Abstract# 6742: PV-10 triggers immunogenic cell death and anti-tumor immunity in head and neck squamous cell carcinoma via endoplasmic reticulum stress and autophagy

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Abstract

Head and neck squamous cell carcinoma (HNSCC) are complex, diverse cancers affecting mucosal linings of the upper aerodigestive tract. PV-10 (10% rose bengal sodium) is an investigational immunotherapeutic agent that has promising anti-tumor activity in multiple solid tumor cancer types. However, the role of PV-10 in HNSCCs is unknown. Our *in vitro* findings reveal that PV-10 induces cytotoxicity in both mEER and MTE-RAS cells. Notably, PV-10 promotes an increase in reactive oxygen species (ROS), leading to a significant rise in late apoptotic cells. Our results suggest that PV-10 induces immunogenic cell death (ICD) in both mEER and MTE-RAS *in vitro* and *in vivo* including the release of Damage-associated molecular pattern molecules (DAMPs) such as HMGB1, ATP, calreticulin, HSP-70, and HSP-90 fostered by ROS-based endoplasmic reticulum (ER) stress and apoptosis. Intralesional (IL) PV-10 injection caused significant tumor regression and a complete response in some mice. These findings hold a promise for potential therapeutic avenues to manage HNSCC.

Introduction

- HNSCC, affecting the upper aerodigestive tract, ranks as the sixth most prevalent cancer globally. The primary risk factors include human papillomavirus infection (HPV+ HNSCC), or carcinogens found in tobacco and/or the consumption of excessive alcohol (HPV- HNSCC).
- Despite available treatments, recurrent and metastatic HNSCC has poor prognosis, necessitating innovative therapies.
- ICD, a multifaceted cell-death process, involves release of DAMPs upon cellular stress, recruiting DCs and facilitating uptake of tumor antigens.
- PV-10, an IL immunotherapeutic agent, has exhibited ICD and substantial anti-tumor activity in multiple solid tumors, with minimal adverse effects. Its potential in HNSCC remains unclear.
- We evaluated PV-10's role in HNSCC both *in vitro* and *in vivo*. Our findings demonstrate that PV-10 effectively induces release of DAMPs, promoting ICD through activation of ER stress, autophagy, and apoptosis, leading to a significant HNSCC tumor regression.

Methods and Materials

- Cell viability was assessed using Alamar blue (AB) cell viability assay in murine mEER and MTE-RAS HNSCC tumor cell lines.
- To evaluate the immunomodulatory and apoptotic properties of PV-10, we employed flow cytometry, Immunofluorescence (IF), and luminescence-based assays, DCFDA, Alexa Fluor 488 Annexin V/dead cell apoptosis assay.
- The immunoblotting and multiplex immunohistochemistry (mIHC) were performed to gain insights into the underlying mechanisms of PV-10.

Results

PV-10 induces ROS-mediated apoptosis in HNSCC cells

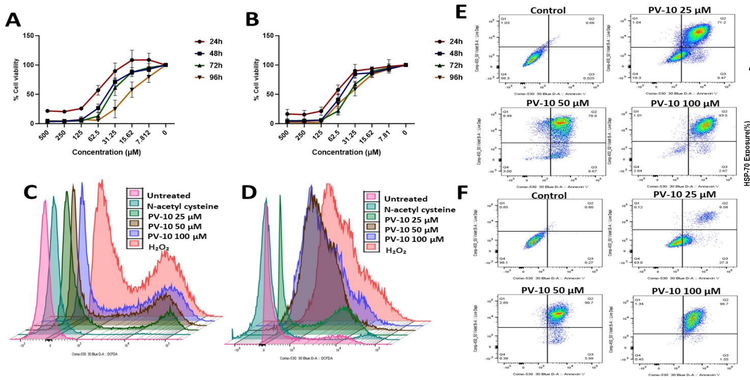

Figure 1. (A&B) AB cell viability assay results for mEER and MTE-RAS cells. **(C&D)** Representative plots of DCFDA fluorescence intensity in mEER and MTE-RAS cells. **(E&F)** Apoptosis assessment in mEER and MTE-RAS cells using Alexa Fluor 488 Annexin V and DAPI staining.

PV-10 induces the surface expression of calreticulin in HNSCC cells

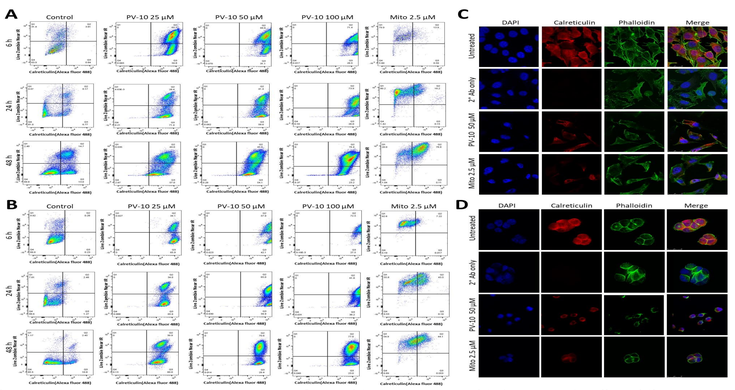

Figure 2. Flow cytometric analysis was performed in **(A)** mEER and **(B)** MTE-RAS cells, while IF was carried out in **(C)** mEER and **(D)** MTE-RAS cells.

PV-10 induces extracellular ATP and HMGB1 release in HNSCC cells

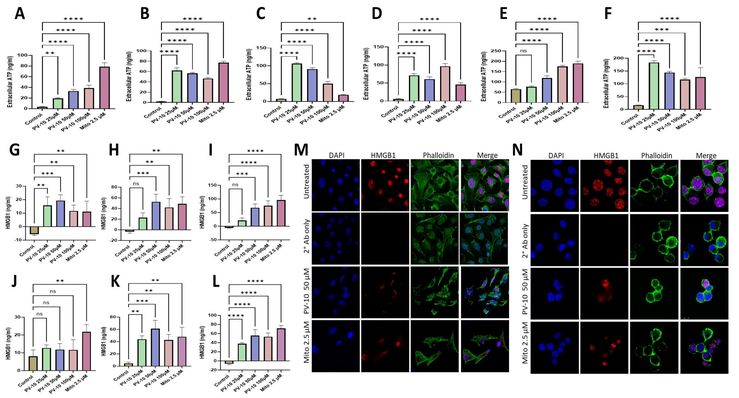

Figure 3. Quantification of extracellular ATP levels from **(A-C)** mEER and **(D-F)** MTE-RAS as well as HMGB1 levels from **(G-I)** mEER and **(J-L)** MTE-RAS at 6h, 24h, and 48h. while IF was carried out in **(M)** mEER and **(N)** MTE-RAS cells to analyze HMGB1 expression.

Surface Expression of HSP-70 and HSP-90 in PV-10-Induced ICD in HNSCC

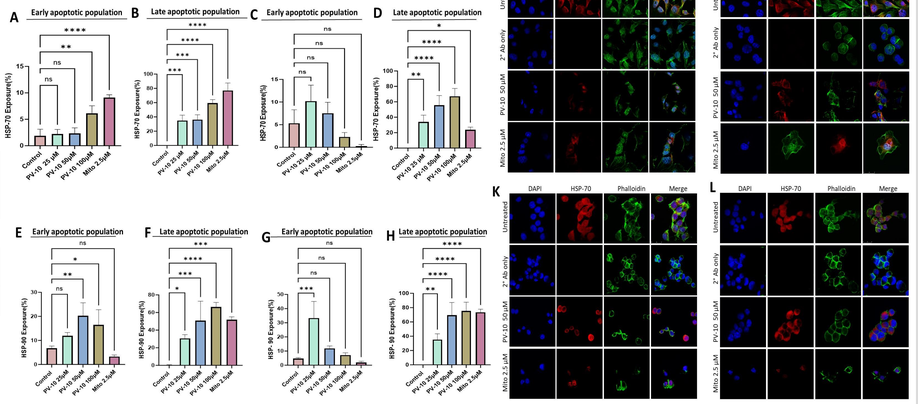

Figure 4. The graphs illustrate the surface HSP-70 expression in **(A)** early apoptosis (EA) and **(B)** late apoptosis (LA) of mEER cells, and in **(C)** EA and **(D)** LA of MTE-RAS cells. Additionally, the surface HSP-90 expression is shown in **(E)** EA and **(F)** LA of mEER cells, and in **(G)** EA and **(H)** LA of MTE-RAS cells and IF was carried out to analyze HSP-70 and HSP-80 expressions in **(I&J)** mEER cells and **(K&L)** MTE-RAS cells.

IL PV-10 Injection promotes tumor regression in HNSCC by inducing ER stress, triggering autophagy, and initiating apoptosis.

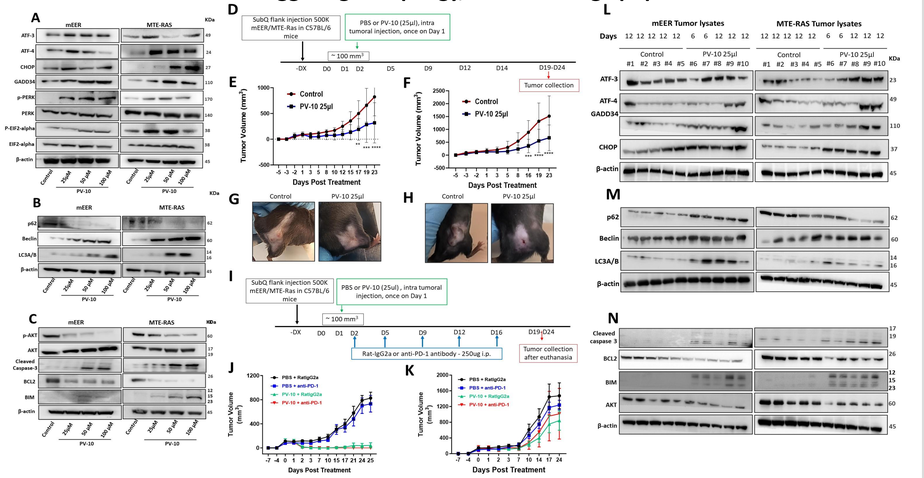

Figure 5. Immunoblotting analysis was performed to evaluate the effect of PV-10 treatment on **(A-C)** ER stress, autophagy, and apoptosis markers. **(D-K)** *In vivo* assessment of tumor regression in mEER and MTE-RAS mouse models post IL treatment of PV-10. **(L-N)** Analysis of ER stress markers, autophagy, and apoptosis pathways in mEER and MTE-RAS tumors

Conclusions

- PV-10, an IL immunotherapeutic agent, demonstrates significant anti-tumor activity in HNSCC.
- Our *In vitro* studies show PV-10 induces potent ICD in HNSCC cells.
- Our *In vivo* experiments show IL PV-10 lead to substantial tumor regression and complete responses in some mice.
- These findings underscore the significance of PV-10-induced ICD which may offer a novel and promising approach for managing HNSCC and potentially pave the way for improved survival rates and reduced adverse events

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