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A novel class of small molecules for oral application to enhance tumor-reactive T cell cytotoxicity against melanoma

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Introduction

The introduction of targeted immunomodulating agents has transformed cancer treatment over the last decade by demonstrating unprecedented efficacy in patients who respond. However, limited clinical response rates as well as adverse events of presently used biologics underline the need to identify alternative low molecular weight modalities in cancer immunotherapy. Here we report for the first time on the discovery of a novel class of low molecular weight compounds for oral application that selectively enhance tumor-reactive T cell cytotoxicity.

Methods

Hit-to-lead development of compound hit series A is being performed based on medicinal chemistry to investigate structure-activity-relations. Newly synthetized compounds are tested for EC50 potency on stimulated T cells. *In vitro* killing efficiency is assessed in co-culture assays of T cells with melanoma. Furthermore, absorption, distribution metabolism and excretion (ADME) profiling and pharmacokinetics (PK) behavior are investigated to select candidate compounds for proof-of-concept studies in a B16-SIY melanoma and EO771 breast cancer mouse model. Activation of T cells in tumor draining lymph nodes is assessed by flow cytometry.

Conflict of interest

Some of the authors are shareholders of invIOs GmbH and APEIRON Biologics AG.
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Figures were created with GraphPad Prism and Biorender.com.

Results

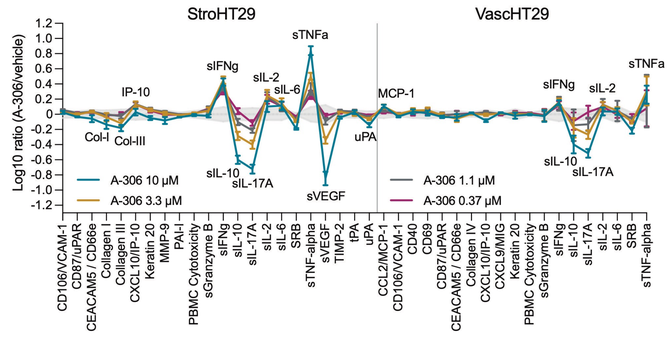

Figure 1. A-306 elicits an inflammatory biomarker signature in human *in vitro* cell cultures that mimic a suppressive TME. Biomarker expression of A-306 treated cancer cell line, primary immune and tissue cell co-cultures compared to vehicle control was measured using BioMAP assay. The grey region represents the 95% significance envelope generated from historical vehicle controls.

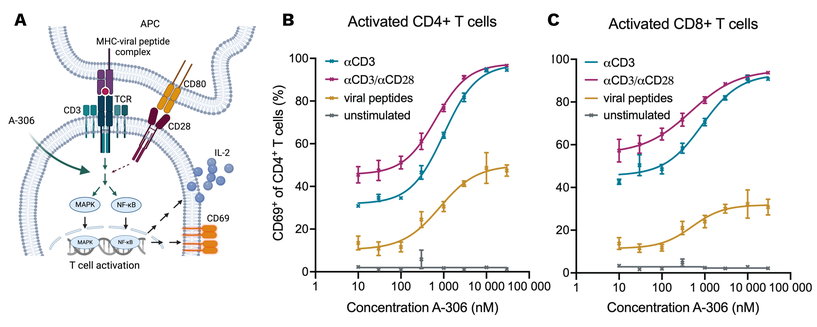

Figure 2. A-306 enhances viral peptide specific T cell activation from healthy individuals. A: T cell activation by viral peptide antigen presenting cells (APC) and A-306 compound addition. **B:** CD69 expression on purified human CD4+ T cells stimulated with anti-CD3, anti-CD3/CD28 or CEFx viral peptide pool and different concentrations of A-306. **C:** as in B except for CD8+ T cells.

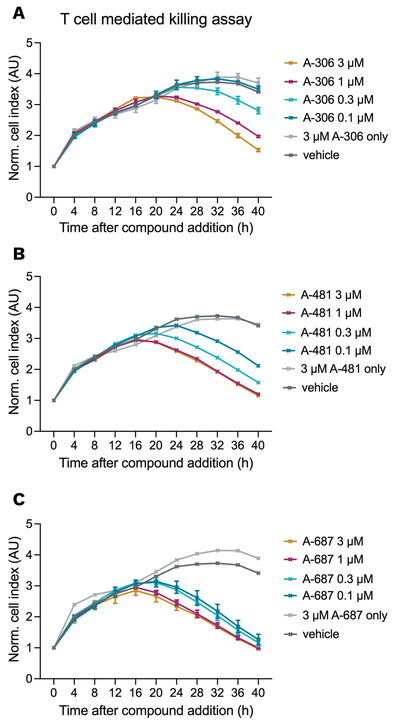

Figure 3. A-306, A-481 and A-687 treated T cells eliminate M21 melanoma cells in a dose-dependent manner. A: M21 cell growth upon anti-CD3/CD28 stimulated T-cell co-culture and different concentrations A-306. **B:** as in A except for A-481. **C:** as in A except for A-687.

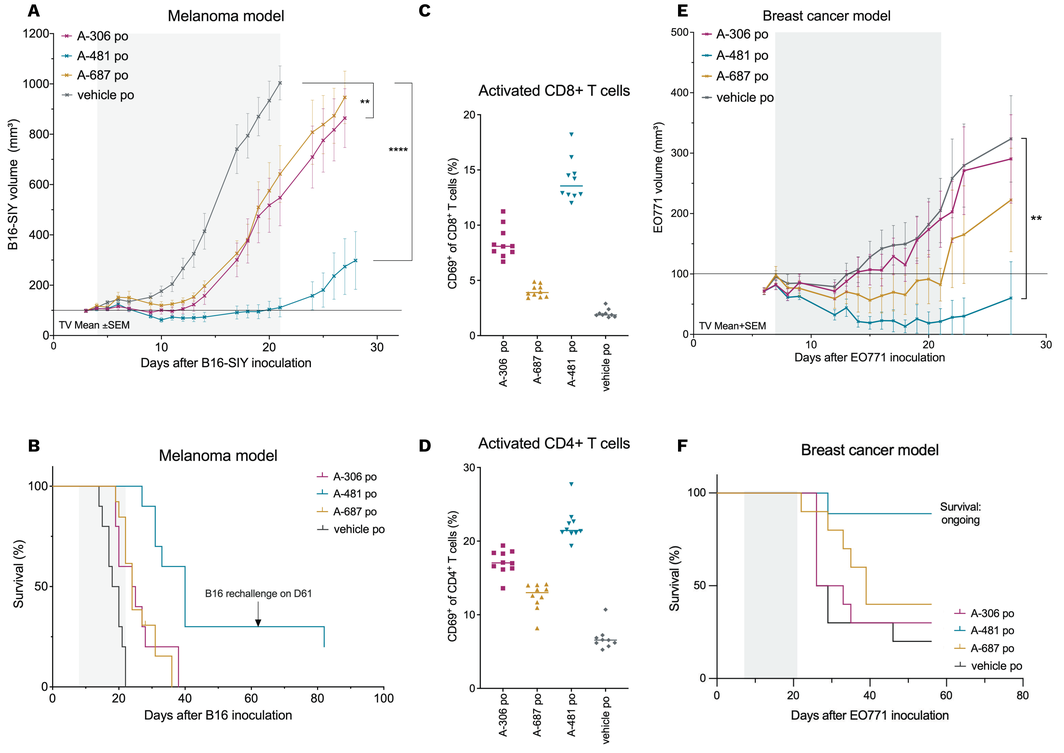

Figure 4. Front runners A-481 and A-687 show efficacy in murine melanoma and breast cancer models beyond end of treatment. A: Average tumor volumes ± SEM of B16-SIY melanoma bearing mice receiving po treatment with A-306 (30 mg/kg D4 + 10 mg/kg QD), A-481 (20 mg/kg D4 + 10mg/kg QD) or A-687 (10mg/kg QD) starting from day 4 until day 21. **B:** Survival of mice receiving po treatment as in A. **C:** CD69+ CD8+ T cells in tumor draining lymph nodes in melanoma bearing mice as in A, measured on Day 13. **D:** as in C except CD4+ T cells. **E:** Average tumor volumes ± SEM of EO771 tumor bearing mice receiving po treatment as in A. **F:** Survival of mice receiving po treatment as in E. The grey area represents the treatment period from day 4 until day 21.

Results

A-306 elicits an inflammatory biomarker signature in human cell *in vitro* cultures that mimic a suppressive tumor microenvironment (TME). A-306 enhances virus-specific T cell activation in cells from healthy individuals and uncouples TCR-specific immune cell activation from CD28 co-stimulation. All 3 tested compounds show dose-dependent tumor cell killing *in vitro*. In a murine B16-SIY melanoma and EO771 breast cancer model oral single-agent administration is well tolerated and shows good bioavailability in lymphoid organs and plasma (data not shown). Oral application of front runners A-481 or A-687 results in significant tumor growth inhibition and prolonged survival beyond the end of treatment (D21). These long-term surviving mice show a distinct activation pattern in T cells from tumor draining lymph nodes indicative of anti-tumor immunity.

Conclusion

For the first time we report on the discovery of a novel class of small molecules possessing high potential for selective anti-tumor activation of the immune system upon oral single-agent administration. Medicinal chemistry efforts resulted in front runner compounds A-481 and A-687, which significantly inhibit tumor growth in a B16-SIY melanoma and EO771 breast cancer mouse model. Furthermore, A-481 and A-687 lead to prolonged survival beyond the end of treatment and display a good safety profile.

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