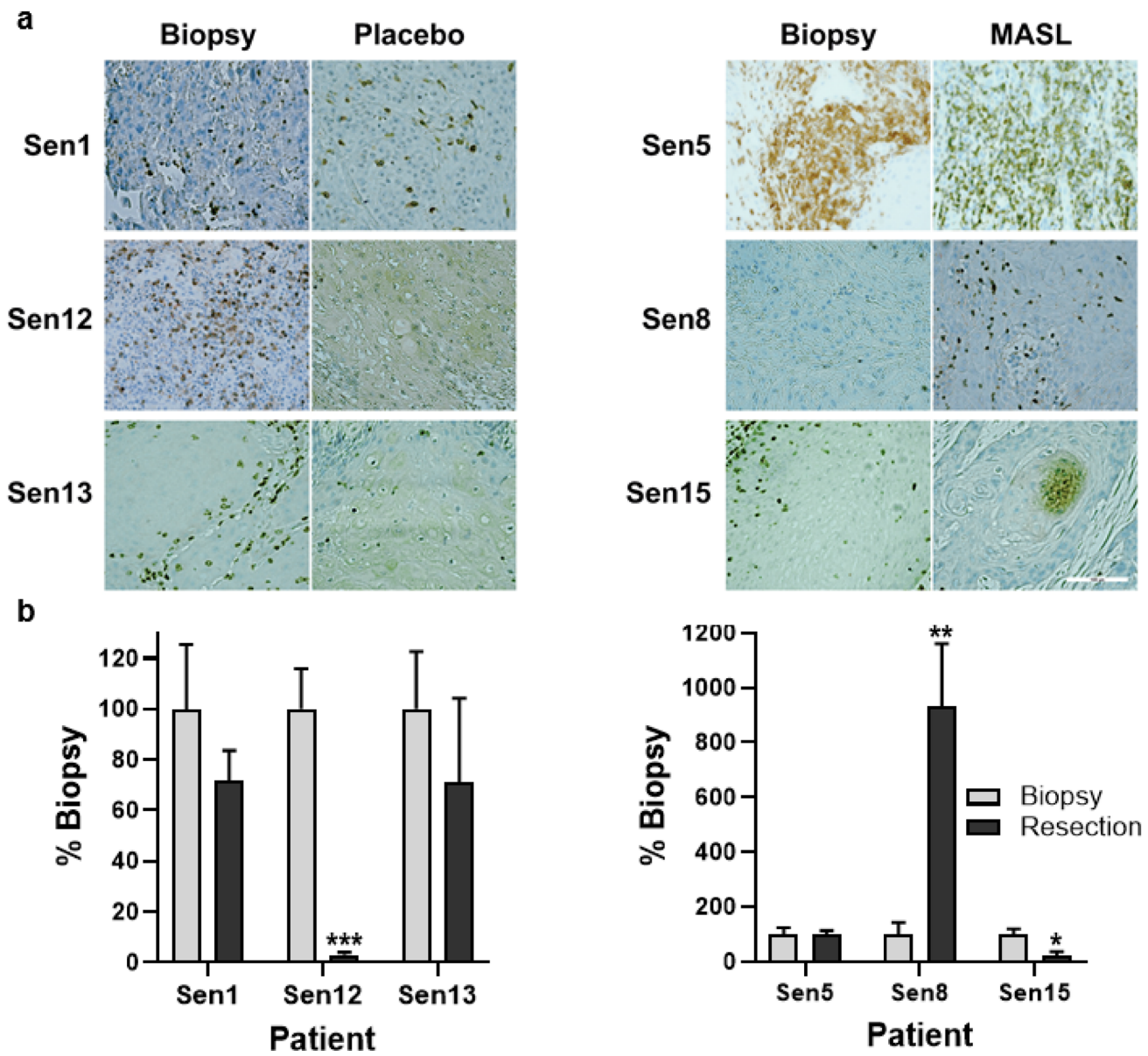

Fig. 2 Effect of MASL on CD3 immune cell infiltration into OSCC lesions. Surgical samples from OSCC tumor tissue in patients were examined by hemotoxilin staining and immunohistochemistry to detect CD3 labeled T lymphocytes. **a** Biopsies and resections from placebo and MASL treated patients enrolled in clinical trial as indicated (bar = 100 microns). **b** Effects of placebo or MASL treatment on T lymphocyte infiltration into patient OSCC tumor tissue were quantitated as percent of T cells found in a 0.4 mm^2 field in resections compared to biopsies (mean + SEM, $n = 6$) with single, double, and triple asterisks indicating $p < 0.05$, $p < 0.01$, and $p < 0.001$ as indicated

MASL inhibited Sen8 and Sen18 cell motility and viability even though they do not express PDPN in culture. These data indicate that MASL targets other receptors in addition to PDPN to inhibit OSCC cell motility and growth (see Discussion).

Near-infrared photoimmunotherapy (NIR-PIT) is being utilized to develop effective anticancer treatments, and is being used to treat head and neck squamous cell carcinoma (Kobayashi et al. 2021; Miyazaki et al. 2023; Mohiuddin et al. 2023; Nakajima and Ogawa 2024). We utilized patient derived OSCC cells obtained from this study to evaluate the ability of antibodies that target to kill human OSCC cells by NIR-PIT. Cell surface PDPN expression on HSC2 and SB2 cells, but not Sen8 cells was confirmed by flow cytometry as shown in Fig. 7a. Accordingly, near-infrared (NIR) exposure killed HSC2 and SB2 cells incubated with IR700 conjugated NZ-1 antibody in a dose responsive manner, while PDPN deficient Sen8 cells were not affected as shown in Fig. 7b.

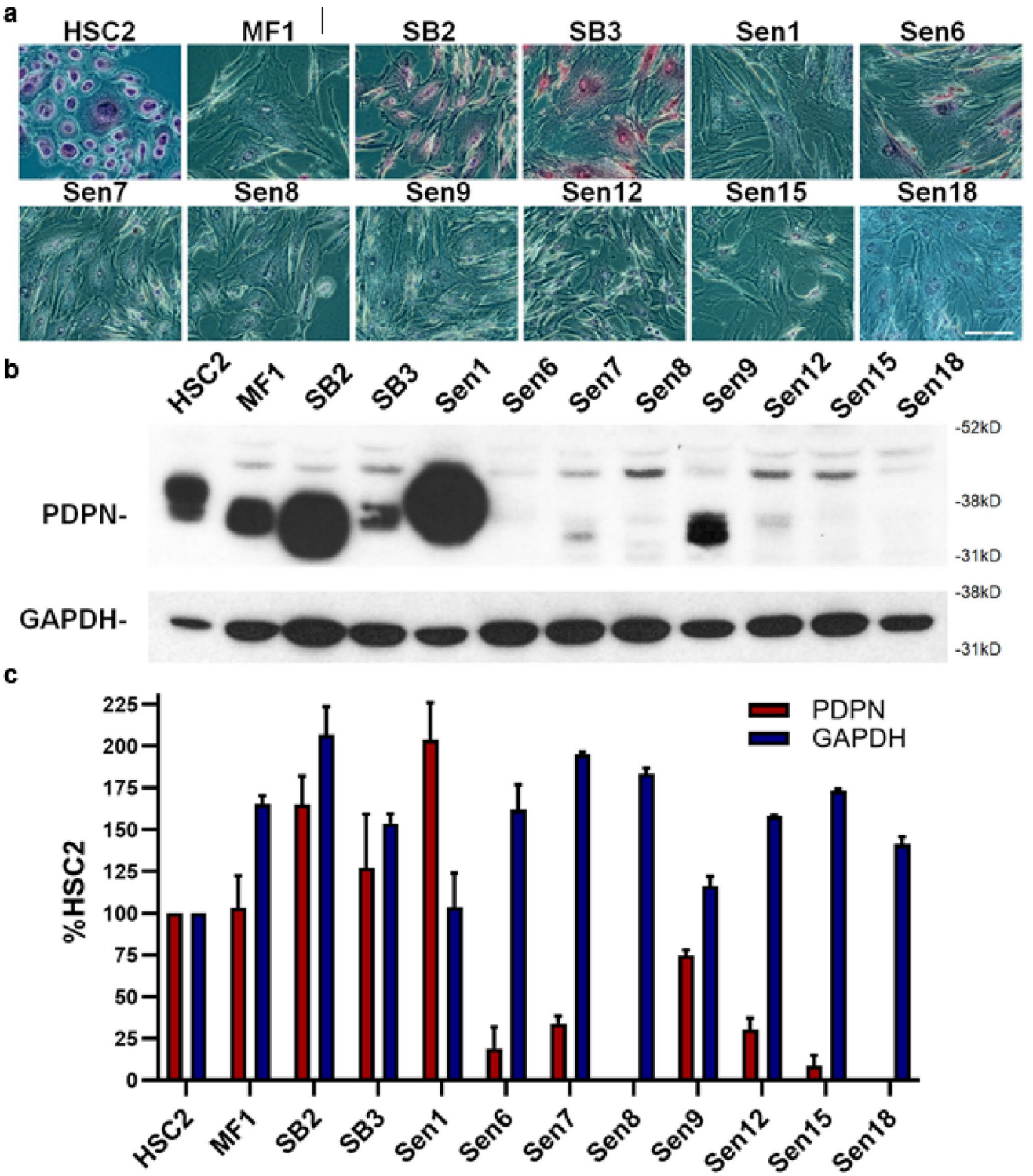

Fig. 3 Patient ex vivo oral lesion cell morphology and protein expression profiles. **a** Patient cells were adapted to culture, grown on glass slides, H&E stained, and visualized with phase contrast microscopy (bar = 100 microns). **b** PDPN and GAPDH expression were evaluated by Western blotting. **c** PDPN and GAPH expression were quantitated relative to HSC2 cells

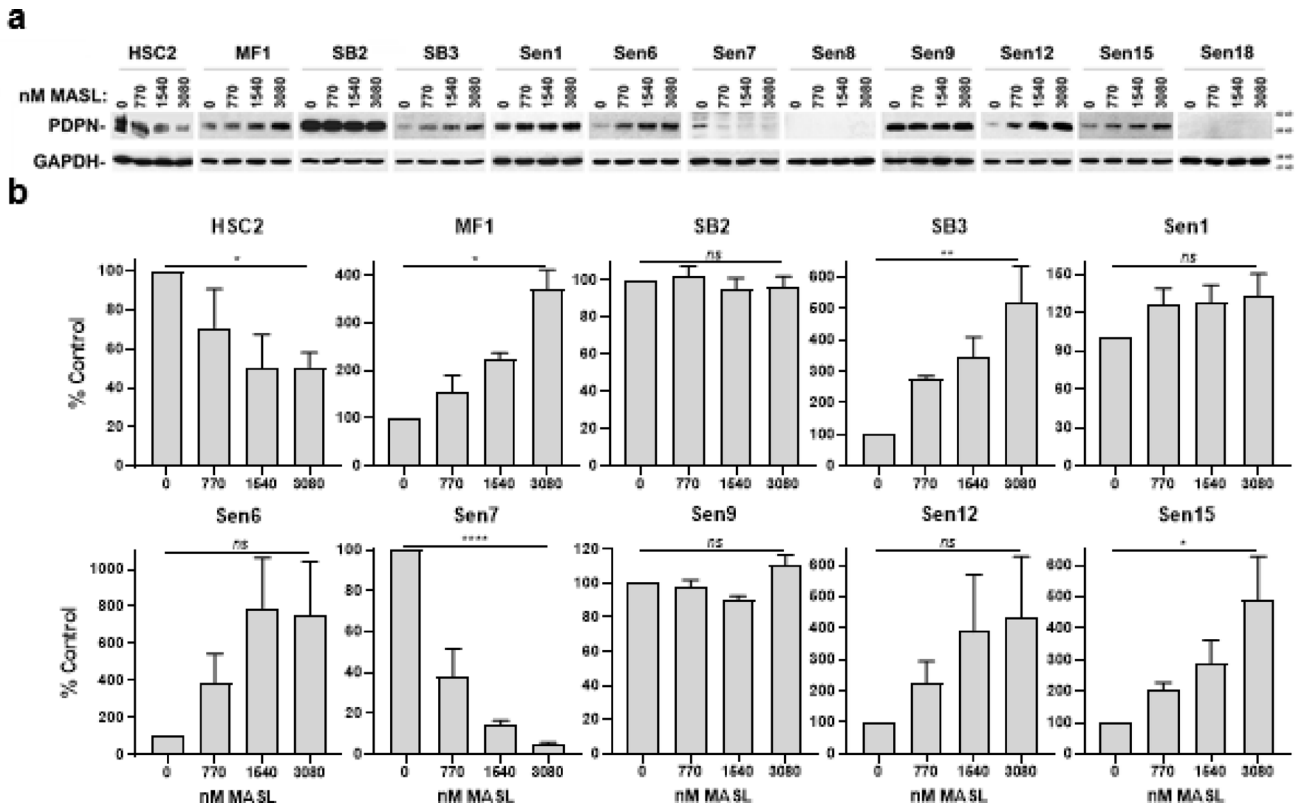

Fig. 4 Effects of MASL on patient oral lesion cell PDPN expression in culture. **a** Western blotting was used to examine PDPN and GAPDH expression in patient derived cells treated with 0 nM, 770 nM, 1540 nM, and 3080 nM MASL for 24 h with position of molecular weight markers shown as indicated. **b** PDPN expression was quantitated as percent of nontreated controls (mean + SEM, $n = 3$) for each patient cell line as indicated

Discussion

OSCC cells respond variably to standard chemotherapies, adding to the challenge of treating oral cancer malignancies (Baskar et al. 2023; Parmar et al. 2021). The PDPN receptor has been identified as a functionally relevant biomarker and chemotherapeutic target on OSCC cells (Retzbach et al. 2018; Sayuddin et al. 2024; Sheehan et al. 2022). MASL has emerged as a potential anticancer agent that targets sialic acid residues found on the PDPN receptor. Indeed, MASL can target PDPN to inhibit OSCC cell growth and motility (Hamilton et al. 2021; Ochoa-Alvarez et al. 2015; Retzbach et al. 2018; Yin et al. 2024). This clinical trial was designed to investigate the effects of MASL on OSCC cells in oral cancer patients. All patient OSCC cells expressed PDPN in situ, and PDPN expression correlated with degree of dysplasia as shown in Fig. 1. This study was limited to one 100 mg MASL dose by oral administration. This limited treatment did not induce any adverse effects on participants, nor did it produce any notable effects on PDPN expression or OSCC cell morphology in patient lesions.

In addition to OSCC cells, PDPN has been identified as a co-inhibitory receptor expressed by tumor infiltrating lymphocytes (Chihara et al. 2018; Peters et al. 2015). We, therefore, hypothesized that MASL might target PDPN to increase lymphocyte activity and patient anticancer immune responses. MASL appeared to significantly increase lymphocyte cell infiltration into OSCC tumor fields in lesions from one patient out of 3 patients examined, while no increase was found in lesions from placebo treated patients as shown in Fig. 2. These data from a single oral MASL dose suggest the need for further studies to more accurately evaluate the potential of this lectin to support an anticancer immune response.

Cells cultured from 9 out of 11 oral lesions taken from patients enrolled in this study expressed PDPN detectable by Western blotting. Interestingly, however, MASL significantly decreased the motility and viability of all OSCC cells produced in this study regardless of their PDPN expression as shown in Figs. 5 and 6. These results indicate that MASL can bind other receptors. Indeed, many cancers exhibit increased expression of sialic acid residues compared to their normal precursors (Bull et al. 2014; Habeeb et al. 2024; Zhu et al. 2024).

While MASL can target PDPN to inhibit OSCC cell migration and viability (Hamilton et al. 2021; Ochoa-Alvarez et

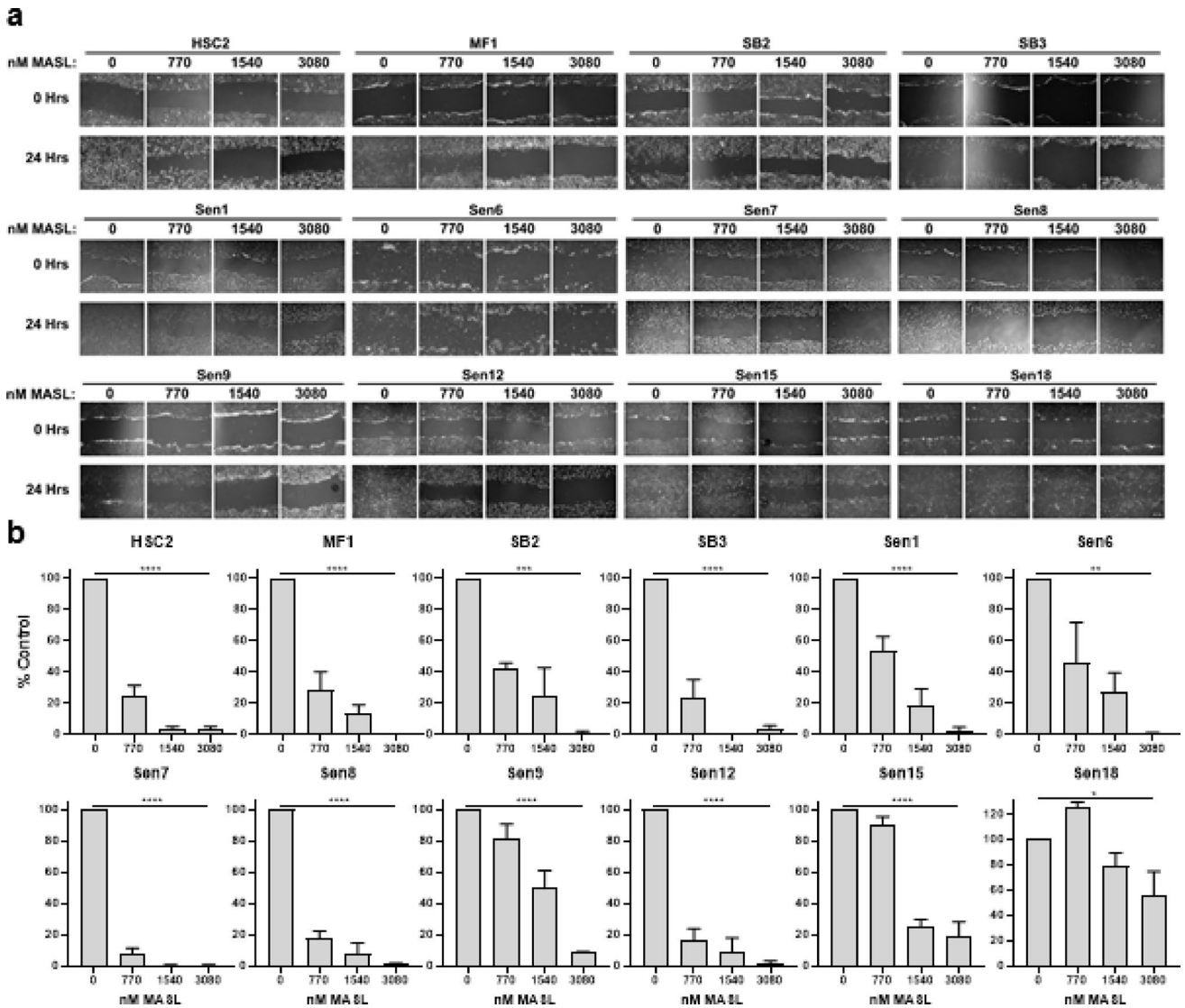

Fig. 5 Effects of MASL on patient oral lesion cell migration in culture. **a** Scratch assay was used to evaluate migration of patient derived cells treated with 0 nM, 770 nM, 1540 nM, and 3080 nM MASL for 24 h was evaluated as indicated (bar = 100 microns). **b** Migration was quantitated as percent of nontreated controls (mean + SEM, $n = 3$) for each patient cell line with double, triple, and quadruple asterisks indicating $p < 0.01$, $p < 0.001$, and $p < 0.0001$ by ANOVA respectively

al. 2015; Retzbach et al. 2018; Yin et al. 2024), some OSCC cells might survive MASL treatment. Indeed, results from this study indicate that MASL can increase PDPN expression in some OSCC cells as shown in Fig. 4. We, therefore, examined the potential of NIR-PIT to target PDPN in order to kill OSCC cells. An IR700 conjugated EGFR antibody (cetuximab-IR700, RM-1929) is being used to successfully treat head and neck cancer patients (Miyazaki et al. 2023). In addition, preclinical studies indicate that IR700 conjugated anti-mouse PDPN antibodies can be activated by NIR-PIT to inhibit murine OSCC cells in syngeneic mice (Kato et al. 2023). Here, we confirm that NIR-PIT can also employ anti-human antibodies to target and kill human OSCC cells in culture as shown in Fig. 7.

Taken together, results from this study confirm that OSCC cells express PDPN which correlates with morphological dysplasia in human oral cancer patient lesions. In addition, most OSCC cells continue to express PDPN in culture. Interestingly, MASL can inhibit the motility and viability of human OSCC cells regardless of PDPN expression. A single 100 mg oral dose of MASL was found to be safe with no adverse effects reported in this limited Phase 1 trial. While this dose did not alter PDPN expression or OSCC cell morphology in situ, data suggest that this limited MASL exposure appeared to promote lymphocyte infiltration into one of these patient oral lesions. These results suggest that additional studies are warranted to further investigate the potential of MASL to inhibit oral cancer progression alone

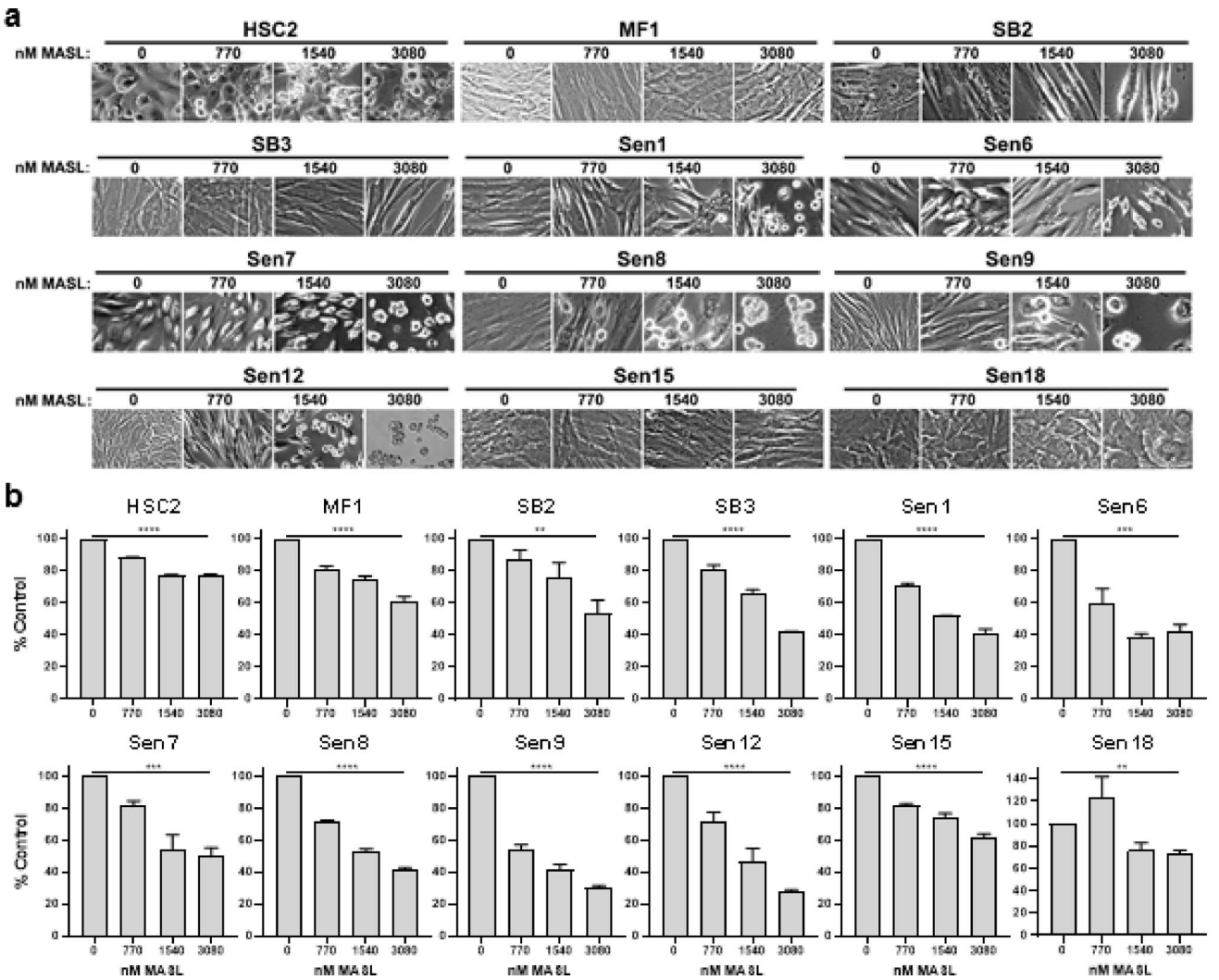

Fig. 6 Effects of MASL on patient lesion cell viability in culture. **a** Patient oral lesion cells treated with 0 nM, 770 nM, 1540 nM, and 3080 nM MASL for 24 h which were visualized by phase contrast microscopy indicated (bar = 50 microns). **b** AlamarBlue assay was used to evaluate viability of these cells, and quantitated as percent of nontreated controls (mean + SEM, $n = 3$) for each patient cell line with double, triple, and quadruple asterisks indicating $p < 0.01$, $p < 0.001$, and $p < 0.0001$ by ANOVA as indicated

or in combination with other therapies including standard cytotoxic chemotherapy agents (Hamilton et al. 2021) and NIR-PIT (Kato et al. 2023; Miyazaki et al. 2023).

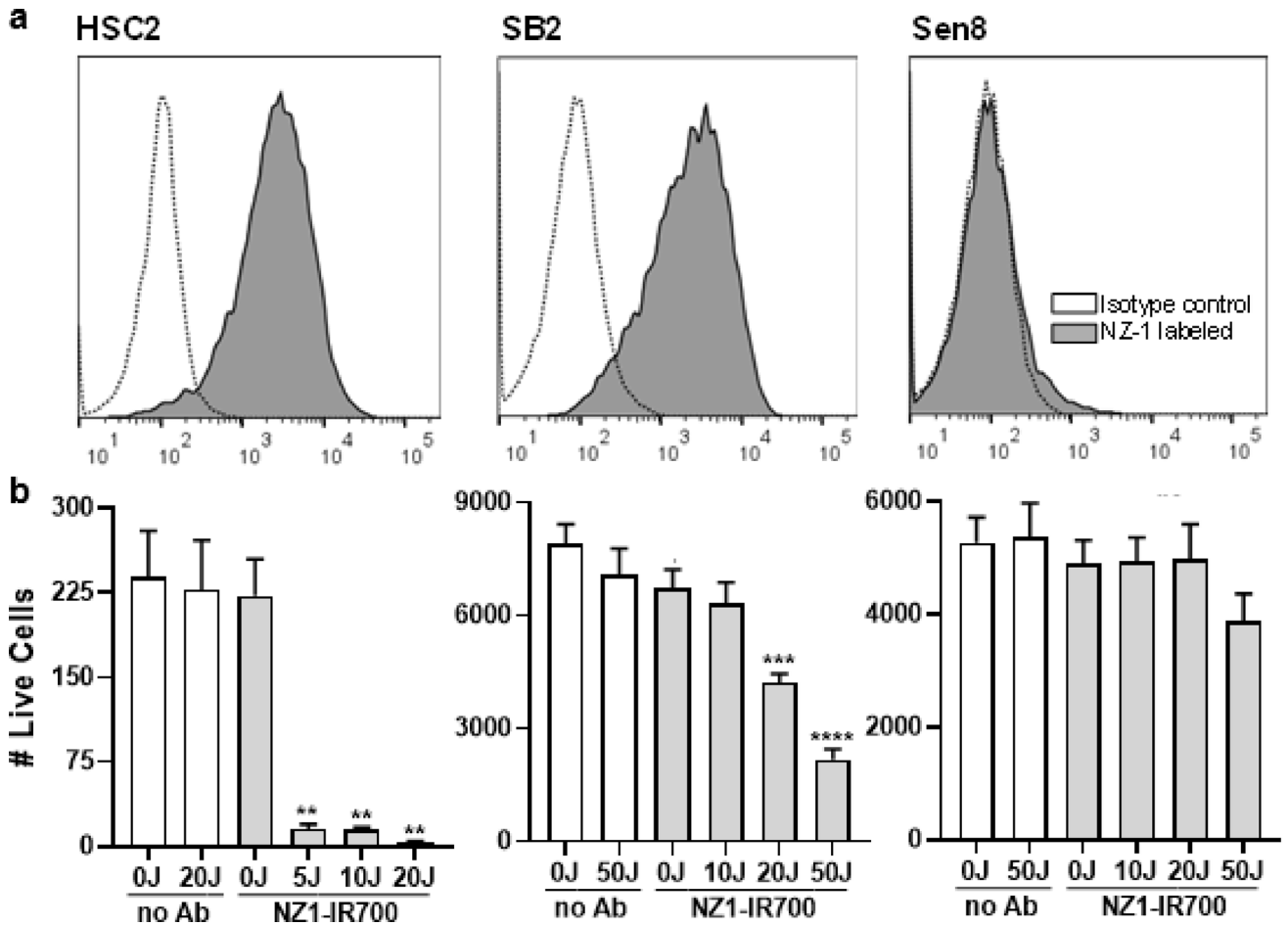

Fig. 7 NIR-PIT targets PDPN to kill human OSCC cells. **a** OSCC cells were incubated with PE labeled anti-PDPN Ab (clone NC-08) or isotype Ab control to analyze cell surface PDPN by flow cytometry as indicated. **b** Cells were incubated with or without IR700 conjugated NZ-1 Ab, exposed to NIR light, labeled with viable stain, and analyzed by flow cytometry to determine NIR-PIT effects on cell viability as indicated. Double, triple, and quadruple asterisks indicate $p<0.01$, $p<0.001$, and $p<0.0001$ compared to untreated controls by t-test as indicated

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Author contributions A.C.Y. and C.J.H. performed cell culture and protein analysis shown in Figs. 3, 4, 5 and 6.T.J.H. and E.J.B. assisted with cell culture and protein analysis shown in Figs. 3, 4, 5 and 6. D.I.S. and A.J.S. performed IHC and analysis shown in Figs. 1 and 2. D.R., E.K., G.M., S.B., and R.M.S. took care of patients, performed surgery, and assisted with tissue preparation needed for tissue and cell culture analysis throughout the manuscript. E.C. attended to patients during administration procedures. M.K.K. and Y.K. generated antibodies used for NIR-PIT shown in Figure 7. H.K. and A.F. performed NIR-PIT analysis shown in Figure 7. M.F. and G.S.G. directed all aspects of the study including patient tissue and cell culture analysis and generated the original manuscript. All authors reviewed and revised the manuscript for submission. G.S.G, Y.K., and H.K. provided funding to perform this study.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare the following relationships which may be considered as potential competing interests: GSG has intellectual property and ownership in Sentrimed Inc. ACY and CJH have ownership and received financial support from Sentrimed Inc. which is developing agents including MASL that target podoplanin to treat diseases including cancer and arthritis. The other authors declare no conflicts of interest.

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References

Baskar G et al (2023) Recent and advanced therapy for oral cancer. Biotechnol Bioeng 120:3105–3115. https://doi.org/10.1002/bit.28452

Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A (2024) Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries CA. Cancer J Clin 74:229–263. https://doi.org/10.3322/caac.21834

Bull C, Stoel MA, den Brok MH, Adema GJ (2014) Sialic acids sweeten a tumor's life. Cancer Res 74:3199–3204. https://doi.org/10.1158/0008-5472.CAN-14-0728

Carpintero-Fernandez P, Varela-Eirin M, Lacetera A, Gago-Fuentes R, Fonseca E, Martin-Santamaria S, Mayan MD (2020) New therapeutic strategies for osteoarthritis by targeting Sialic acid receptors. Biomolecules 10:40637. https://doi.org/10.3390/biom10040637

Chihara N et al (2018) Induction and transcriptional regulation of the co-inhibitory gene module in T cells. Nature 558:454–459. https://doi.org/10.1038/s41586-018-0206-z

D'Cruz AK, Vaish R, Dhar H (2018) Oral cancers: current status. Oral Oncol 87:64–69. https://doi.org/10.1016/j.oraloncology.2018.10.013

Furusawa A et al (2022) CD29 targeted near-infrared photoimmunotherapy (NIR-PIT) in the treatment of a pigmented. Melanoma Model Oncoimmunology 11:2019922. https://doi.org/10.1080/2162402X.2021.2019922

Habeeb IF, Alao TE, Delgado D, Buffone A Jr. (2024) When a negative (charge) is not a positive: sialylation and its role in cancer mechanics and progression. Front Oncol 14:1487306. https://doi.org/10.3389/fonc.2024.1487306

Hamilton KL et al (2021) Effects of Maackia amurensis seed lectin (MASL) on oral squamous cell carcinoma (OSCC) gene expression and transcriptional signaling pathways. J Cancer Res Clin Oncol 147:445–457. https://doi.org/10.1007/s00432-020-03456-8

Hamilton KL, Greenspan AA, Shienbaum AJ, Fischer BD, Bottaro A, Goldberg GS (2022) Maackia amurensis seed lectin (MASL) ameliorates articular cartilage destruction and increases movement velocity of mice with TNFalpha induced rheumatoid arthritis. Biochem Biophys Rep 32:101341. https://doi.org/10.1016/j.bbrep.2022.101341

Hellmig TJ et al (2023) Amur maackia (Maackia amurensis) seed lectin history and potential effect on cancer progression, inflammation, and viral infection. In: Rajendram R, Preedy V, Patel V (eds) Ancient and traditional foods, plants, herbs and spices used in Cancer. Taylor and Francis, Oxfordshire

Hwang BO et al (2021) Platelet CLEC2-podoplanin axis as a promising target for oral cancer. Treat Front Immunol 12:807600. https://doi.org/10.3389/fimmu.2021.807600

Kamoto S et al (2020) Phase i/ii clinical trial of the anti-podoplanin monoclonal antibody therapy in dogs with malignant melanoma. Cells 9:2529. https://doi.org/10.3390/cells9112529

Kato T et al (2023) Near-infrared photoimmunotherapy targeting podoplanin-expressing cancer cells and cancer-associated fibroblasts. Mol Cancer Ther 22:75–88. https://doi.org/10.1158/1535-7163.MCT-22-0313

Kobayashi H, Furusawa A, Rosenberg A, Choyke PL (2021) Near-infrared photoimmunotherapy of cancer: a new approach that kills cancer cells and enhances anti-cancer host immunity. Int Immunol 33:7–15. https://doi.org/10.1093/intimm/dxaa037

Krishnan H et al (2018) Podoplanin: an emerging cancer biomarker and therapeutic target. Cancer Sci 109:1292–1299. https://doi.org/10.1111/cas.13580

Miyazaki NL, Furusawa A, Choyke PL, Kobayashi H (2023) Review of RM-1929 Near-Infrared photoimmunotherapy clinical efficacy for unresectable and/or recurrent head and neck squamous cell carcinoma cancers. (Basel) 15:5117. https://doi.org/10.3390/cancers15215117

Mohamad I, Glaun MDE, Prabhash K, Busheri A, Lai SY, Noronha V, Hosni A (2023) Current treatment strategies and risk stratification for oral carcinoma. Am Soc Clin Oncol Educ Book 43:e389810. https://doi.org/10.1200/EDBK_389810

Mohiuddin TM, Zhang C, Sheng W, Al-Rawe M, Zeppernick F, Meinhold-Heerlein I, Hussain AF (2023) Near infrared photoimmunotherapy: a review of recent progress and their target molecules for cancer therapy. Int J Mol Sci 24:32655. https://doi.org/10.3390/ijms24032655

Monteiro L, Mariano LC, Warnakulasuriya S (2024) Podoplanin could be a predictive biomarker of the risk of patients with oral leukoplakia to develop oral cancer: a systematic review and meta-analysis. Oral Dis 30:207–215. https://doi.org/10.1111/odi.14378

Nakajima K, Ogawa M (2024) Near-infrared photoimmunotherapy and anti-cancer immunity. Int Immunol 36:57–64. https://doi.org/10.1093/intimm/dxad042

Nayak AR et al (2025) Maackia amurensis seed lectin (MASL) structure and sequence comparison with other Maackia amurensis lectins. J Biolog Chem 301:108466. https://doi.org/10.1016/j.jbc.2025.108466

Nicoletto RE et al (2024) Effects of cadherin mediated contact normalization on oncogenic Src kinase mediated gene expression and protein phosphorylation Scientific reports 14:23942 https://doi.org/10.1038/s41598-024-75449-3

Nylander AN et al (2017) Podoplanin is a negative regulator of Th17 inflammation. JCI Insight 2:91321. https://doi.org/10.1172/jci.insight.92321

Ochoa-Alvarez JA et al (2015) Antibody and lectin target Podoplanin to inhibit oral squamous carcinoma cell migration and viability by distinct mechanisms. Oncotarget 6:9045–9060

Parmar A et al (2021) Interventions for the treatment of oral cavity and oropharyngeal cancer: chemotherapy. Cochrane Database Syst Rev 12:CD006386. https://doi.org/10.1002/14651858.CD006386.pub4

Peters A, Burkett PR, Sobel RA, Buckley CD, Watson SP, Bettelli E, Kuchroo VK (2015) Podoplanin negatively regulates CD4+effector T cell responses. J Clin Investig 125:129–140. https://doi.org/10.1172/JCI74685

Quintanilla M, Montero-Montero L, Renart J, Martin-Villar E (2019) Podoplanin in inflammation and cancer. Int J Mol Sci 20:707. https://doi.org/10.3390/ijms20030707

Retzbach EP et al (2018) Podoplanin emerges as a functionally relevant oral cancer biomarker and therapeutic target. Oral Oncol 78:126–136. https://doi.org/10.1016/j.oraloncology.2018.01.011

Sayuddin E, Taher M, Arzmi MH, Burhanudin NA, Rostam MA (2024) The role of Podoplanin inhibitors in controlling oral

cancer progression. Arch Oral Biol 157:105841. https://doi.org/10.1016/j.archoralbio.2023.105841

Sheehan SA et al (2021) Evidence that Maackia amurensis seed lectin (MASL) exerts pleiotropic actions on oral squamous cells with potential to inhibit SARS-CoV-2 infection and COVID-19 disease progression. Exp Cell Res 403:112594. https://doi.org/10.1016/j.yexcr.2021.112594

Sheehan SA, Retzbach EP, Shen Y, Krishnan H, Goldberg GS (2022) Heterocellular N-cadherin junctions enable nontransformed cells to inhibit the growth of adjacent transformed cells. Cell Commun Signal 20:19. https://doi.org/10.1186/s12964-021-00817-9

Shinada M et al (2023) Podoplanin promotes cell proliferation, survival, and migration of canine non-tonsillar squamous cell carcinoma. J Vet Med Sci 85:1068–1073. https://doi.org/10.1292/jvms.23-0062

Singh K, Agrawal L, Gupta R, Singh D, Kathpalia M, Kaur N (2024) Lectins as a promising therapeutic agent for breast cancer: A review. Breast Dis 43:193–211. https://doi.org/10.3233/BD-230047

Suzuki H, Kaneko MK, Kato Y (2022) Roles of Podoplanin in malignant progression of tumor. Cells 11:575. https://doi.org/10.3390/cells11030575

Takemoto A et al (2022) Targeting Podoplanin for the treatment of osteosarcoma clinical cancer research: an official. J Am Assoc Cancer Res 28:2633–2645. https://doi.org/10.1158/1078-0432.CCR-21-4509

Yin AC et al (2024) Maackia amurensis seed lectin (MASL) and soluble human Podoplanin (shPDPN) sequence analysis and effects on human oral squamous cell carcinoma (OSCC) cell migration and viability. Biochem Biophys Res Commun 710:149881. https://doi.org/10.1016/j.bbrc.2024.149881

Yoneda H et al (2024) Antipodoplanin antibody enhances the antitumor effects of CTLA-4 Blockade against malignant mesothelioma by natural killer cells. Cancer Sci 115:357–368. https://doi.org/10.1111/cas.16046

Zhu W, Zhou Y, Guo L, Feng S (2024) Biological function of Sialic acid and sialylation in human health and disease. Cell Death Discov 10:415. https://doi.org/10.1038/s41420-024-02180-3

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