Adenovirus Encoding TNFα and IL-2 Induces a Tertiary Lymphoid Structure Signature in Immune Checkpoint Inhibitor Refractory Head and Neck Cancer

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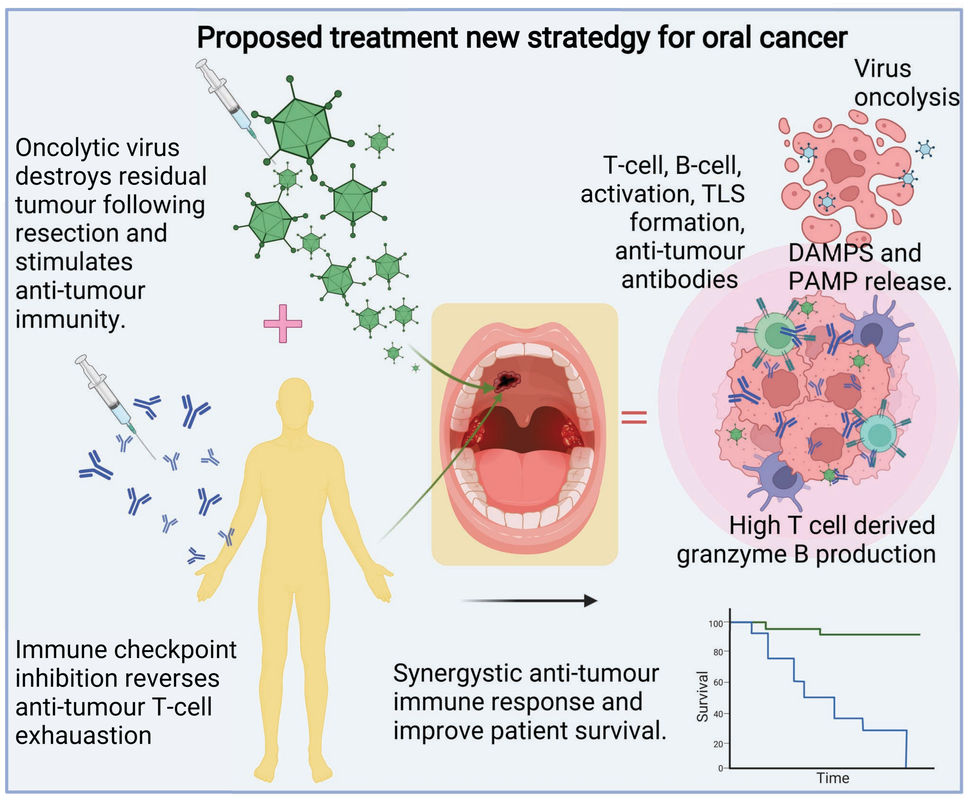
INTRODUCTION



• Head and neck cancer is the sixth most common cancer worldwide and is anticipated to increase 30% by 2030 to 1.08 million new cases annually [1,2].

• Approximately 20% of HNSCC patients receive measurable benefits from treatment with immune checkpoint inhibitors (ICI). Patients often present or develop resistance ICI [3].

• Oncolytic viruses (OVs) are a class of immunotherapeutic agents, capable of inducing a potent immune response, through selective lysis of tumor cells, release of tumor-associated antigens and facilitation of epitope spreading [4]. OVs can be further modified to contain immunostimulatory transgenes, designed to further amplify the antitumor immune response. Adenoviruses make up one family of well-characterized OVs with these capabilities, yet also have distinct inherencies, including an ability to prime CD4+ T cells with Granzyme-mediated cytotoxic function and PD-1 sensitivity [5,].

• Here, we aimed to validate TILT-123 can also improve the response to ICI (anti-PD-1 and anti-PD-L1) in head and neck squamous cell carcinoma and improve survival outcomes in heterotopic murine models of oral cancer.

• We also set out to provide new insights into the mechanism of action of TILT-123 with ICI, by proteomic and transcriptomic analysis of the immune compartment of the secondary lymhpoid organs and tumour microenvironment.

METHODS

• HNSCC cell lines were infected with TILT-123 at three different infectious ratios (10, 100 and 1000VP/cell). Cell viability was measured at day 3, 5 and 7 by MTS. In parallel cells and supernatant were collected for transgene expression (IL-2 and TNFa) and virus replication (E1a).

• ICI naive syngeneic mouse (C57BL/6) model of oral cancer using indolent and aggressive mouse cell liness MOC1 and MOC2 respectivley. Cells were engrafted heterotopically and tumours grown to 4mm in diameter. Mice were then treated with either PBS (i.t.), anti-PD-1/PD-L1 (i.p.), Ad5-CMV-TNFa/IL-2 (i.t.) or anti-PD-1/PD-L1 (i.p.) + Ad5-CMV-TNFa/IL-2 (i.t.). Tumours wre measured using digital calipers and survival followed for 100 days after first treatment.

• ICI refractory syngeneic mouse (C57BL/6) model of oral cancer using MOC1 cell line. 4mm tumours were treated with ICI until 8mm (our 'refractory' classification). Refractory tumours were then treated with anti-PD-1/PD-L1 (i.p.), Ad5-CMV-TNFa/IL-2 (i.t.) or anti-PD-1/PD-L1 (i.p.) + Ad5-CMV-TNFa/IL-2 (i.t.). In addition, Ad5-Luc was used in a serperate experiment to evaluate mechanism of action.

• Treated ICI refractory mice were euthanized for evaluation of mechanism of action, 10 days post first treatment or followed up for survival. $CD3^+$ cells were isolated and evaluated for cytotoxic profile using an IFN-γ and Granzyme b ELISpot, $CD45^{+/-}$ tumour cells were isolated and analysed by bulk RNA-Seq and secondary lymphoid organs analysed by flow cytometry (for T cell profiling) and ELISpot. Tumours were also evaluated for TLS by immunohistochemistry.

CONCLUSION

1. Oncolytic Adenoviruses armed with IL-2 and TNFα are able to significantly improve therapeutic outcome in ICI sensitive, primary resistant and acquired resistant models of oral cancer, likely through immunogenic viral oncolysis and induction of a cytolytic immune response associated with tertiary lymphoid structure formation.

2. Tertiary lymphoid structures (TLS) are transient ectopic lymph node-like structures that develop in response to chronic inflammaton. Pre-existing TLS are assocaited with improved clinical outcomes for patients treated with ICI.

3. We demonstrate in all three models, a synergistic effect when combining virus with anti-PD-1 or anti-PD-L1, evident at a mechanistic level and by improvement to overall survival.

RESULTS

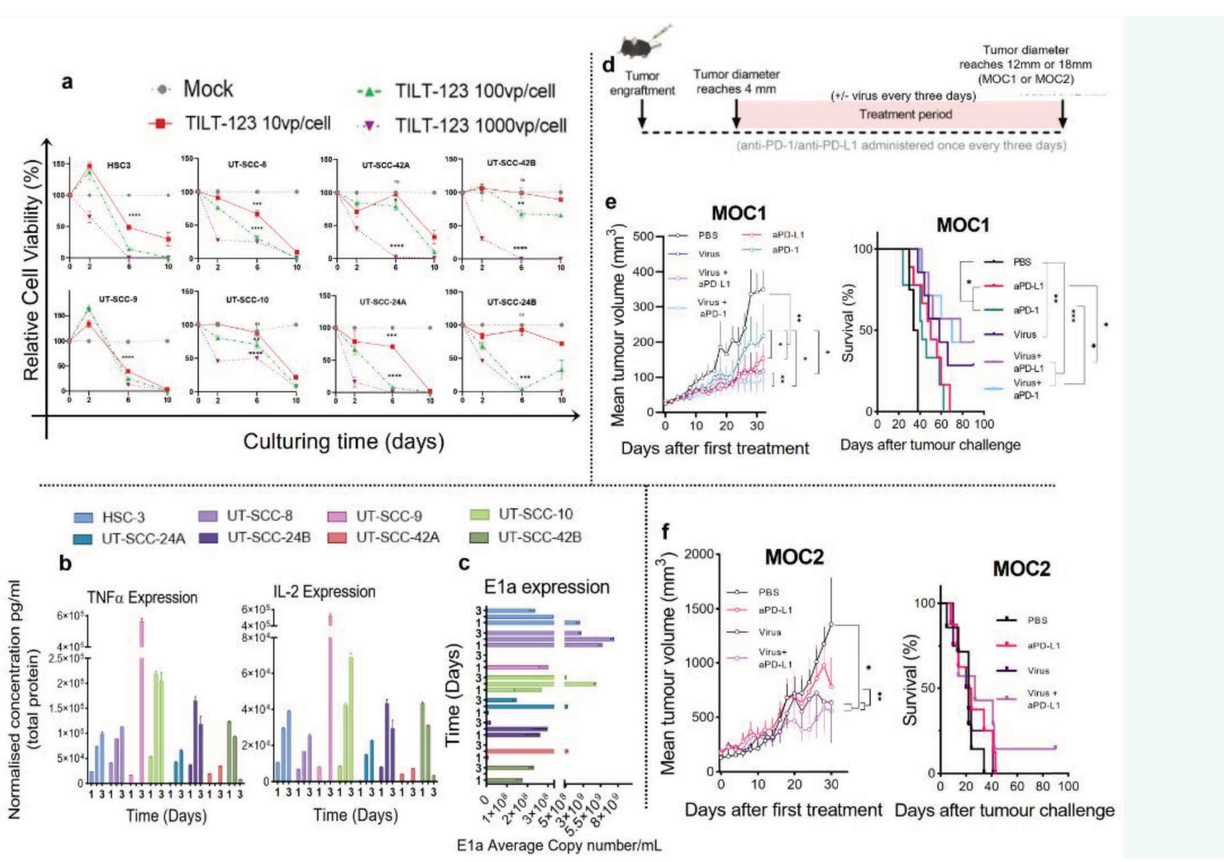


Figure 1. TILT-123 replicates in all HNSCC cell lines screened and the antitumor response in ICI treatment naïve murine model is improved when combining ICI with non-replicative Ad5-CMV-mTNFα/mIL-2. (**a**) Lytic activity of TILT-123 measured by MTS (**b**) transgene expression of TILT-123 over three days measured by Cytokine Flex set, (**c**) TILT-123 replication measuerd by qPCR of adenovirus E1a (**d**) treatment schedule of mouse experiments, (**e**) 30 day tumour growth control of MOC1 tumours, and 100 day survival, (**f**) 30 day tumour growth control of MOC2 tumours, and 100 day survival. All data sets are presented as means ± SEM and significance represented as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001, ns, not significant.

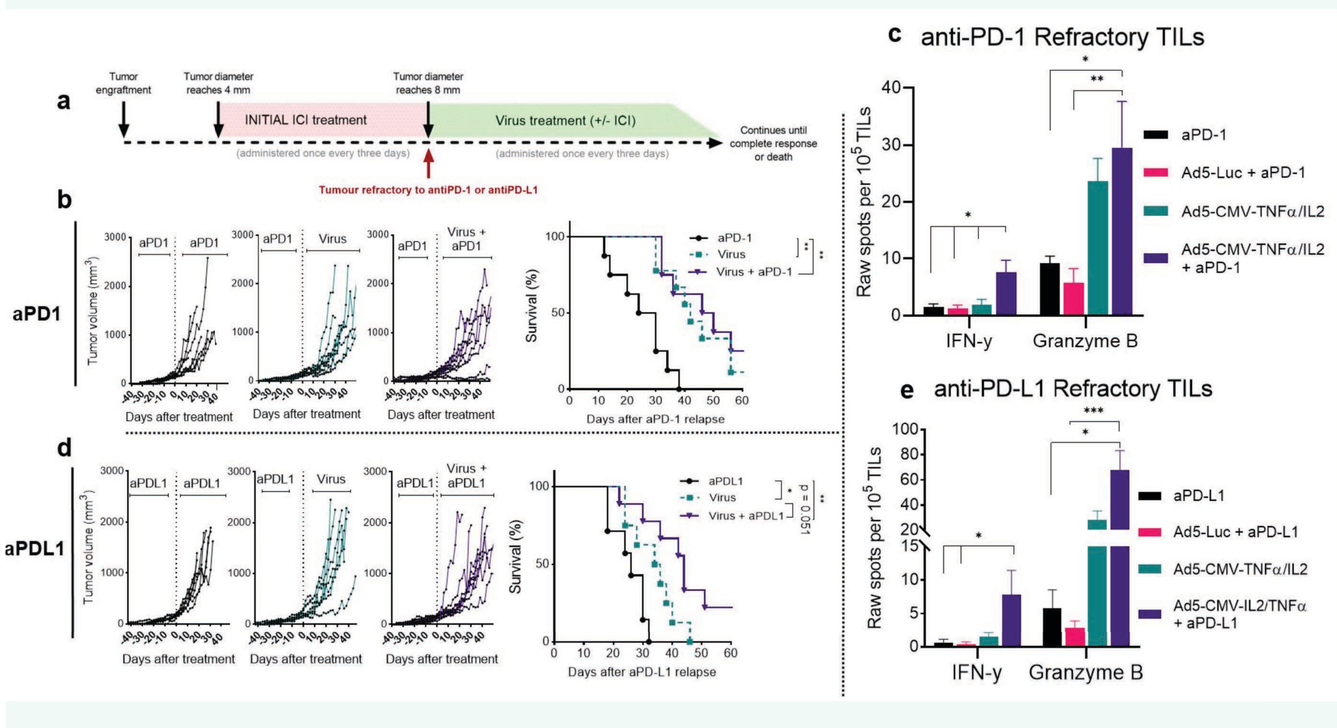


Figure 2. Non-replicative Ad5-CMV-mTNFα/mIL-2 improves the anti-tumor response in ICI refractory MOC1 tumors and continued ICI treatment is beneficial to the response. (**a**) Experiment design. C57BL/6J mice were subcutaneously injected with MOC1 cells into the right flank. When tumors reached 4-5mm then 100ug of either anti-PD-1 or anti-PD-L1 was injected every three days intraperitoneally. When tumors progressed over 8mm, animals were assigned to a group where they were treated with either continued anti-PD-1/PD-L1, with 1×10^9 VPs (non-replicative Ad5-CMV-mTNFα/mIL-2) intratumorally, or in combination. An additional virus backbone control group (Ad5-Luc) was included for immune cell analysis. Treatment frequency as indicated. (**b, d**) Individual tumor growth curves and Kaplan–Meier survival analysis for **b** anti-PD-1 and d anti-PD-L1 refractory. (**c, e**) Corresponding treatment group tumor derived $CD3^+$ cytotoxic functional analysis measured by Granzyme B and IFN-γ dual-ELISpot. All data are shown as means ± SEM and significance is represented as *p < 0.05, **p < 0.01, ***p < 0.001, ns, not significant.

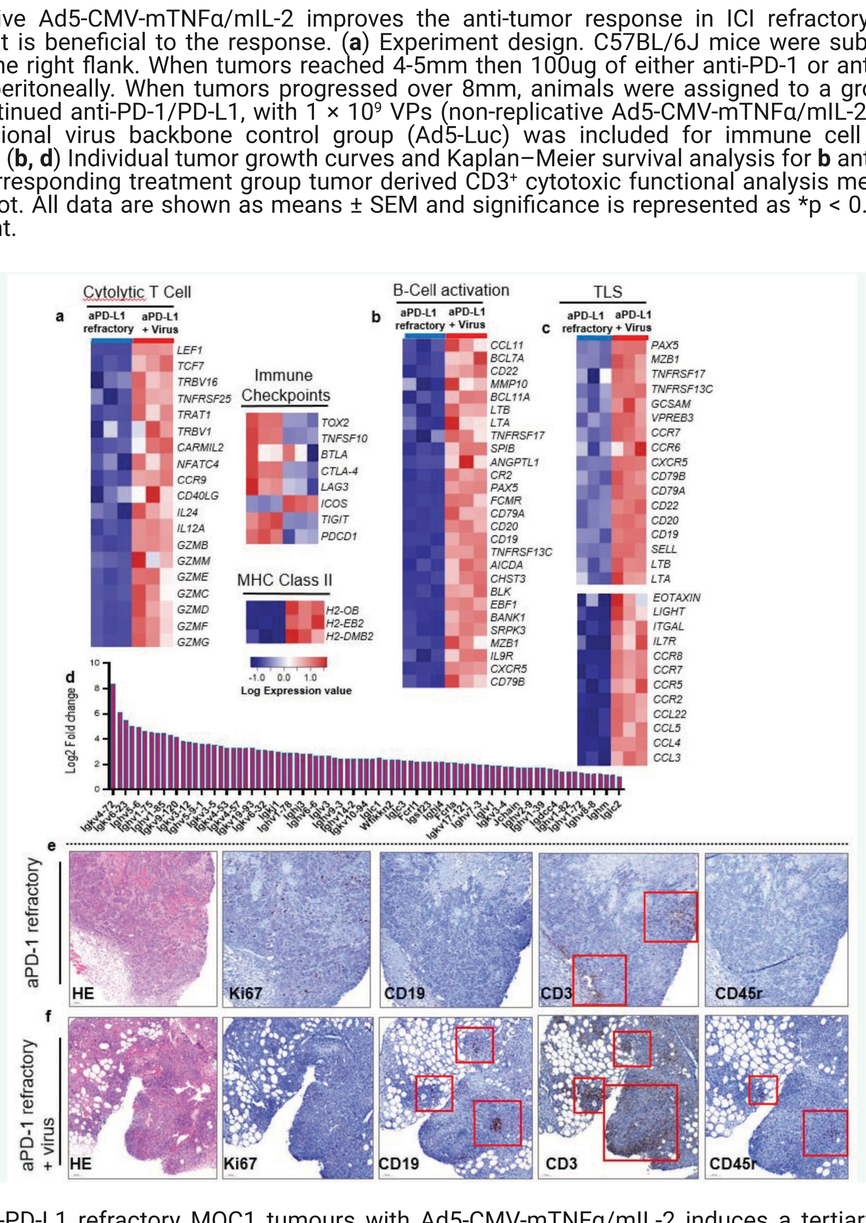


Figure 3. Treating anti-PD-L1 refractory MOC1 tumours with Ad5-CMV-mTNFα/mIL-2 induces a tertiary lymphoid structure gene signature within the tumour. Heat maps for significantly differentially expressed genes between anti-PD-L1 refractory tumors and anti-PD-L1 refractory tumors treated with Ad5-CMV-mTNFα/mIL-2. Heat maps are organized into (**a**) T cell activation genes, immune checkpoints and MHC genes (**b**) B-cell activation genes, (c) typical tertiary lymphoid structure genes. (**d**) Fold change increase of significantly differentially expressed genes related to immunoglobulin synthesis between anti-PD-L1 refractory tumors and anti-PD-L1 refractory tumors treated with Ad5-CMV-mTNFα/mIL-2. (**e**, **f**) Immunohistochemistry of tertiary lymphoid structure related markers (HE, Ki67, CD19, CD3 and CD45r) from anti-PD-1 refractory tumors treated with (**f**) or without (**e**) non-replicative Ad5-CMV-mTNFα/mIL-2. Red boxes highlight positively stained areas (brown) for respective marker indicated in the bottom left of each panel.

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