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Preclinical In Vivo Characterization of the Anti-tumor Activity of a Non-blocking PD-1 Antibody Fused to Attenuated IL-2

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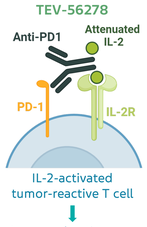
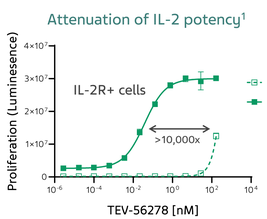
TEV-56278 demonstrates potential as an effective anti-tumor immunotherapy, both as a monotherapy, and in combination with Anti-PD1

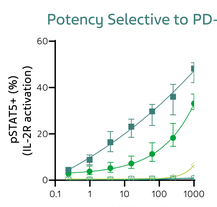
Background

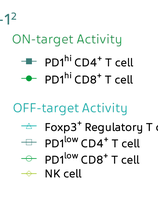
- **TEV-56278** is a fusion protein developed by Teva, combining a non-blocking anti-PD1 antibody with an attenuated IL-2 variant. This design targets IL-2 to PD-1+ T cells, aiming to enhance anti-tumor immunity while minimizing systemic IL-2 side effects.
- IL-2 is crucial for immune regulation, as it enhances T cell proliferation and activation, thereby boosting anti-tumor immunity. However, its clinical use is limited by systemic toxicity and a narrow therapeutic window (Atkins et al., 1999). TEV-56278 features an attenuated IL-2 moiety, developed to mitigate these risks.
- PD-1 expression is significantly higher in tumor-infiltrating T cells compared to circulating and other tissue-resident T cells (Ahmadzadeh et al., 2009). TEV-56278's design allows the IL-2 variant to target PD-1-expressing cells while preserving PD-1 receptor functionality. This enables treatment both as a monotherapy and in combination with an anti-PD1 antibody.
- Here, we describe the impact of TEV-56278 and its murine surrogate, mAnti-PD1-IL2, on tumor progression and tumor-infiltrating lymphocytes in pre-clinical tumor models.



TEV-56278 Selectively Activates IL-2R in PD-1+ Cells





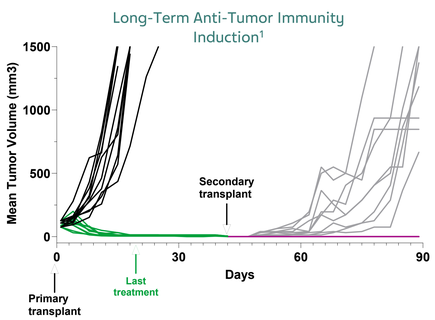


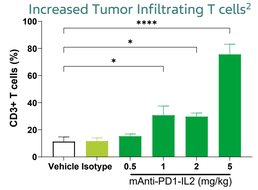
*IL-2R=interleukin-2 receptor

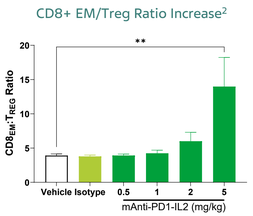
1. Proliferation assay (luminescence) for IL-2 activity on IL-2R positive, hPD-1 negative, and hPD-1 positive engineered cells after culturing with various concentrations of TEV-56278. Results are presented as mean ± SD of 3 technical replicates.
2. pSTAT5 (%) quantified by flow cytometry analysis in purified activated (PD1^{hi}) and non-activated (PD1^{low}) CD8+, CD4+ T cells (high PD-1), regulatory T cells, and NK cells after culturing with various concentrations of TEV-56278 (n = 4 donors, mean ± SD).

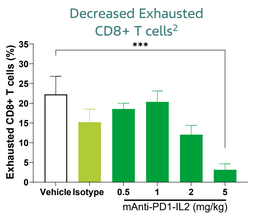
Syngeneic Mouse Tumor Model

mAnti-PD1-IL2 Induces Long Term Protective Immunity and Favorable Immune Shift Within the Tumor Microenvironment



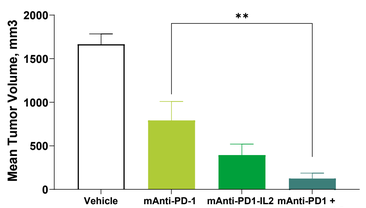






CD8+ T effector memory cells (EM) distinct by CD3+/CD8+/CD44+/CD62L-; Regulatory T cell (Treg) distinct by CD3+/CD4+/CD25+/FoxP3+; CD8+ exhausted T cells distinct by CD3+/CD8+/CD44+/CD62L-/CD69+/ki67+/PD-1+/Tim3+

MC38 Murine Tumor Model



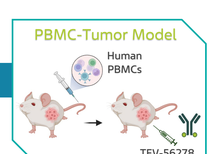
1. Long-term immunity rechallenge study: MC38 tumor bearing mice were treated with mAnti-PD1-IL2 at 5 mg/kg, IP, twice weekly, following the inoculation of a primary tumor transplant (green, n = 10). mAnti-PD1-IL2 complete responders (10/10) were subsequently re-inoculated with the MC38 tumor (purple, n = 8-10).
2. Single dose escalation study: MC38 tumor bearing mice were treated with mAnti-PD1-IL2 at 0.5, 1, 2, and 5 mg/kg, IP, n = 5. Tumors were analyzed by flow cytometry on day 8. 1-way ANOVA, -/+ SEM.
3. Combination study: MC38 tumor bearing mice were treated with mAnti-PD1-IL2 and mAnti-PD1, IP, at 1 mg/kg and 2 mg/kg, respectively, weekly, n = 8-10. Mean tumor volume was measured at day 29 (study endpoint). 1-way ANOVA, -/+ SEM.

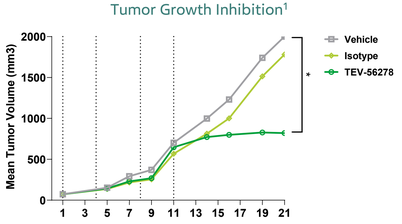
*p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0. 001, ****p ≤ 0.0001

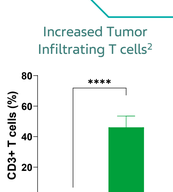
Humanized Mouse Tumor Models

TEV-56278 Inhibits Tumor Growth

PBMC-Tumor Model







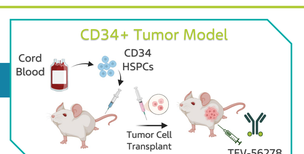
NCG mice with A2058 human melanoma tumors were engrafted with PBMCs from two healthy donors and treated with either human IgG1 isotype or TEV-56278 (5mg/kg, IP) twice weekly (n = 16).

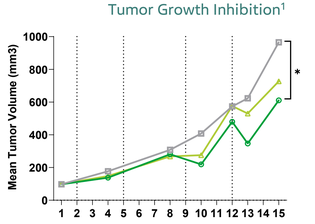
1. Mean tumor volume measurements: Paired t-test, one-tailed. *p ≤ 0.05
2. Flow cytometry analysis: Tumors were analyzed on day 21. Unpaired t-test, -/+ SEM. ****p ≤ 0.0001

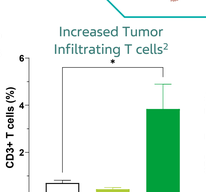
*PBMCs = Peripheral blood mononuclear cells

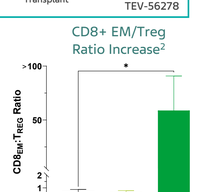
TEV-56278 Enhances Anti-Tumor Immunity

CD34+ Tumor Model









BRGSF-HIS (CD34+) mice with A2058 human melanoma tumors were treated with either Nivolumab or TEV-56278 (5 mg/kg, IP) twice weekly (n = 8-10). Five CD34 donors were equally distributed between the groups.

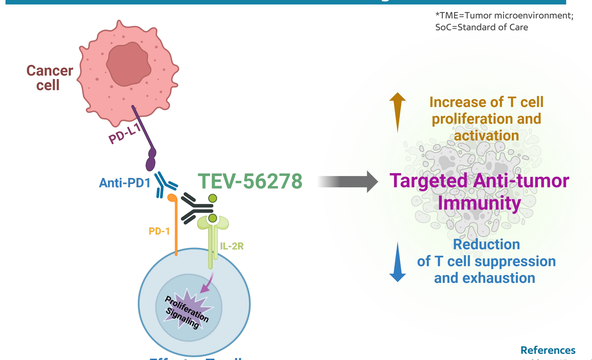
1. Mean tumor volume measurements. Paired t-test, one-tailed. *p ≤ 0.05
2. Flow cytometry analysis. Tumors were analyzed on day 15. Unpaired t-test, -/+ SEM. *p ≤ 0.05.

CD8+ EM distinct by CD3+/CD8+/CCR7-/CD45RO+; Treg distinct by CD3+/CD4+/CD25+/FoxP3+

*HSPCs = Hematopoietic stem/progenitor cells

TEV-56278 Promotes TME Reprogramming Driving Anti-Tumor Immunity

*TME=Tumor microenvironment; SoC=Standard of Care



TEV-56278 and the surrogate agent mAnti-PD1-IL2 enhance tumor-infiltrating T cells, promote tumor regression, establish durable immune memory, and can be combined with SoC PD-1 inhibitors

To see the poster on the TEV-56278 phase 1 clinical study design (abstract 671) click on this QR code.

Acknowledgments
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Disclosures
Illustrative figures were created with BioRender.com. All authors are current or former Teva employees.

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