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High resolution FTIR Imaging of Oral Tongue Squamous Cell Carcinoma: new insights on tumor staging

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WHAT IS KNOWN…

Oral Tongue Squamous Cell Carcinoma (OTSCC)

- 40% of all oral cancer cases;
- originating from the stratified squamous epithelium of oral mucosa, with connective tissue invasion;
- pathological staging (from Stage I to Stage IV) mainly based on morphological features (primary tumor size; invasion of adjacent tissues; metastasis both in regional lymph nodes and other organs).
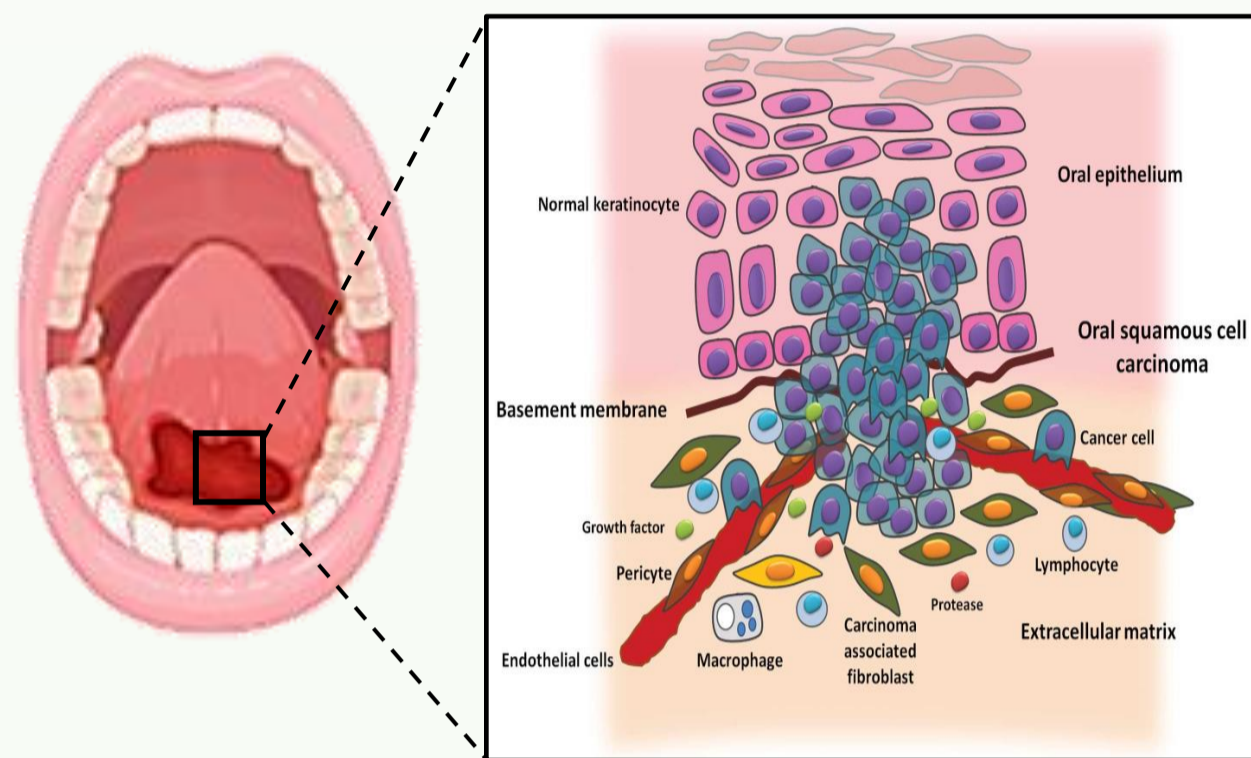
Fourier Transform InfraRed Spectroscopy

- Reliable, fast and non-destructive analytical technique;
- coupling morphological and chemical analyses of specific tissue areas;
- no specific sample processing.

THE PROBLEM!

The presence of subgroups of patients with diagnosis of OTSCC classified within the same tumor stage, not properly responding to therapeutic protocols and showing a poor outcome.

MATERIALS & METHODS

Samples collection. N. 29 FFPE biopsy samples, including N. 26 with histological diagnosis of OTSCC (from Stage I to Stage IV), and N. 3 with no OTSCC (taken as control samples, H). Adjacent sections (4-μm thickness) for histological (H&E) and FTIRI analyses.

Histological analysis. Haematoxylin & Eosin (H&E) sections observed by an Olympus BM50 Optical Microscope.

FTIRI analysis. Bruker INVENIO-R interferometer equipped with a Hyperion 3000 Vis-IR microscope and a Focal Plane Array detector. IR maps (164×164 μm² size, 4096 spectra, 2.56×2.56 μm² spatial resolution) acquired in transmission mode in the 4000–900 cm^{-1} spectral range (256 scans; 4 cm^{-1} spectral resolution) (OPUS 7.5 software package, Bruker Optics, Ettlingen, Germany).

Statistical analysis. Principal Component Analysis (PCA) (OriginPro 2023 software, OriginLab Corporation, Northampton, MA, USA). One-way analysis of variance (ANOVA) and Tukey's multiple comparison test (software Prism6, Graphpad software, Inc., San Diego, CA, USA).
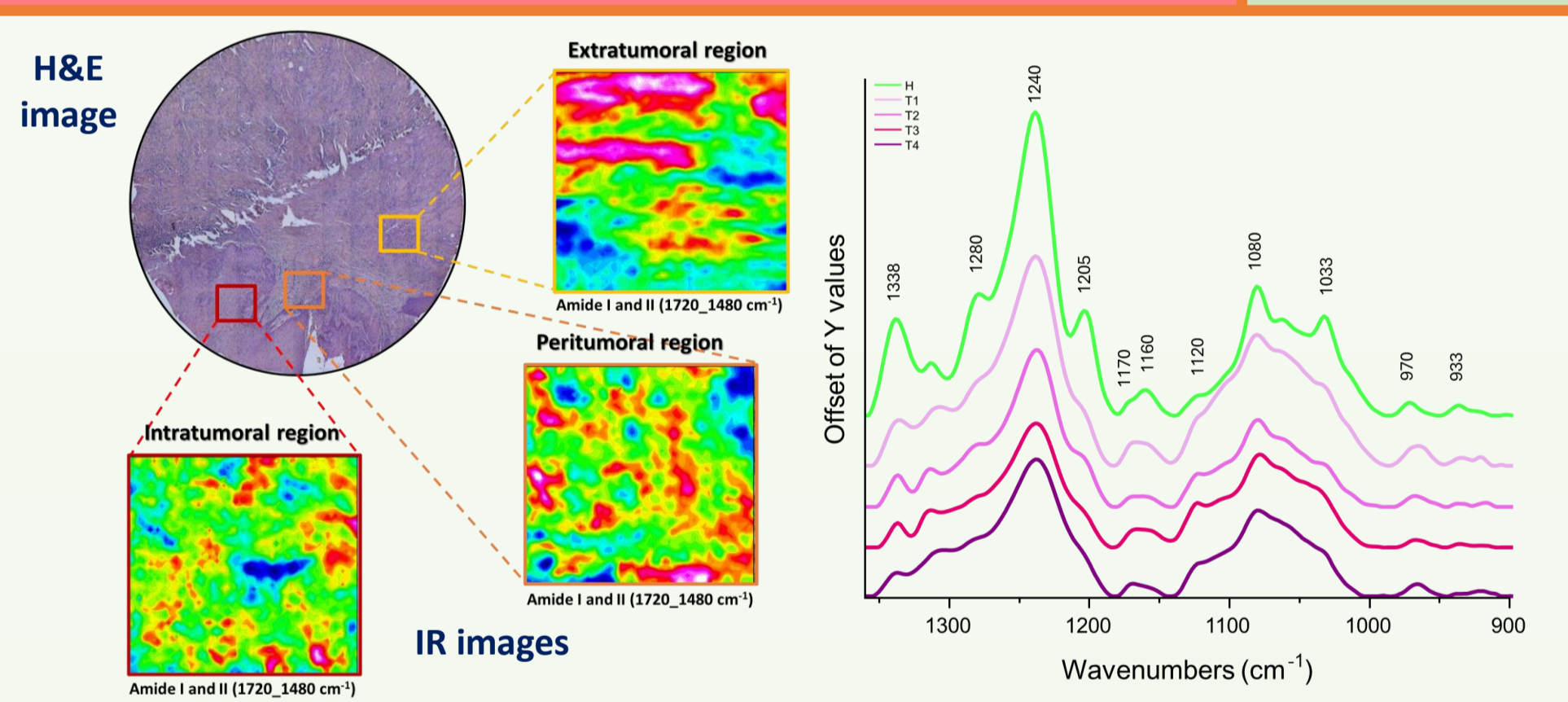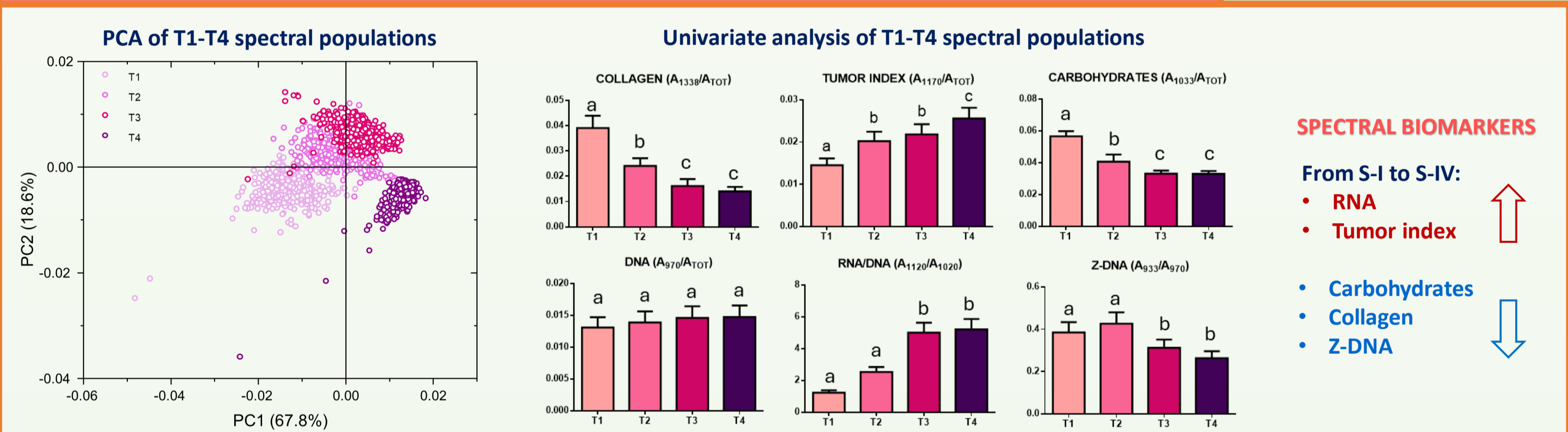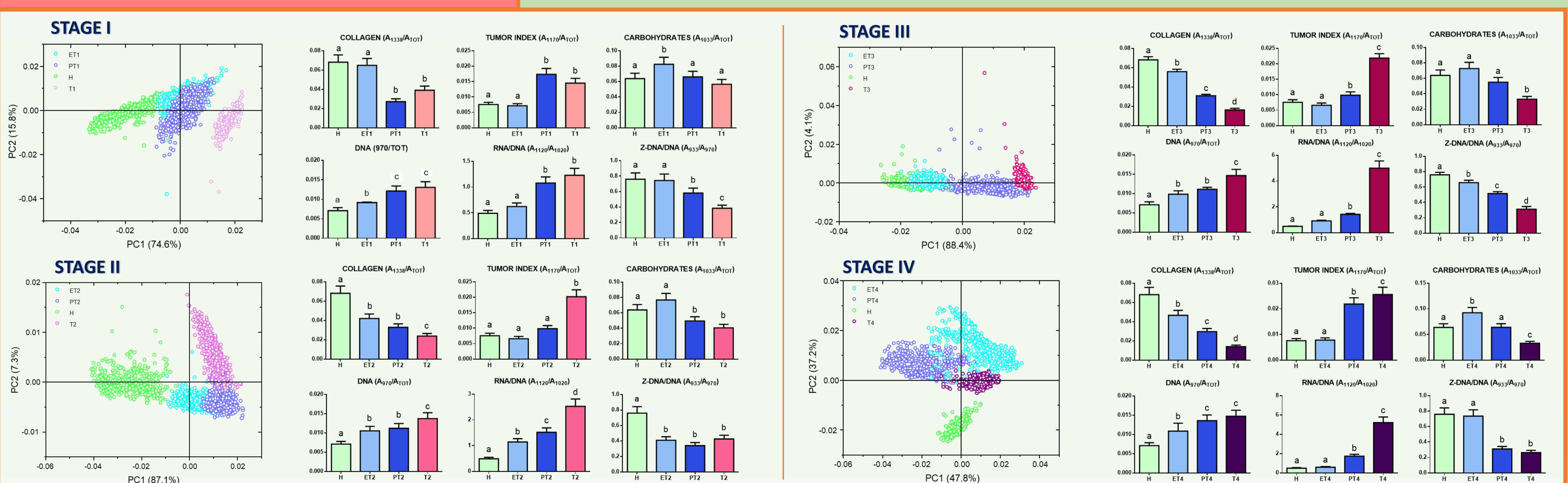
AIMS

(1) Improving the **prognostic and predictive potential of tumor staging characterization** through the identification of new reliable **spectral markers** related to the different pathological stages (from T1 to T4);

(2) Deepening knowledge on tumor growth mechanisms verifying the involvement of the **peritumoral** (up to ca. 100 μm from the tumor; from PT1 to PT4) and **extratumoral** (ca. 500-600 μm from the tumor; from ET1 to ET4) **regions** in relation with the tumor staging.

IMAGING AND SPECTRAL ANALYSIS

INTRATUMORAL REGION: identification of spectral markers

SPECTRAL BIOMARKERS

From S-I to S-IV:
- RNA ↑
- Tumor index ↑
- Carbohydrates ↓
- Collagen ↓
- Z-DNA ↓

EXTRA- AND PERITUMORAL REGIONS

STAGE I

STAGE II

STAGE III

STAGE IV

CONCLUSIONS

FTIRI coupled with multivariate and univariate analyses highlighted what follows:
(1) a different macromolecular composition of the tumor mass in relation with the stage, providing reliable spectral markers which could improve the morphological staging characterization;
(2) similar spectral features between the tumor mass and the region very close to it (named peritumoral), mainly in advanced stages (III and IV) respect to early ones (I and II), letting hypothesize the presence of a major tumor involvement in this region.

REFERENCES

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