



Hollings Cancer Center
An NCI-Designated Cancer Center

Risk Stratification of Oral Premalignant Lesions Using Molecular Fluorescent Imaging

Dauren Adilbay, MD, Ph.D.

Assistant Professor
Department of Otolaryngology/HNS

Disclosures

- Evergreen Theragnostics – consultant
- Nonfinancial: The presenter has no relevant nonfinancial relationship to disclose

Background

- *"OPMDs – are any oral mucosal abnormality that is associated with a statistically increased risk of developing oral cancer."*
- Leukoplakia,
- Erythroplakia
- Proliferative verrucous leukoplakia
- Oral lichen planus,
- And others: oral submucous fibrosis, palatal lesions in reverse smokers, lupus erythematosus, epidermolysis bullosa, and dyskeratosis congenita.



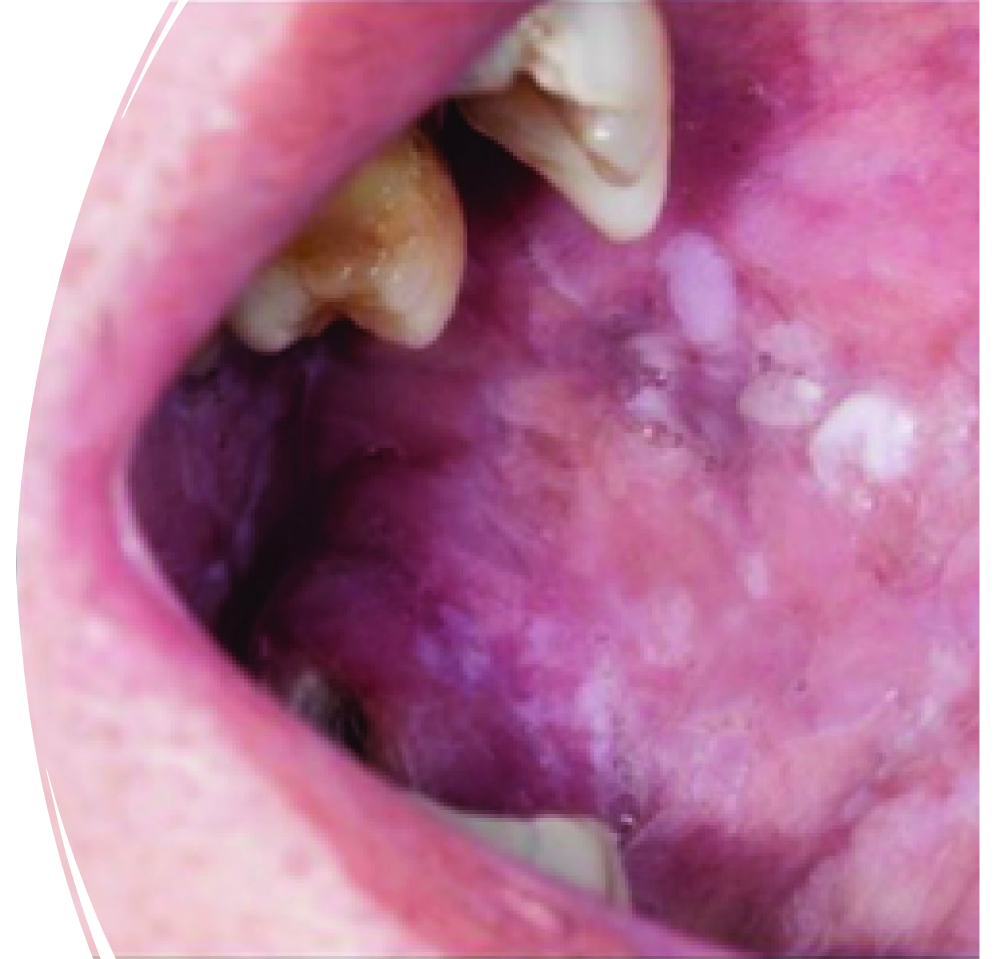
Definition

- leukoplakia: white plaques of questionable risk, having excluded other known diseases or disorders that carry no increased risk for cancer

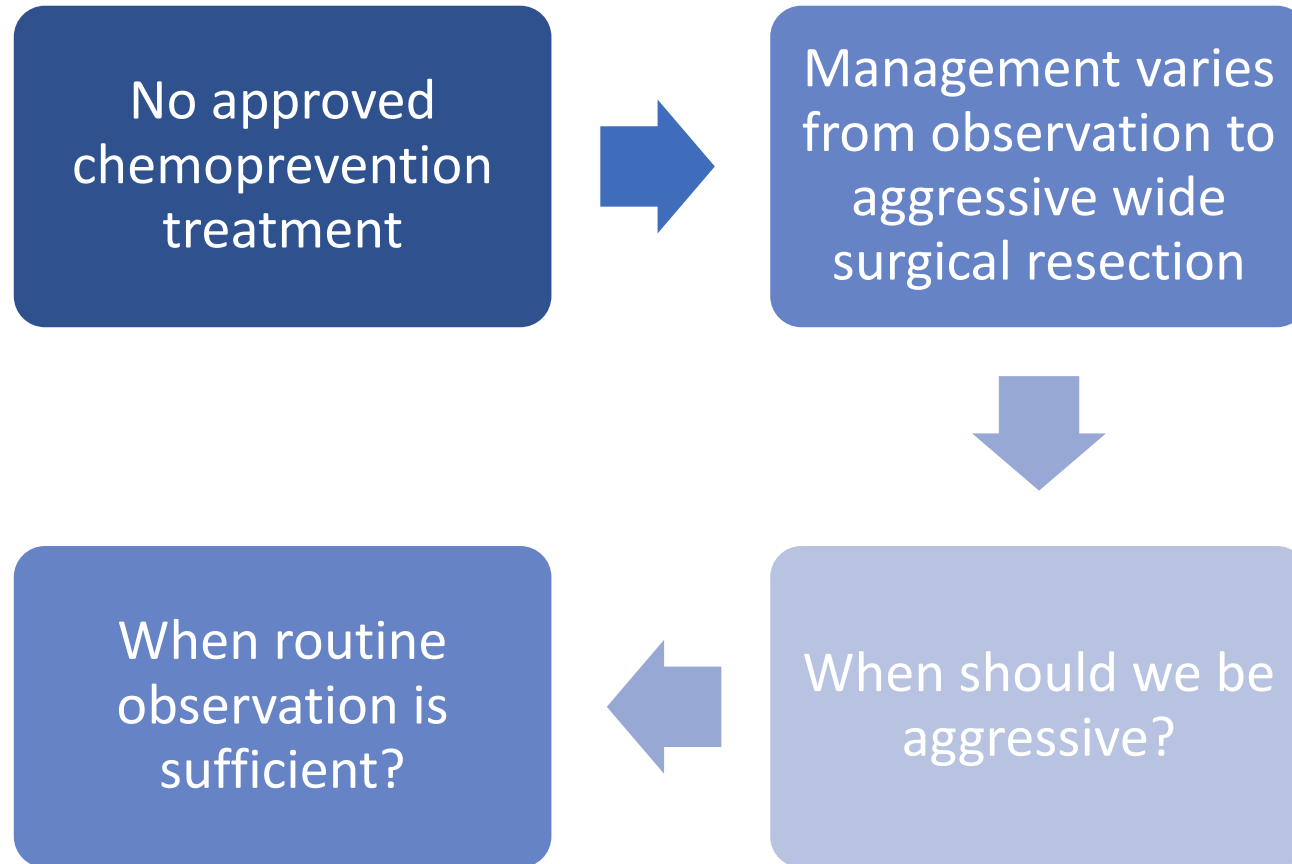


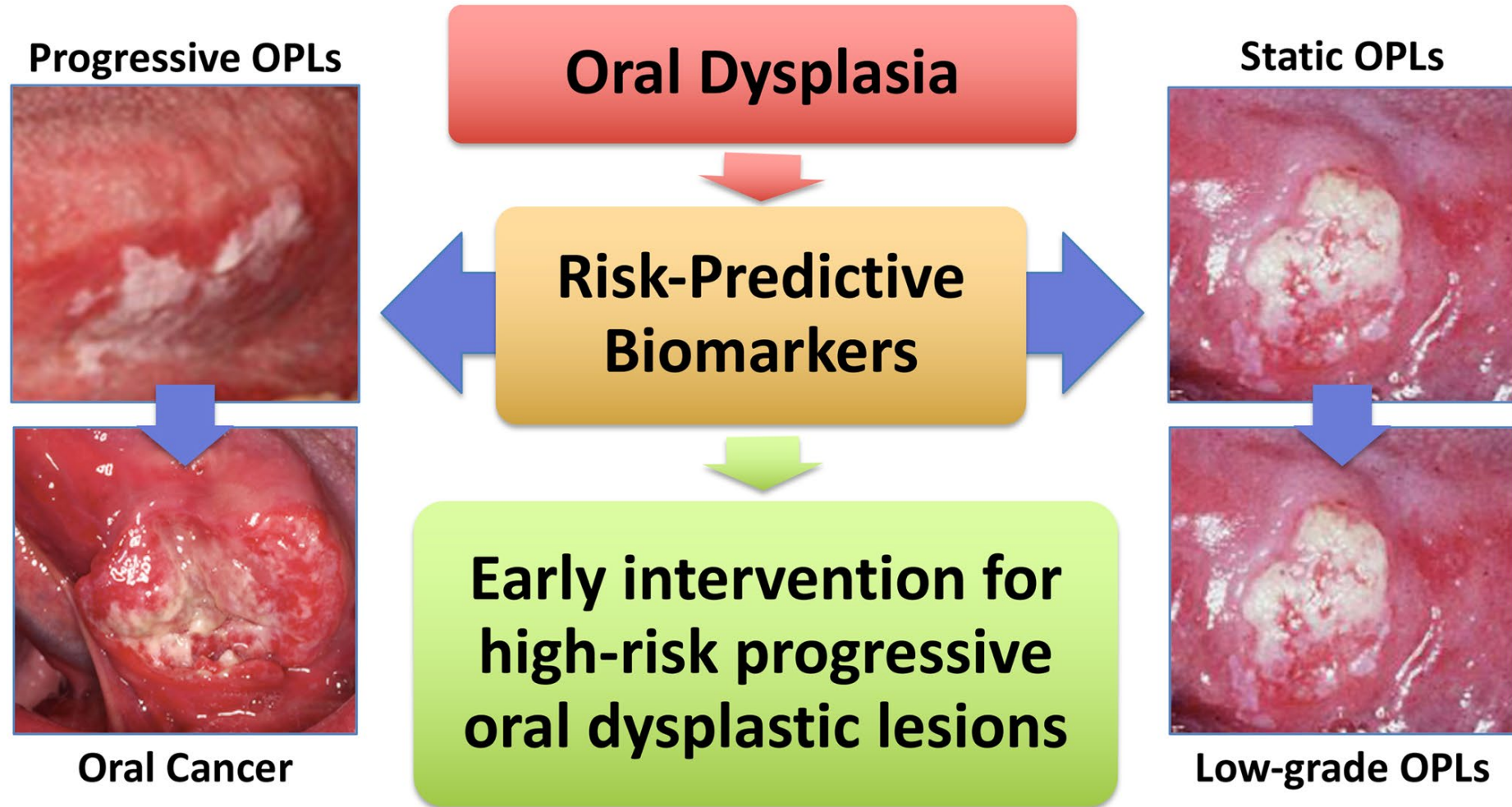
Classification (consensus report)

- Homogenous
- Non-homogenous
 - Nodular leukoplakia: Small polypoid or rounded outgrowths, red or white excrescences.
 - Verrucous leukoplakia: The surface is raised, exophytic, wrinkled, or corrugated
 - Erythroleukoplakia

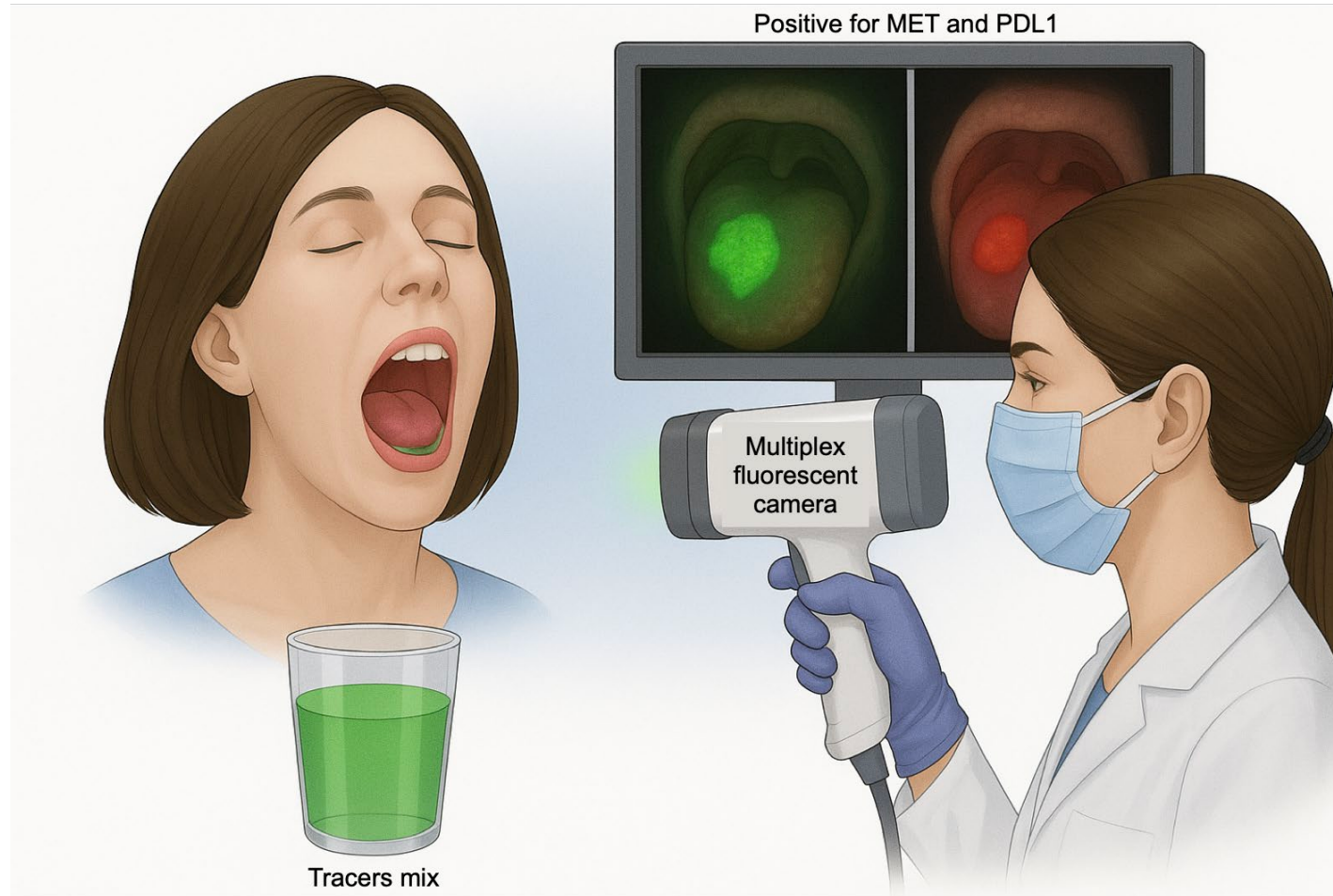


Current management issues





Fluorescent imaging/mapping



Oral premalignant lesions – longitudinal studies

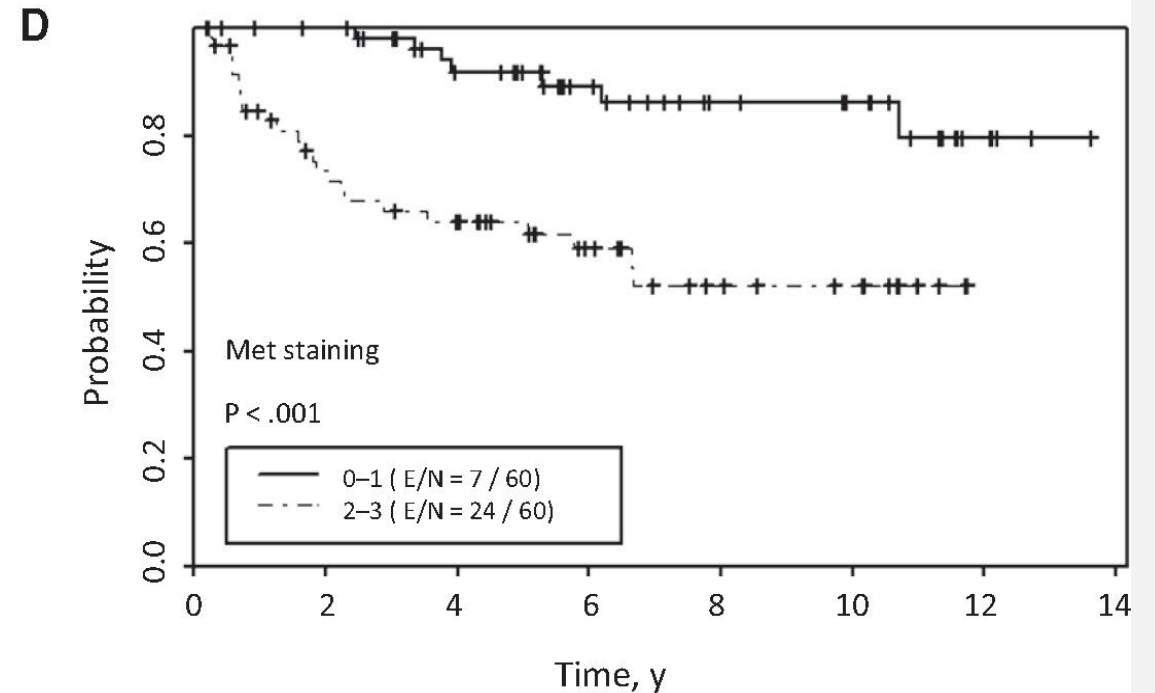
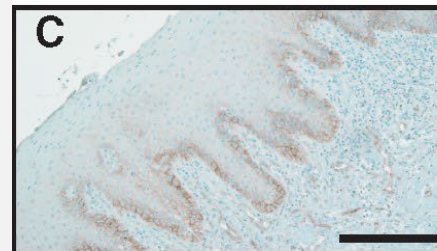
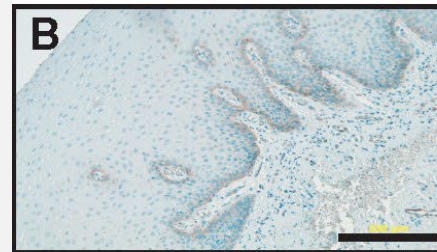
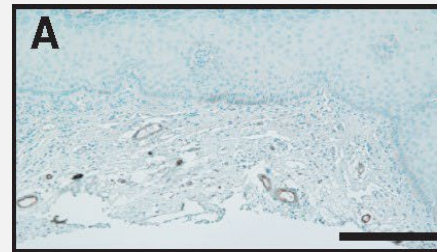
Zhang et al. [21]	COX-2, c-Met, β -catenin, CA9, PDPN, Ki-67, p16, p53, IMP3, c-Jun	Stem Cell Self-Renewal	Oral Leukoplakia (T/N)	11.3 (median)	3.04–29.00 (HR)
Zhang et al. (2017) [22]	Axin2, Snail	Stem Cell Self-Renewal	Oral Leukoplakia (T/N)	10.8 (median)	4.41, 7.47 (HR)
Sakata et al. [24]	SMAD4	Stem Cell Self-Renewal	Oral Leukoplakia (T/N)	Unknown	2.63 (HR)
Ding et al. [25]	Notch1	Stem Cell Self-Renewal	Oral Leukoplakia (T/N)	6.18 (median)	3.4 (HR)
Crawford et al. [36]	Nucleostemin	Stem Cell Self-Renewal	Oral Dysplasia (P/NP)	2–3 (NP), 7–14 (P)	$p = 0.02$ – 0.05
de Vicente et al. [38]	SOX2	Stem Cell Self-Renewal	Oral Leukoplakia (T/N)	6.25 (median)	3.0–5.83 (HR)
Habiba et al. [39]	ALDH1, PDPN	Stem Cell Self-Renewal	LG & HG Oral Dysplasia (T/N)	2.08 (median)	2.91–3.64 (HR)
de Vicente et al. [40]	NANOG	Stem Cell Self-Renewal	LG & HG Oral Dysplasia (T/N)	5.08 (median)	2.01 (HR)
Cruz et al. (1998) [42]	p53	Tumor Suppressor	LG & HG Oral Dysplasia (T/N)	3 (median)	$p = 0.002$
Cruz et al. (2002) [43]	P53	Tumor Suppressor	PMOL (T/N)	5.0 (mean)	29–33% Sensitivity, 83–100% Specificity
Wu et al. [44]	p16	Tumor Suppressor	Oral Leukoplakia (T/N)	Unknown	3.54 (OR)
Baran et al. [47]	MAGE-A	Melanoma Associated Antigen	Oral & Laryngeal Leukoplakia (T/N)	5	96.5% Specificity, 58.2% Sensitivity
Ries et al. [46]	MAGE-A	Melanoma Associated Antigen	Oral Leukoplakia (T/N)	5	$p = 0.0001$
Wu et al. [48]	TGM3	Tumor Suppressor	Oral Leukoplakia (T/N)	4.75 (T), 7.92 (N) (median)	5.55 (HR)
Kaur et al. [49]	S100A7	Cell Cycle & Differentiation	Oral Leukoplakia (T/N)	3.04 (median)	2.36 (HR)
de Vicente et al. [50]	Cortactin, FAK	Tumor Progression & Metastasis	Oral dysplasia- leukoplakia, erythroplakia (T/N)	5 (minimum)	6.30 (HR)
Saintigny et al. [51]	MET	Cell Proliferation	Oral Leukoplakia (T/N)	6.08 (median)	3.84 (HR)
Weber et al. [52]	CD68, CD163	Macrophage Infiltration	Oral dysplasia- mild, moderate, severe (T/N)	5 (full)	55.6–72% Sensitivity, 72.7–73.5% Specificity

Potential markers of malignant transformation

- PDL1
- MET
- NOTCH1
- PDPN



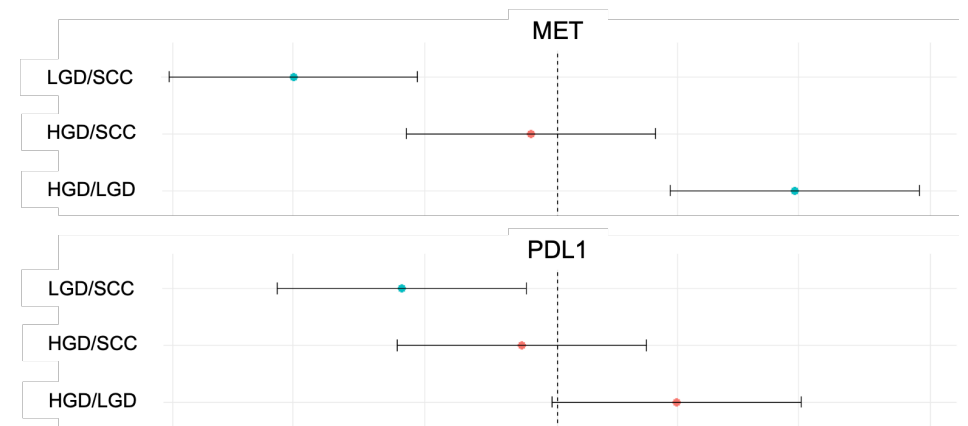
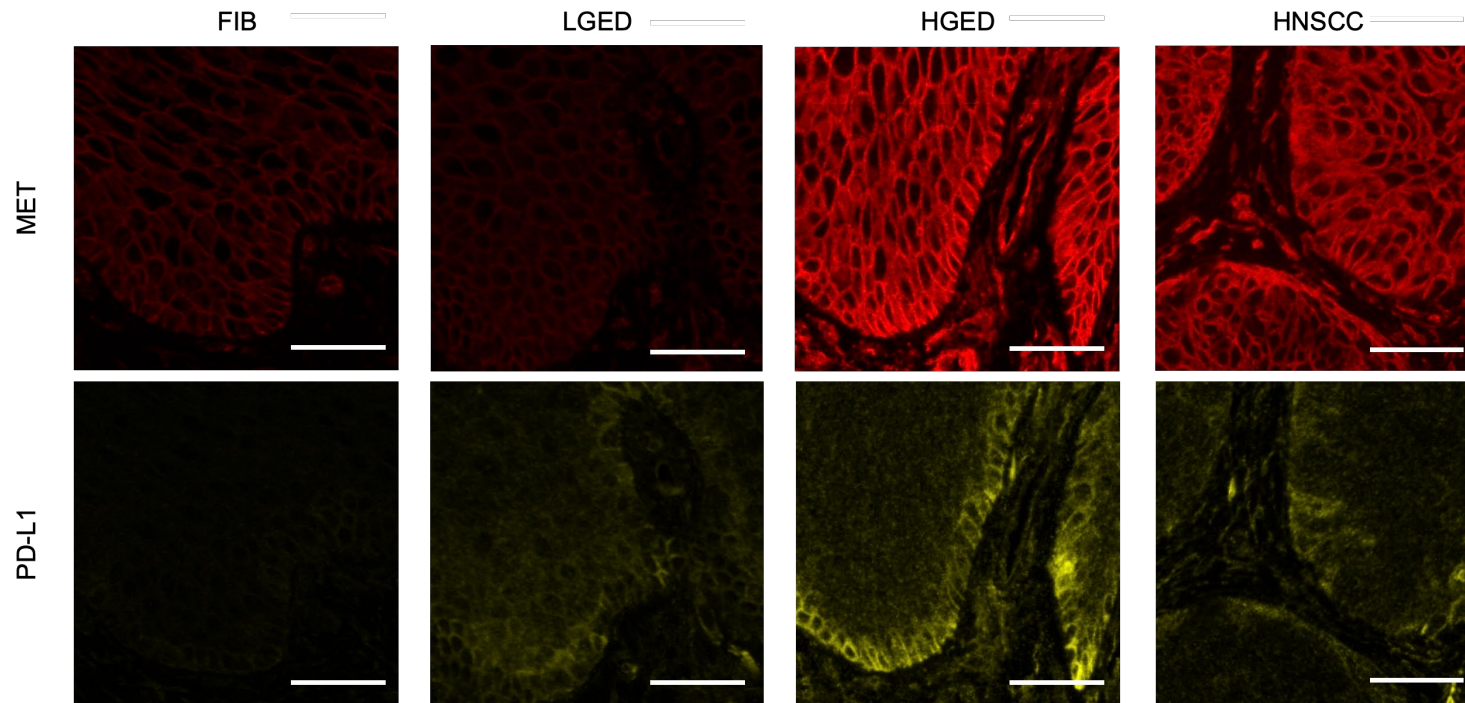
Chase Walton



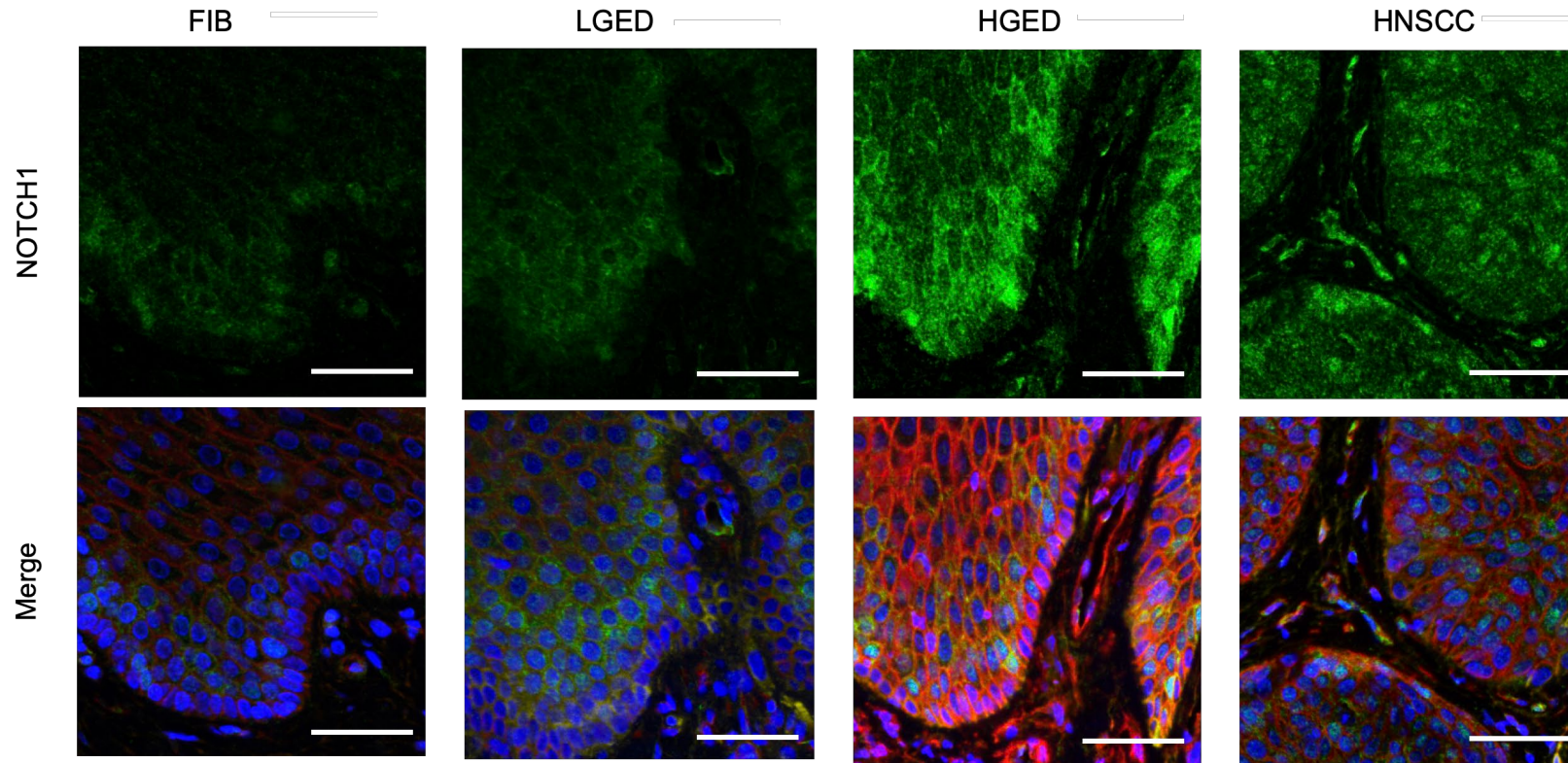
Met staining (No. of patients at risk)

0-1	60	55	43	29	20	17	5	0
2-3	60	39	32	20	12	9	0	0

Multiplex IF on human specimens



Multiplex IF on human specimens



Formulation Method

1

Dye: Protein Ratio (1:1 – 1:50)

- Atezolizumab (ATZ): IR800
- Brontictuzumab (BRO): Cy5
- Onartuzumab (ONT): FI

PBS
pH 8.5
2h R.T

2

Conjugation



FI-ONT



Cy5-Bro



IR800-ATZ

Centrifugation
2.5k rpm/3 min

3

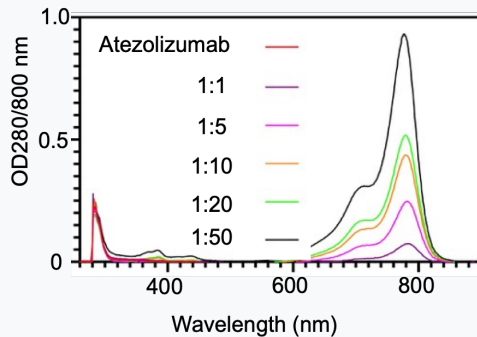
Purification



Zeba Desalting Column

4

Detection

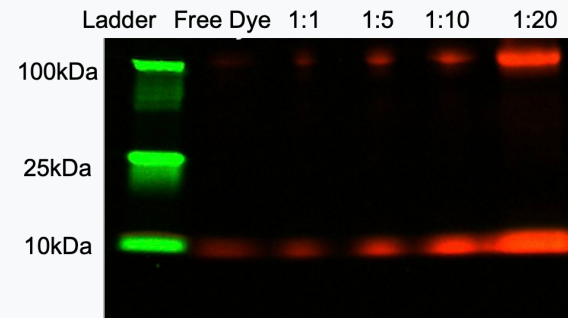


U.V Absorption:

- 280nm (mAb)
- 800 nm (IR800)
- 650 nm (Cy5)
- 550 nm (FI)

5

Confirmation(SDS-PAGE)



6

Dye-Antibody Stock

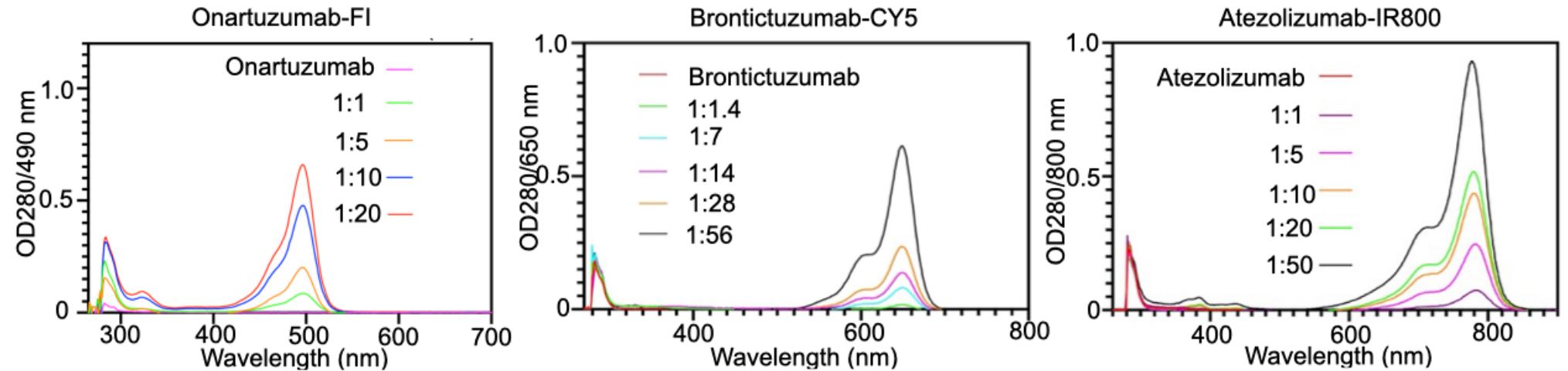


IR800-ATZ Cy5-Bro FI-ONT

Results

Dye: Antibody Ratio

U.V. Absorption

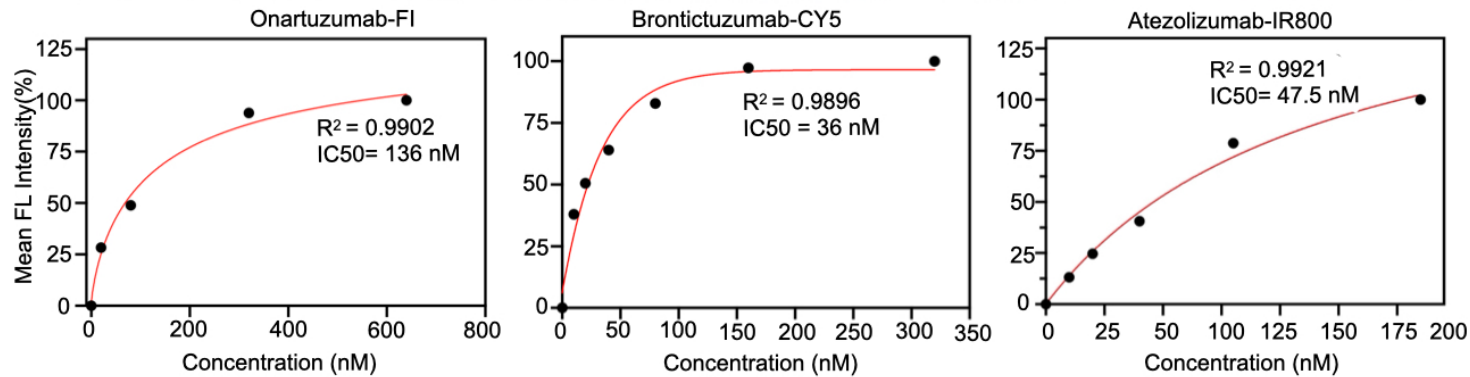
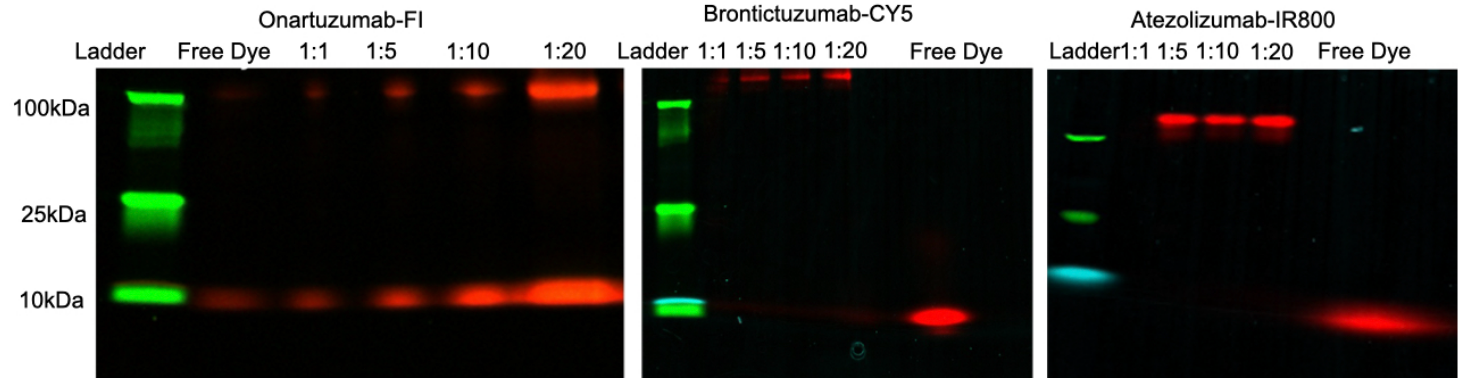


Antibody: Dye Ratio

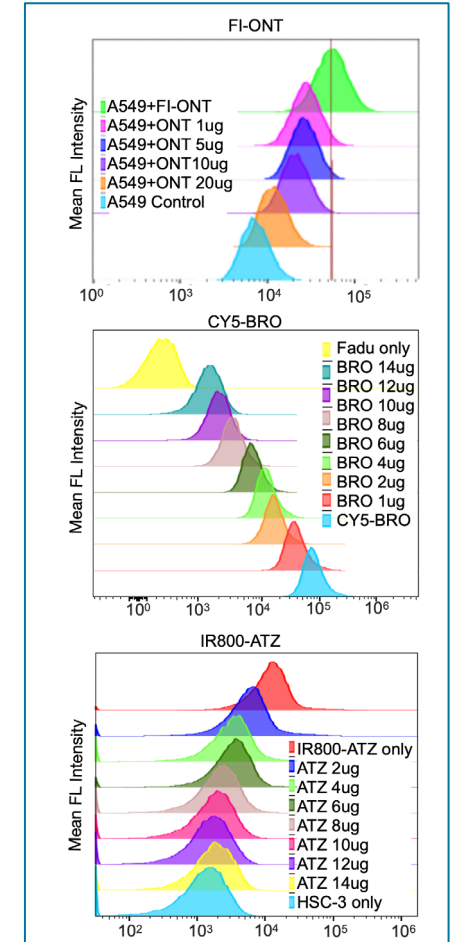
- Atezolizumab (ATZ): IR800 (1:20) = 1.71
- Brontictuzumab (BRO): Cy5 (1:28) = 2.78
- Onartuzumab (ONT): FI (1:10) = 3.4

SDS-PAGE/Binding Efficacy(IC50)

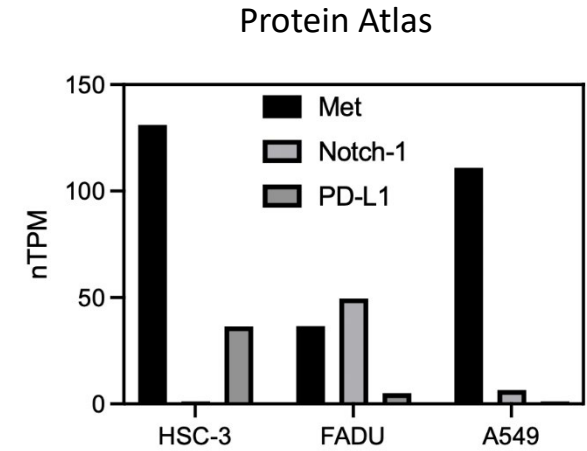
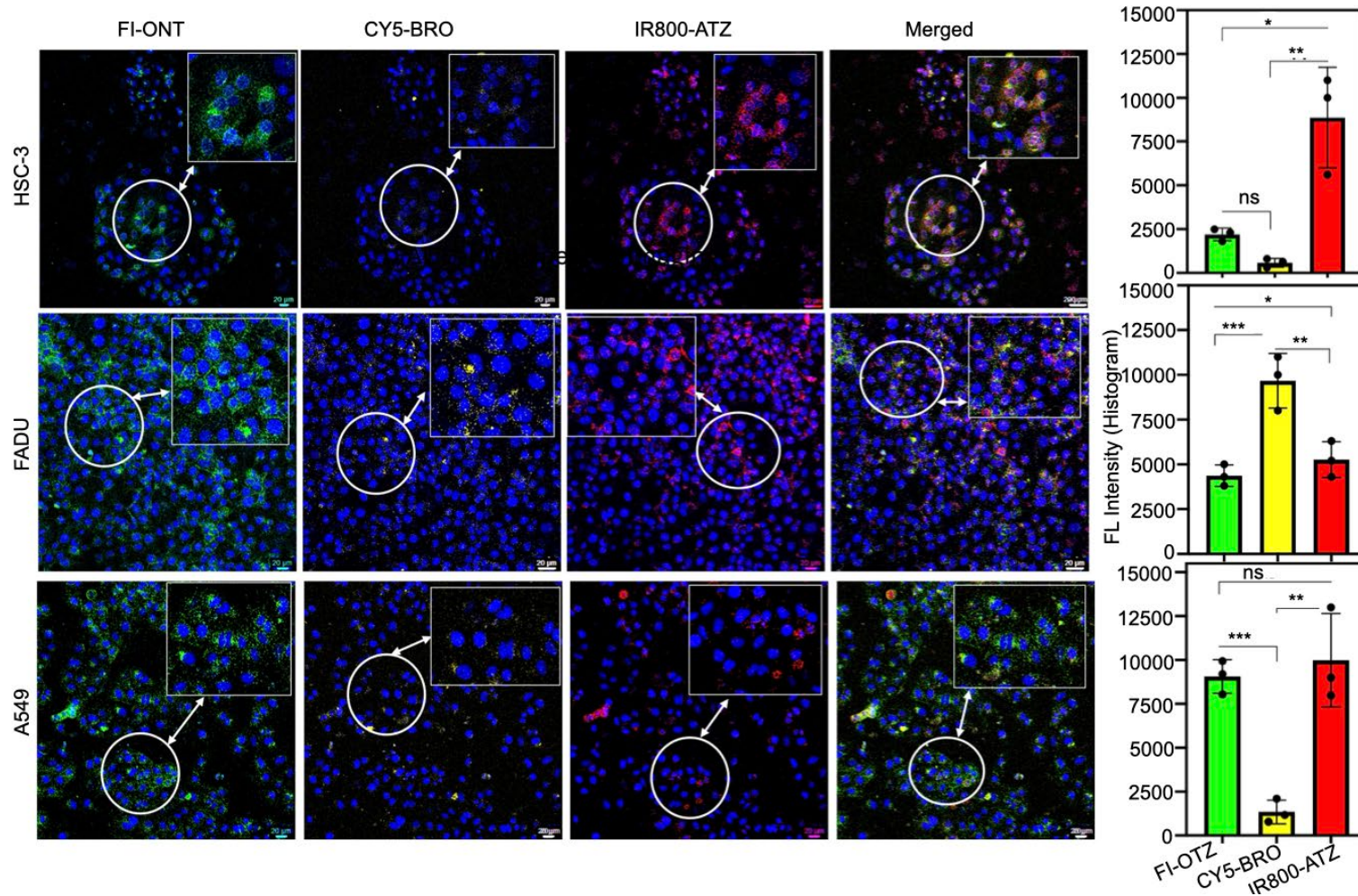
SDS-PAGE



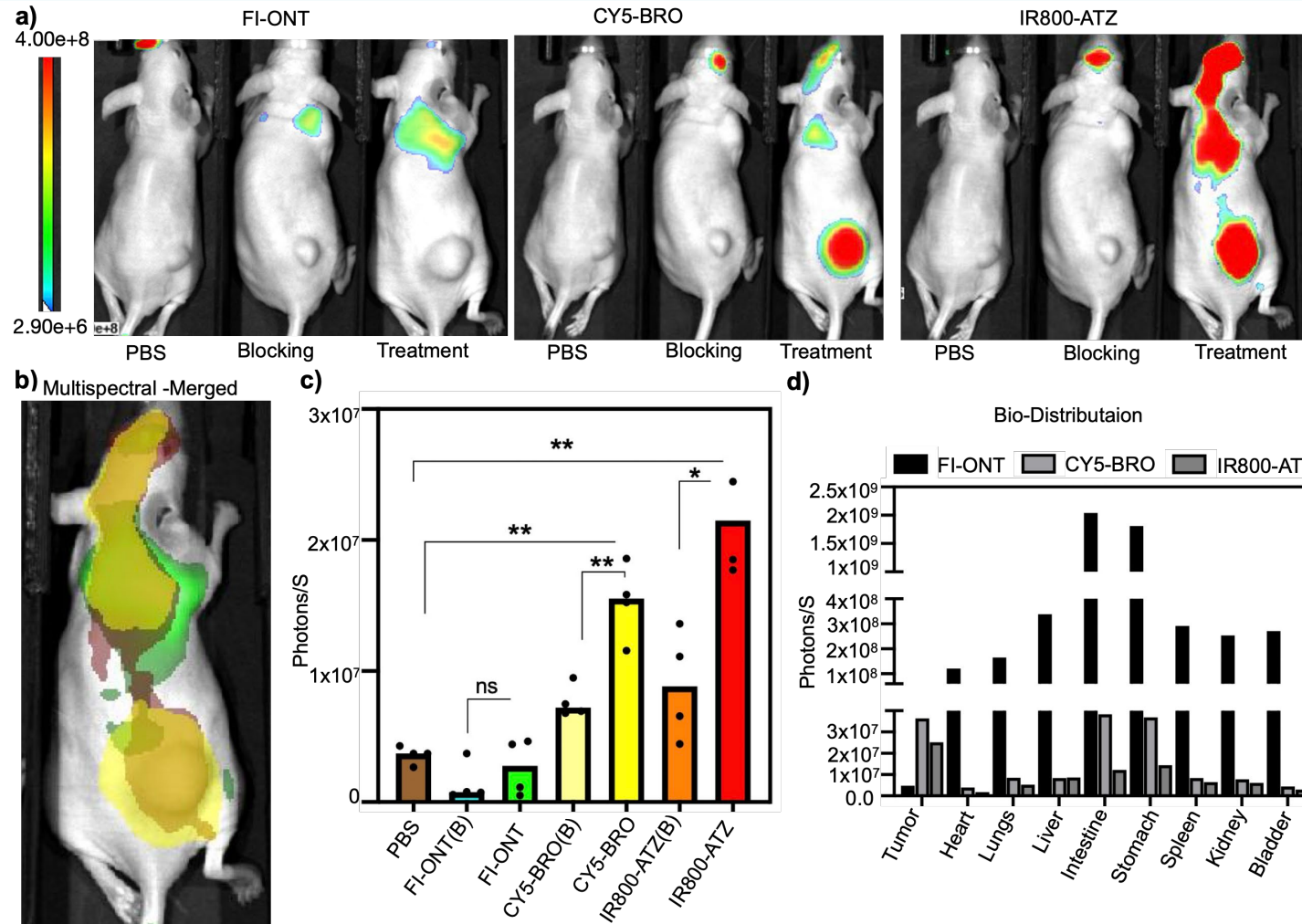
Blocking



Live Cells Multispectral Imaging



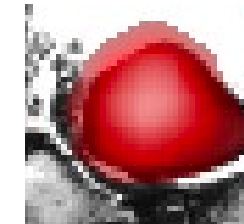
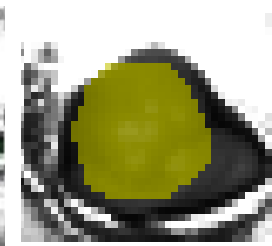
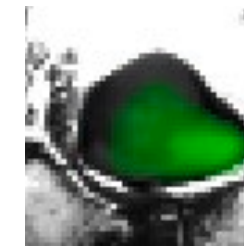
In vivo Multispectral Imaging



Ex Vivo-Tumor

FI-ONT

CY5-BRO



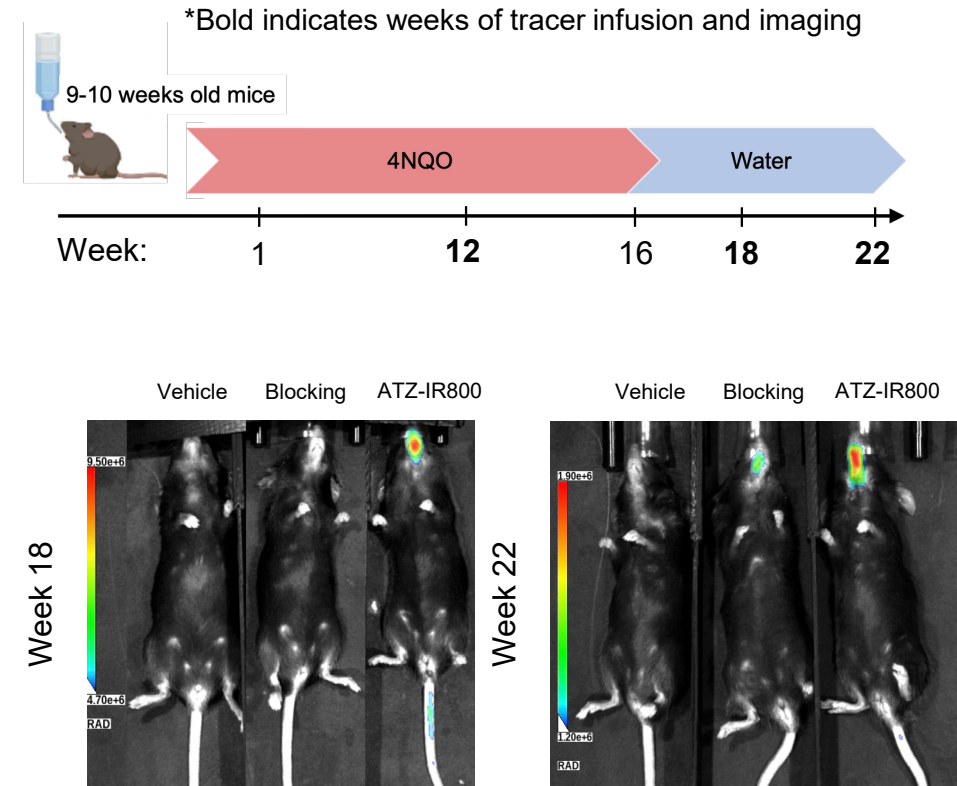
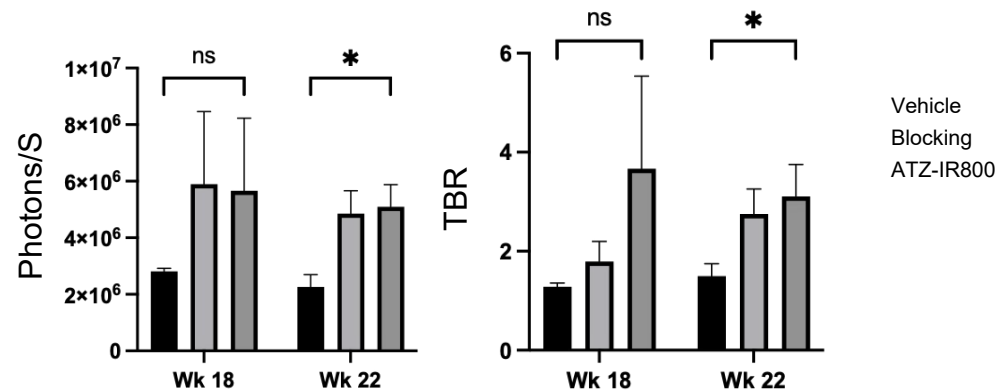
IR800-ATZ



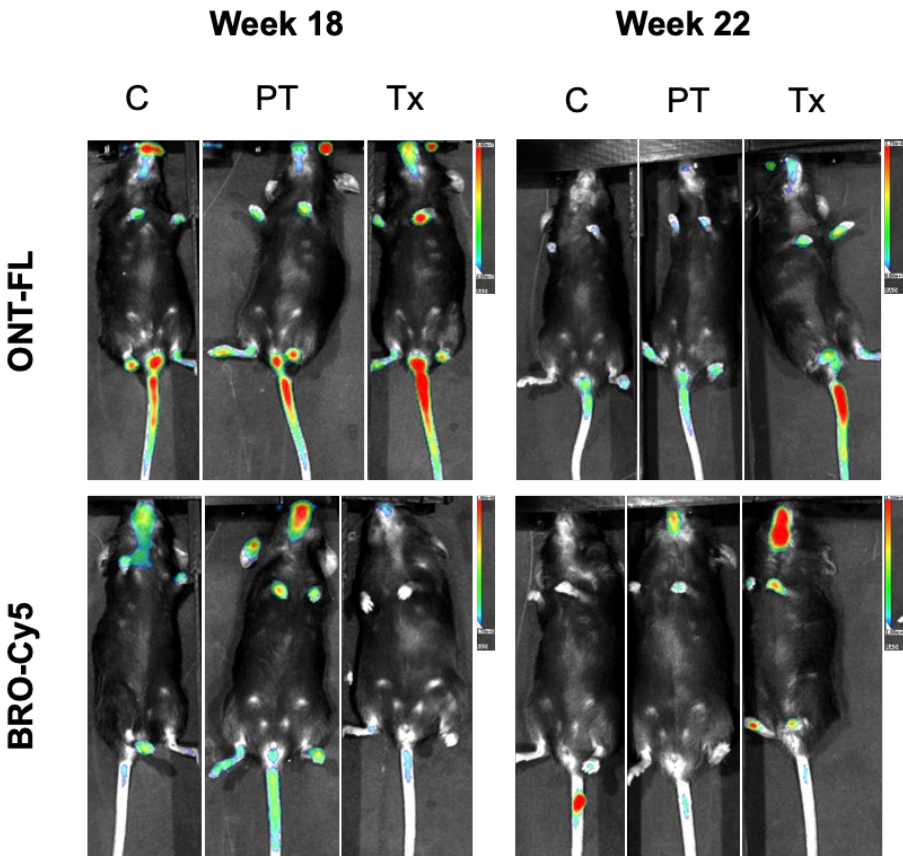
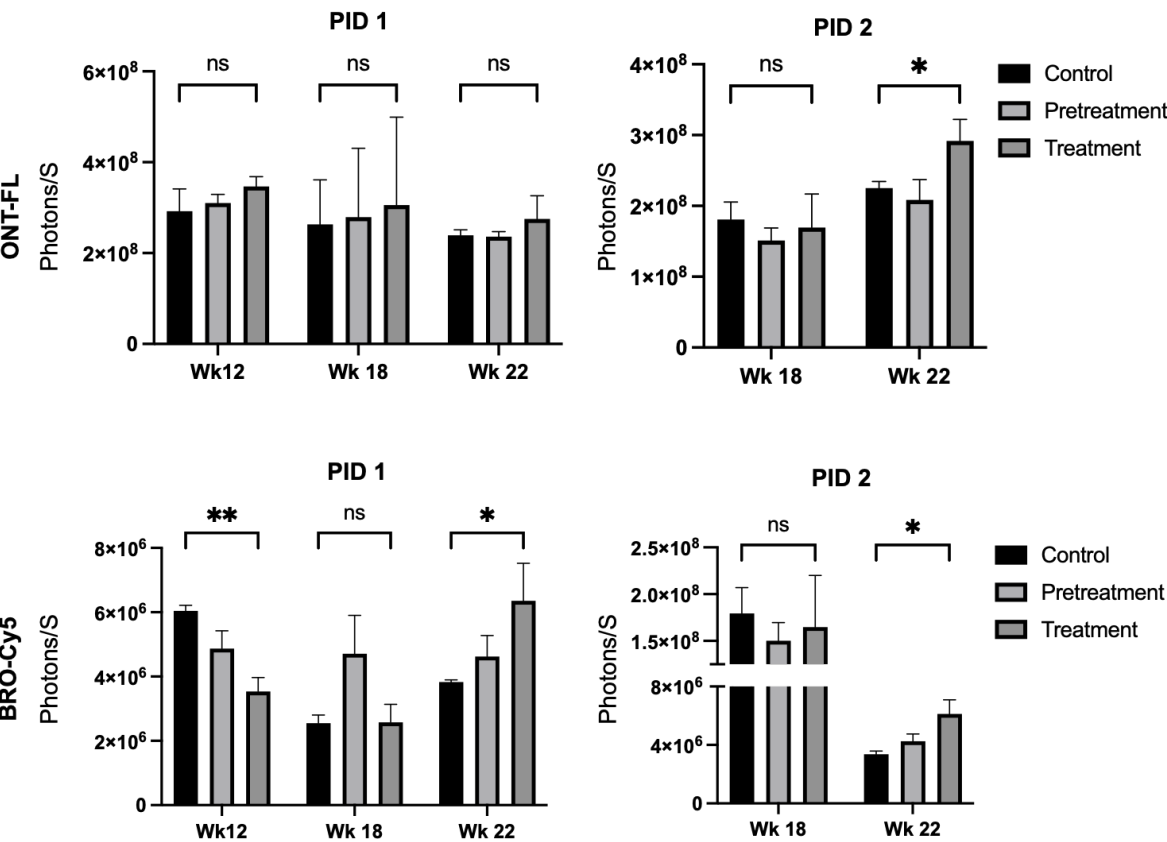
MSI

In vivo MSI in Oral Epithelial Malignancy

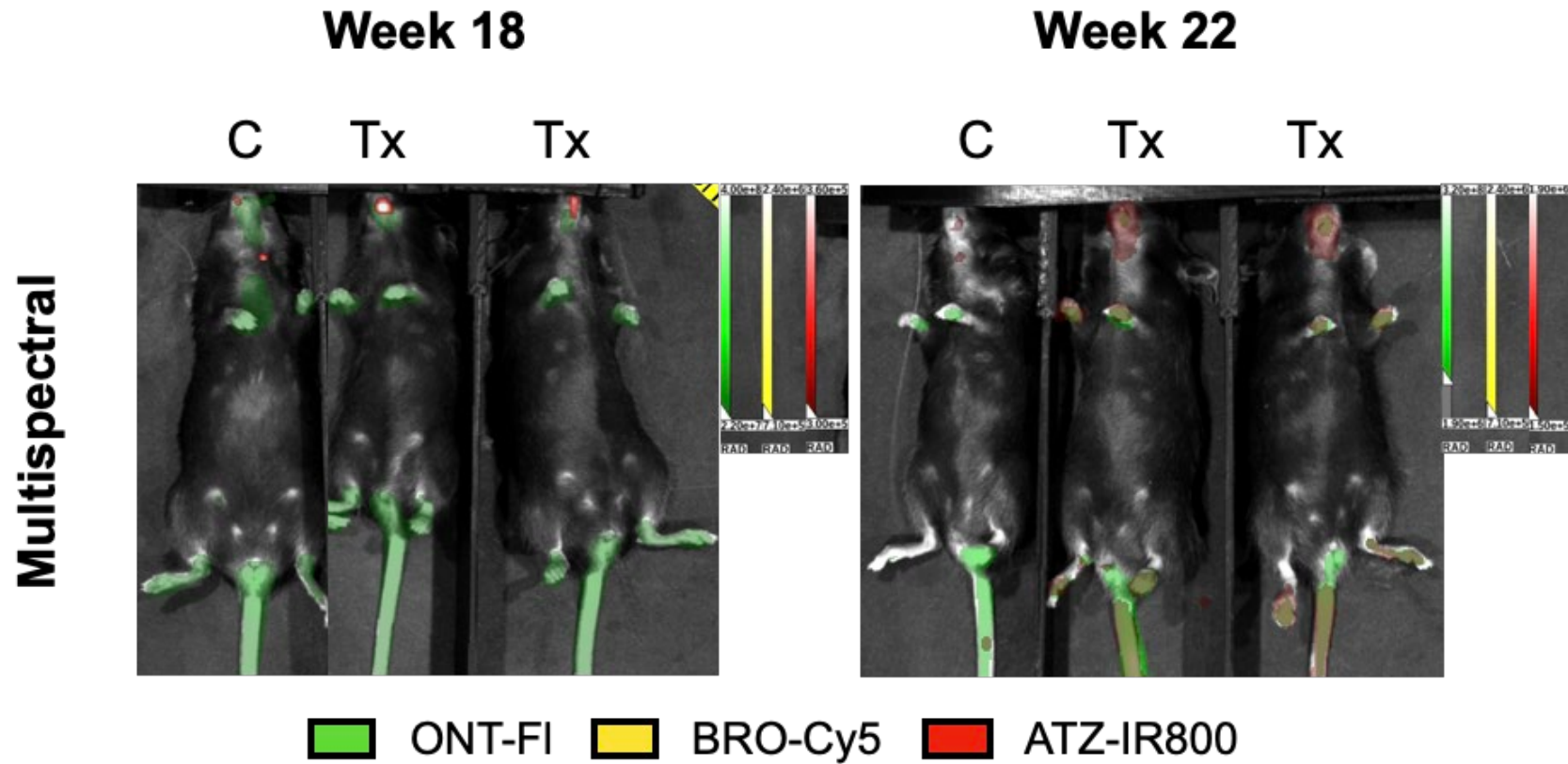
- 4NQO exposure models oral carcinogen exposure akin to habitual cigarette use
- Allows dysplasia progression modeling
 - Week 18: Low to high grade dysplasia
 - Week 22: High grade dysplasia to oral squamous cell carcinoma



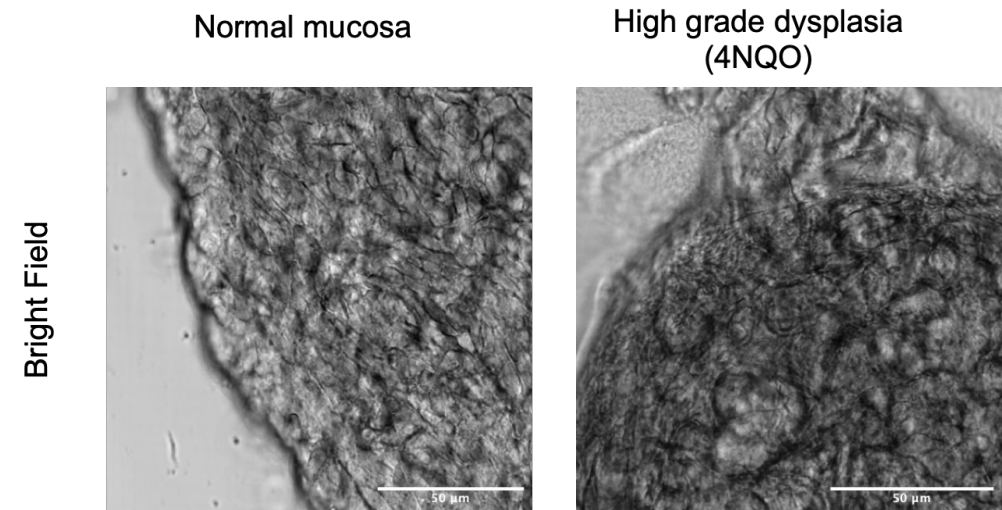
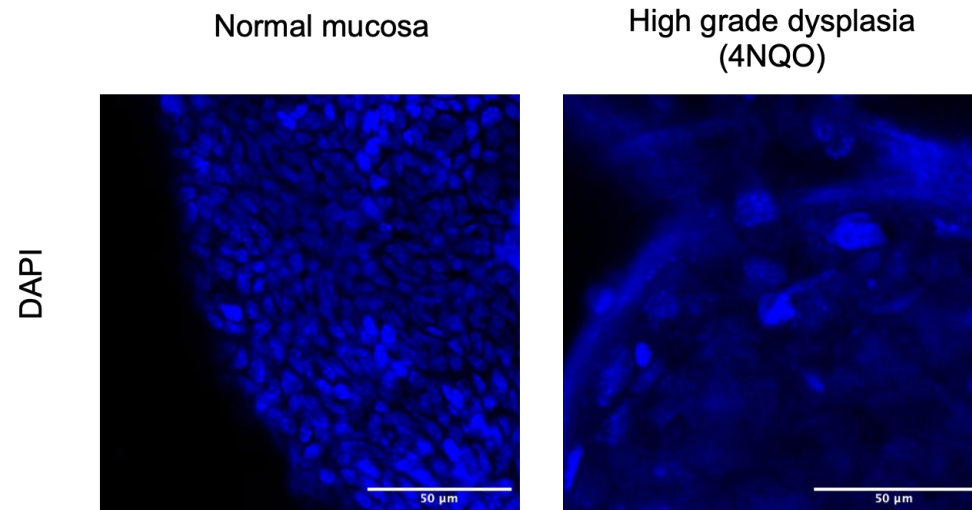
In vivo MSI in Oral Epithelial Malignancy



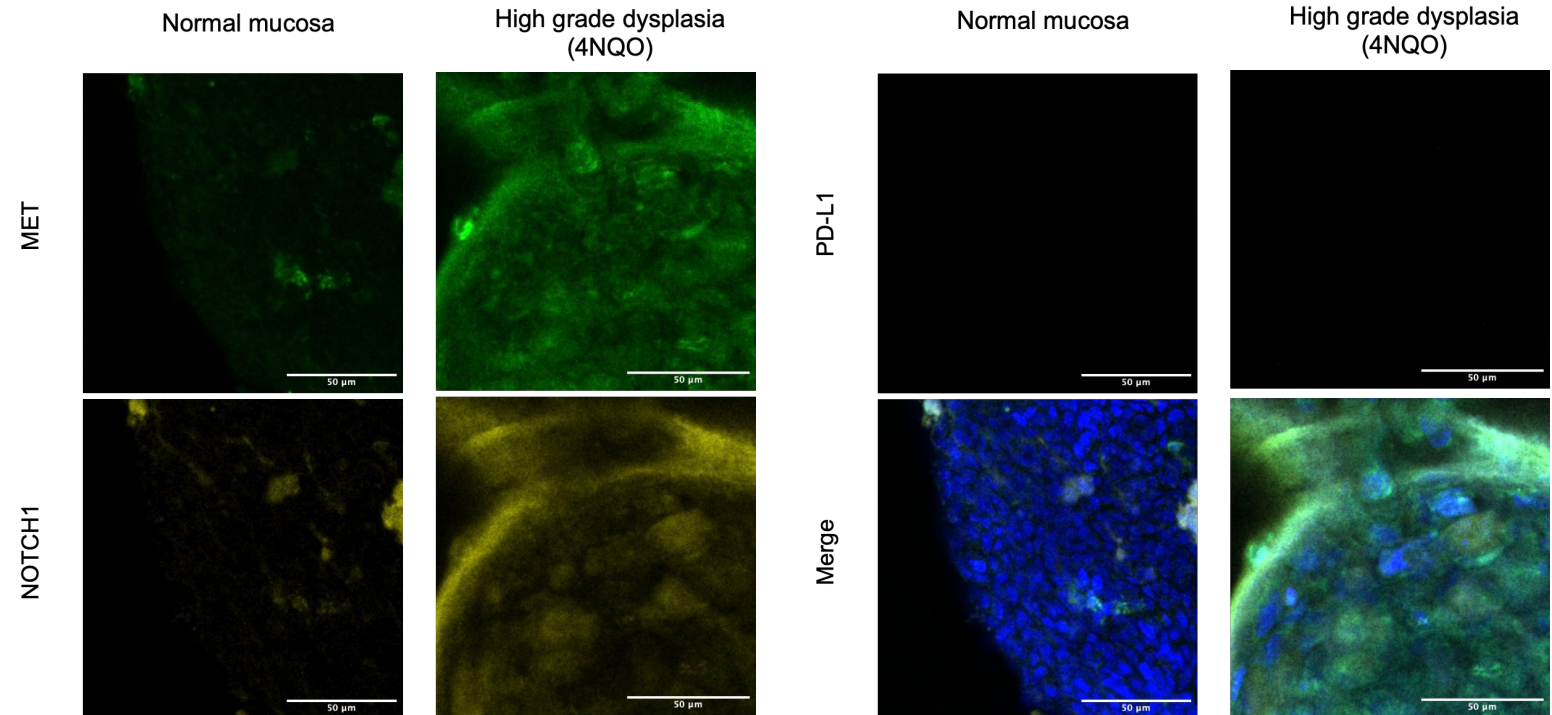
In vivo MSI in Oral Epithelial Malignancy



Mucosal lesion organoids



Mucosal lesion organoids - MSI

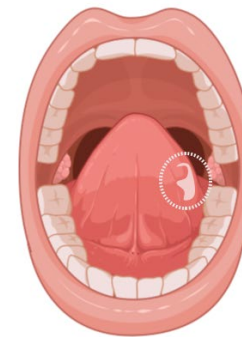


Conclusion

- We have successfully formulated and quantified the FI-OTZ, Cy5-BRO, and IR800-ATZ through the U.V absorption and SDS-PAGE
- These conjugates successfully target MET, NOTCH-1, and PD-L1, respectively—all of which are highly expressed in head and neck mucosal cancer cells.
- In vitro MSI imaging and blocking studies (flow cytometry) showed specificity towards respective antigens.
- In vivo MSI showed precise tumor uptake and specificity of FI-OTZ, Cy5-BRO, and IR800-ATZ

FUTURE DIRECTIONS

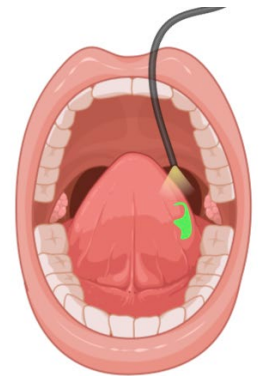
- Tracers has shown promise in early studies, but further exploration of clinical relevance is warranted
- Future study will address the following questions:
 - Sensitivity and specificity of the tracers for low grade, high grade, and malignant lesions
 - Optimal administration route, dose, and timing
 - Safety profile per route of administration



Lesion of Interest
Identified

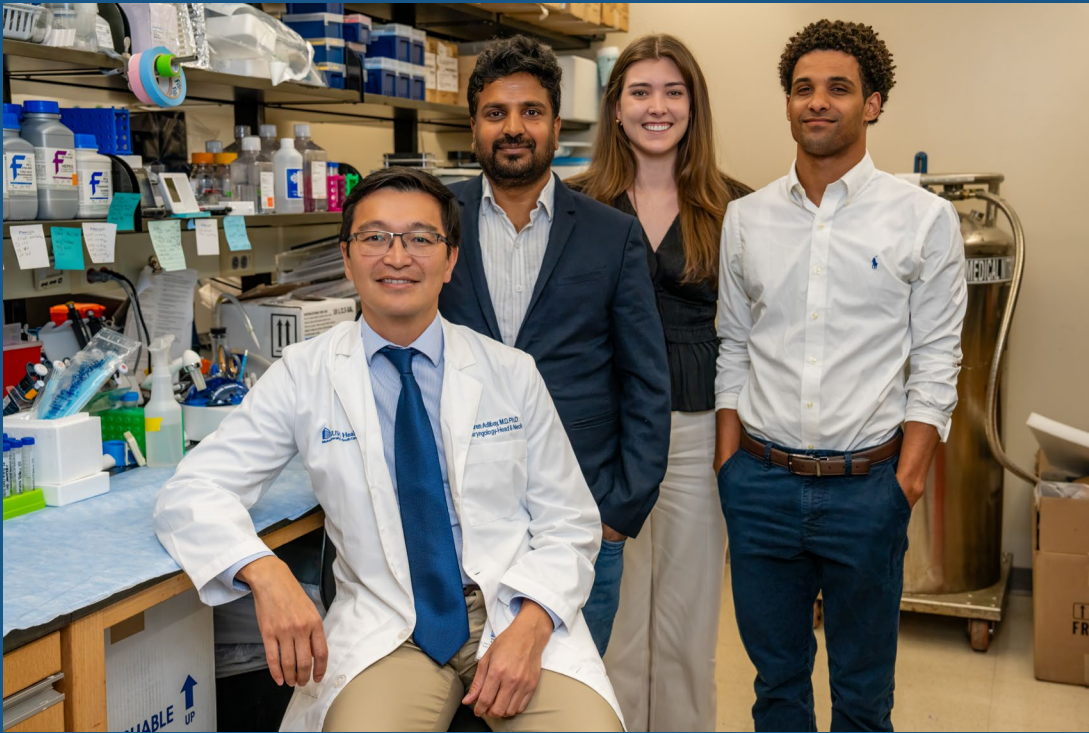


Gargle with ATZ-IR800
Solution



Fluorescence-
Enabled Risk
Stratification

Acknowledgment



Hollings Cancer Center

An NCI-Designated Cancer Center

Dr. Adilbay's Lab Members

Dr. Dauren Adilbay, MD, Ph.D. (PI)
Bhaskar Gurram
Elyse Moore
Stepan Pulukchu



Collaborators

Dr. Evan Graboyes, MD, MPH
Dr. Angela Yoon, MD
Dr. Yuri K. Peterson, PhD.
Dr. Subramanya Pandruvada, PhD



Core Facility (MUSC)

Cell & Molecular Imaging Shared
Resource
Drug Discovery Core (DDC)
Flow Cytometry & Cell Sorting
Division of Lab Animal Resources
Biorepository & Tissue Analysis

