#2508

Clinical significance of PARP7 (*TIPARP*) gene copy number alterations in human non-small cell lung cancer and head & neck carcinomas

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Background

PARP7, encoded by the *TIPARP* gene, is a monoART involved in cellular stress responses and with immunomodulatory functions in cancer. The PARP7 gene is amplified in a subset of squamous cell carcinomas and ongoing clinical studies are assessing its role as an anti-cancer therapeutic target (NCT04053673, NCT05127590 and jRCT2031210373). Here, we analyzed the frequency of *TIPARP* copy number alterations and its association with tumor immune microenvironment (TIME) features and outcomes in non-small cell lung cancer (NSCLC) and head & neck squamous-cell carcinoma (HNSCC) cohorts.

Methods

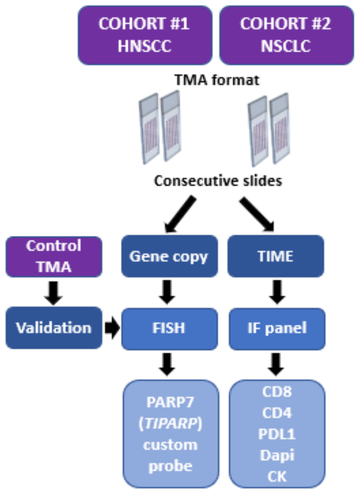

Fig.1 Experimental strategy
The *TIPARP* gene copy number was analyzed using fluorescence in situ hybridization (FISH) with a custom-made dual probe in two retrospective cohorts of HNSCC (Cohort #1, n=83) and NSCLC (Cohort #2, n=124) represented in tissue microarrays. The FISH assay was validated using control cell lines and tumors with known *TIPARP* copy number tested by orthogonal methods.
The TIME was assessed on consecutive tumor sections using a multiplexed quantitative immunofluorescence panel including the markers DAPI, cytokeratin for tumor cells, CD4, CD8 and PD-L1 coupled to computational pathology analysis.

Results

FISH assay validation

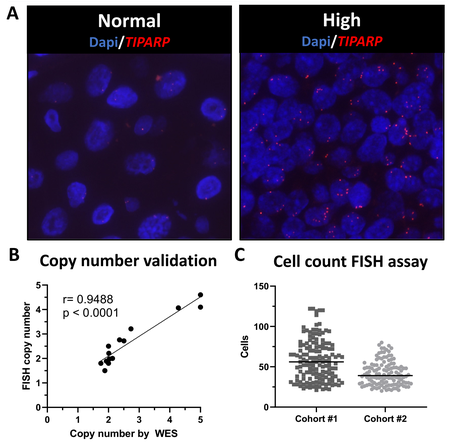

Figure 2. FISH assay validation
A) Representative images of FISH assay for *TIPARP*, image showing the cell nuclei with Dapi (blue) and TIPARP gene (red). The samples correspond to controls with low/normal *TIPARP* gene copy number (left) and high (right). **B)** Correlation between *TIPARP* copy number obtained from the FISH assay and using whole exome sequencing analysis (WES). **C)** Number of cells analyzed per each case across the tumor cohorts.

Cohort Information

Characteristics		Cohort #1 HNSCC Patient No. (%)	Cohort #2 NSCLC Patient No. (%)
Sex	Female Male	21 (25) 62 (75)	149 (60.3) 98 (39.7)
Age, years	<65 >65	55 (66.3) 28 (33.7)	90 (36.6) 156 (63.4)
Smoker	Yes No	63 (77.8) 18 (22.2)	212 (85.8) 35 (14.2)
Histology	Adenocarcinoma Squamous Other	-	169 (69.6) 64 (26.3) 10 (4.1)
HPV (p16)	Positive Negative	85 (88.5) 11 (11.5)	-
AJCC Clinical stage	I-II III-IV	9 (12) 66 (88)	231 (93.9) 15 (6.1)
Treatment		SOC (non IO)	Standard of care Non-immunotherapy

Table 1. Clinicopathologic characteristics of HNSCC and NSCLC cohorts.
For Cohort #2 (NSCLC) the final number of spots was reduced due to elimination of samples with low-quality tissue.

TIPARP/PARP7 gene copy number distribution

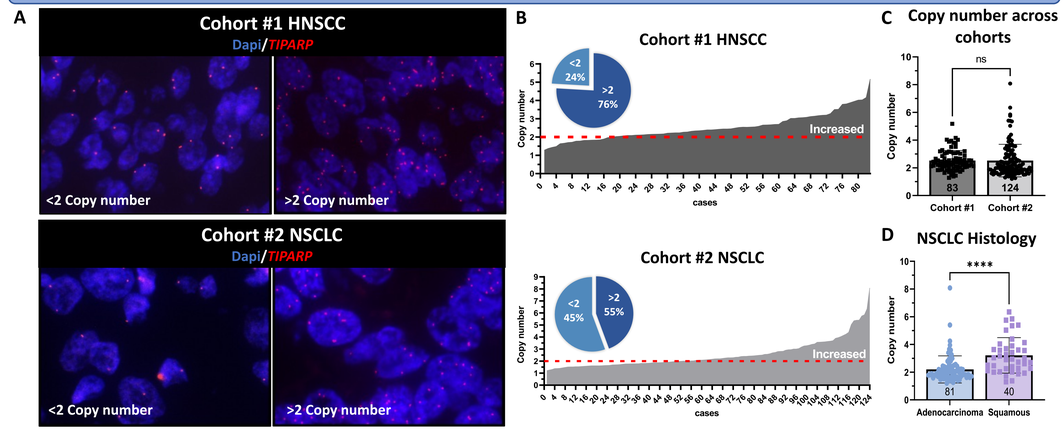

Figure 3. *TIPARP* gene copy number distribution.
A) Representative images of FISH assay for HNSCC (upper panel) and NSCLC (lower panel) with different *TIPARP* copy number. The *TIPARP* signal is shown in red and nuclei are counterstained with DAPI (blue). **B)** *TIPARP* gene copy number distribution in HNSCC samples (upper panel) and NSCLC (lower panel). Cases with increased *TIPARP* copy number (>2 copies) were seen in 76% of HNSCCs and 55% of NSCLCs (85% of them of squamous-cell histology). **C)** The mean number of *TIPARP* copies were comparable in HNSCC and NSCLC cohorts. **D)** A higher *TIPARP* copy number was identified in squamous-cell histology within the NSCLC cohort. No other significant associations between the *TIPARP* copy number and major clinicopathologic variables were found.

TIPARP/PARP7 gene and the TIME association

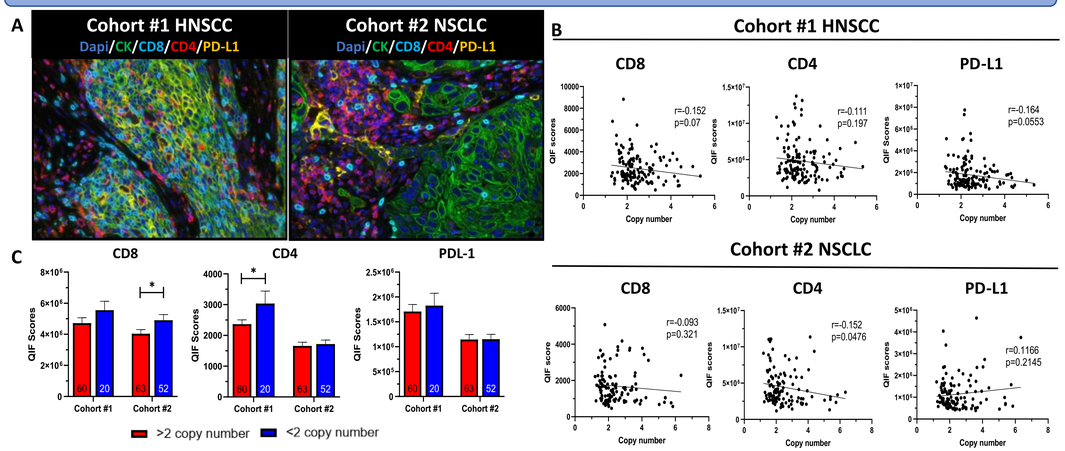

Figure 4. High *TIPARP* gene copy number is associated with lower T-cell infiltration in HNSCC and NSCLC
A) Representative multi-color immunofluorescence images showing Dapi (blue), cytokeratin (green), CD8 (cyan), CD4 (red) and PD-L1 protein (yellow) staining in HNSCC (left) and NSCLC (right). **B)** Association between *TIPARP* gene copy number and CD8, CD4 and PD-L1 protein expression for HNSCC (upper panel) and NSCLC (lower panel). **C)** CD8 (left), CD4 (middle) and PD-L1 (right) protein expression stratified by number of copies of *TIPARP* in HNSCC (cohort #1) and NSCLC (cohort #2).

TIPARP copy number and survival

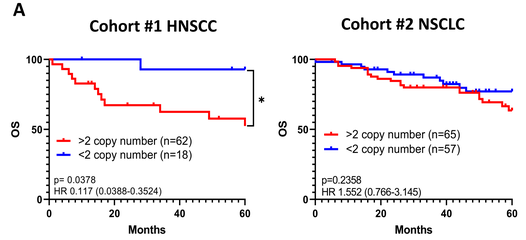

Figure 5. *TIPARP* copy number and 5-year overall survival.
A) Kaplan-Meier graphical analysis of the 5-year overall survival in HNSCC (left) and NSCLC (right) stratified by the number of TIPARP copies. A stratification threshold of 2 gene copies was established to determine cases with normal (blue) or high (red) levels.

Conclusions

- We established a FISH assay to quantitatively determine the *TIPARP* copy number in human fixed tumor specimens.
- 76% of primary HNSCCs and 55% of primary NSCLCs show more than 2 copies of the *TIPARP* gene.
- Using this exploratory cut-point, a higher copy number was observed in lung malignancies with squamous-cell histology.
- Elevated *TIPARP* (PARP7) gene copy number identifies a subset of lung and head & neck squamous-cell carcinomas with unfavorable TIME features. In addition, HNSCC showed an adverse prognosis in cases with elevated *TIPARP* copy number.
- Detection of *TIPARP* gene abnormalities using FISH in tumor biopsy samples is sensitive and has potential as a predictive biomarker for PARP7 targeting therapies.
- Ongoing studies are exploring additional biologically/clinically relevant copy number cut-points and determine the association between *TIPARP* copy number changes and PARP7 protein levels in tumors.

Acknowledgments