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A PRECISION ONCOLOGY EXPERIMENTAL PLATFORM OF SQUAMOUS CELL CARCINOMAS OF THE HEAD & NECK REGION

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OBJECTIVES

Squamous Cell Carcinoma (SCC) accounts for about 90% of head and neck cancers. Especially in the oral cavity, in order to increase the efficacy of radiotherapy in terms of local control and survival, numerous studies have been carried out to verify the efficacy of the association between radiotherapy and other treatments [1-3]. It is also known that overexpression of EGFR in solid carcinomas is related to radio-resistance [4]. The anti-EGFR antibody cetuximab, in addition to inhibiting cell proliferation and inducing apoptosis [5], has the potential to modulate the radio-sensitivity of certain carcinomas [6]. In several preclinical studies conducted on SCC cell lines, the association of this antibody to radiotherapy confirmed the role of cetuximab as a radio-sensitizer [7]. Nevertheless, there are no biological markers that predict response and resistance to EGFR blockade.

The goal of our study was to discover molecular determinants of response to cetuximab for more informed stratification of patients with oral SCC. This was achieved by creating an experimental platform of patients derived xenografts (PDX) at FPO-IRCCS, building from previous experience in colorectal cancer [8,9].

METHODS

Between 2011 and 2022, 298 surgical specimens from patients surgically operated for a primary oral SCC were sampled to obtain viable tissue for ngraftment in mice. Tumor material was collected in IGL1, cut into 25/30 mm^3 pieces and implanted in 2 different male/female NOD-SCID mice. After mass formation, tumors were expanded for 2 generations until production of a cohort of 12 mice. Randomizations for treatment started approximately at 300 mm^3 volume. For each cohort, half of the animals were treated with vehicle and half were dosed with cetuximab (20 mg/kg twice weekly) for 6 weeks. Cut-off values for defined categories of therapies response were as follows: Regression (PR, below the lower line, <35% tumor volume change against pre-treatment volumes) and Progressive Disease (PD, above the upper line, >35%) (Fig1). Keratinocyte differentiation was evaluated by semi-quantitative IHC analysis of cytokeratins 1 and 10 (CK1, CK10) on a cohort of 14 PD and 14 PR PDX samples. For transcriptomic analysis of the PDX vs. pre-implantation human samples, gene expression levels were measured with RNA-sequencing (murine reads were filtered out from xenograft samples using XenoFilter, alignment was performed with STAR v2.7.3 and gene level counts were defined with FeatureCounts, normalized expression levels were obtained with DESeq). Whole exome sequencing (WES) was performed to obtain data on the mutational landscape of tumors.

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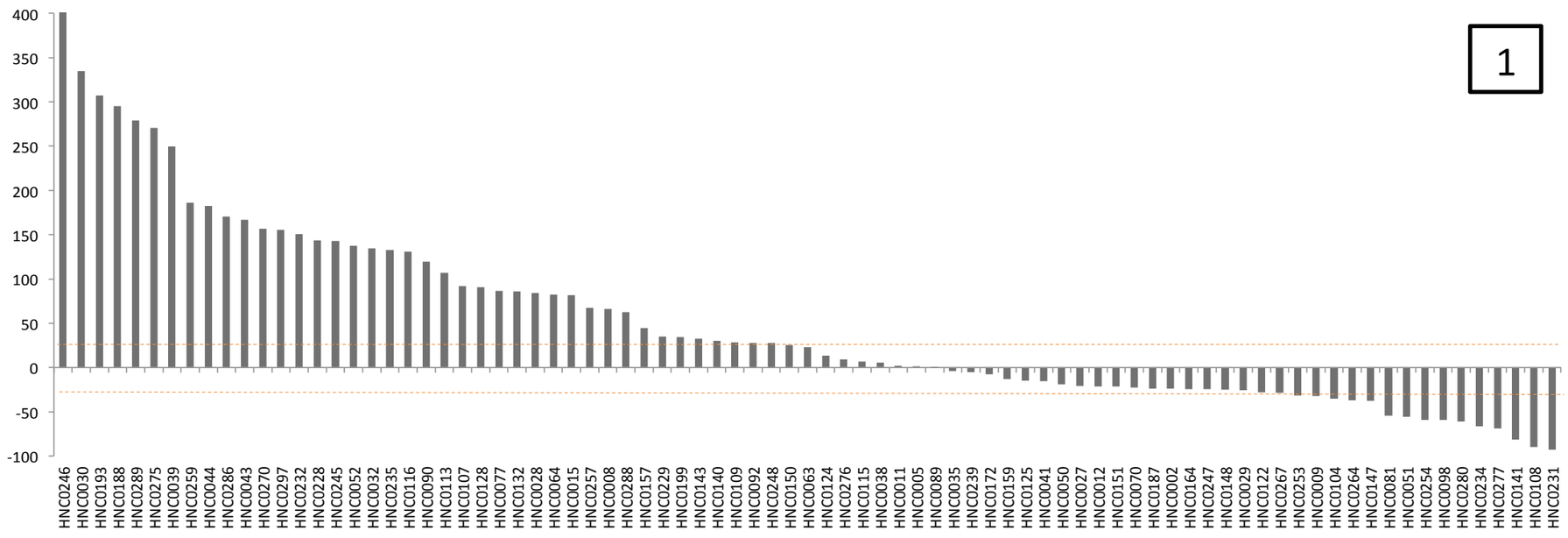

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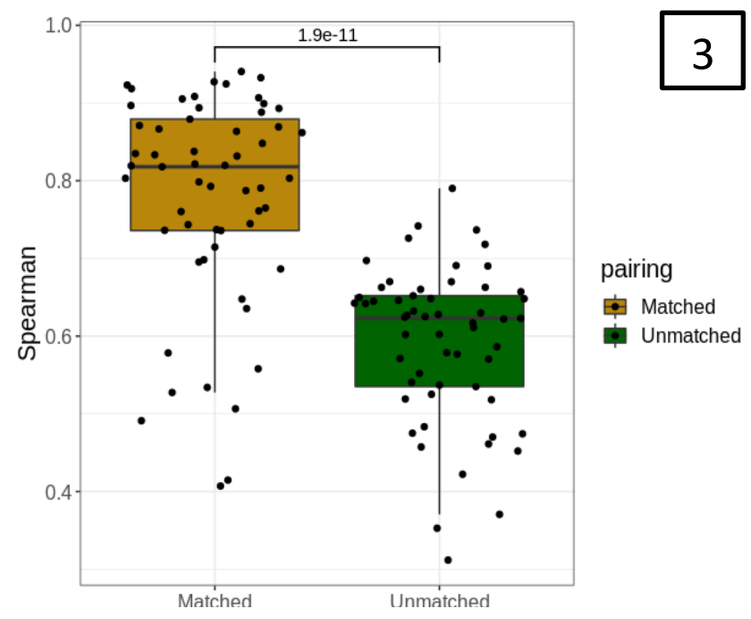

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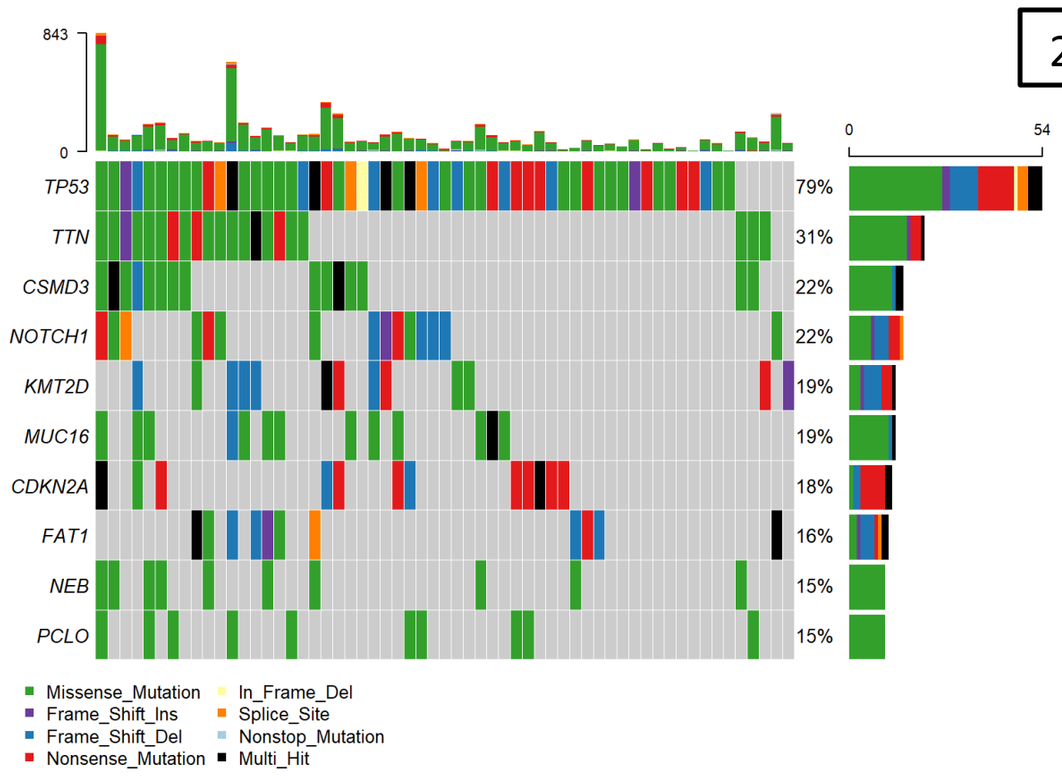

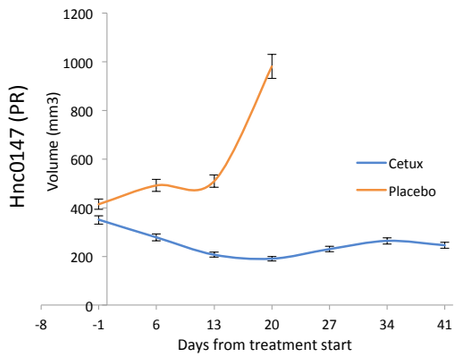

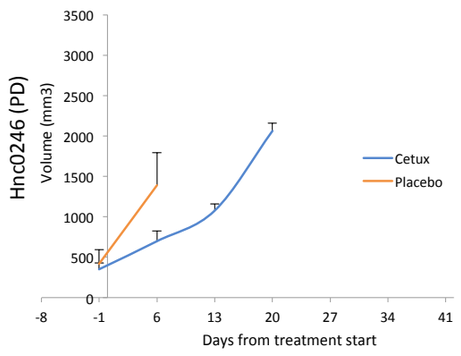

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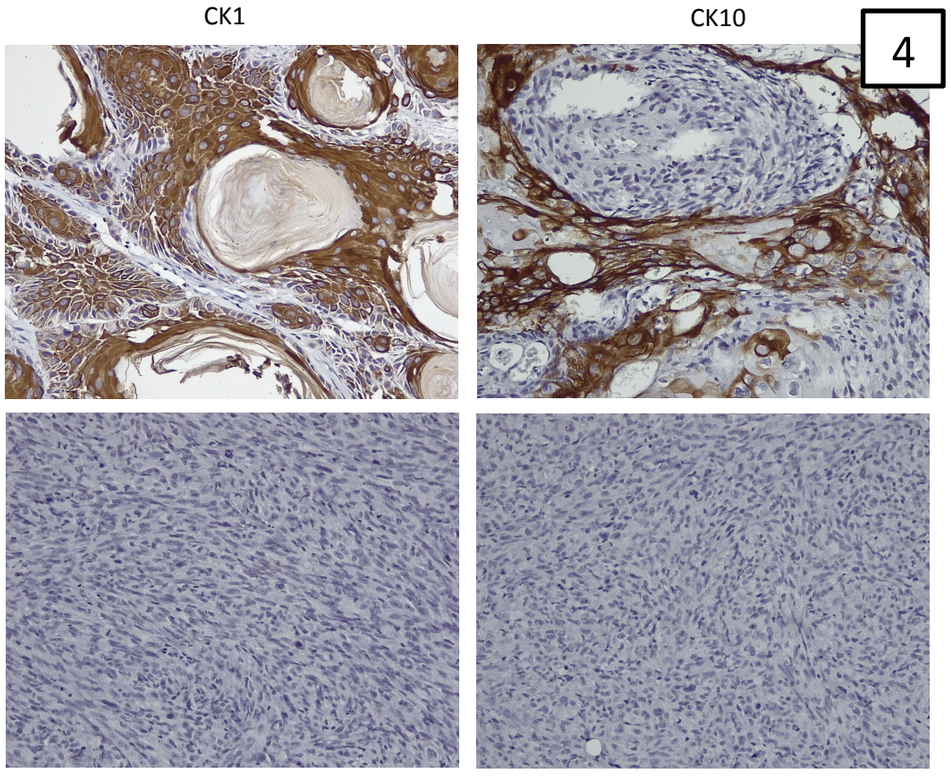

RESULTS

We observed a rate of xenograft engraftment of 47% (140 out of 298). A subgroup of 83 xenografts were treated with cetuximab, obtaining 14/83 (17%) PDXs with PR and 39/83 (47%) PDX with PD (Fig1). We confirm that mutations found in our PDXs are similar in count and frequency to those in public patient datasets (Fig2- WES data analysis). By analyzing expression levels of matched or unmatched pairs of human-xenograft samples, we found that the main transcriptional features defining each individual pre-implantation SCC were maintained in the corresponding PDX model (Fig3-RNAseq of paired PDX and original tumor sample). Pathway analysis indicates that corneal differentiation traits correlate with cetuximab sensitivity: indeed, our PDXs sensitive models showed more intense immunostaining of CK1 and CK10 keratinocyte differentiation markers (Fig4-immunohistochemistry on PDX samples).

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CONCLUSION

Our result show feasibility of propagation and therapeutic annotation of patient-derived SCC samples in mice. In our cohort we observed that CK1 and CK10 are positive biomarkers of cetuximab response in SCC-PDX models. This observation may prove useful to stratify platinum-refractory oral SCC patient to cetuximab treatment.

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