# High and low fractions of intra-tumoral TOX+CD8+ T-cells and their prognostic potential in metastatic melanoma

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## Background

- Tertiary lymphoid structures (TLS) are ectopic immune cell clusters that arise at sites of chronic inflammation, including tumors.[1]
- In metastatic melanoma, TLS presence has been linked to improved survival, but the mechanisms driving this benefit remain unclear.[2]
- TOX (Thymocyte selection-associated HMG-box) is a transcription factor associated with T-cell exhaustion and has been identified in T cells within TLS.[3,4]
- The biological and prognostic role of TOX$^+$ CD8$^+$ T cells within TLS in melanoma metastases is not well understood.

## Objective

To evaluate the prognostic significance of intra-TLS TOX$^+$ CD8$^+$ T cells in cutaneous metastatic melanoma, and their potential link to patient outcomes.

## Methods

- Multiplex immunofluorescence was used to evaluate cutaneous metastatic melanoma samples for the presence of TLS and expression of TOX$^+$ and CD8$^+$ T cells
- A total of 15 TLS-containing specimens were analyzed using Halo Software (Indica Labs) to quantify CD8$^+$ T cells and identify those co-expressing TOX.
- The fraction of CD8$^+$ T cells expressing TOX was calculated within each TLS.
- Fractions were then dichotomized into high (TOX$^{high}$) and low (TOX$^{low}$) TOX expression groups using the Contal-O'Quigley method.
- Survival associations were assessed using log-rank tests.

**Figure 1.**

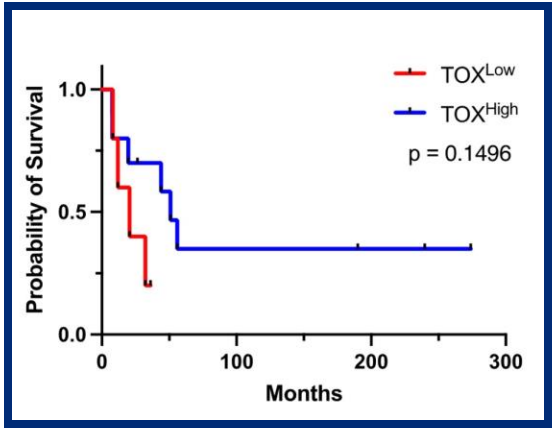


***Figure 1.*** *Survival analysis of TLS+ metastatic melanoma patients harboring low and high fractions of TOX+ T-cells*

## Results

- Survival analysis was performed based on intra-TLS TOX$^+$ CD8$^+$ T cell levels (high vs. low fraction groups).
- Although the difference in overall survival did not reach statistical significance (p = 0.1459), the TOX$^{high}$ group showed a longer median survival than the TOX$^{low}$ group (51 vs. 21 months) in patients with metastatic melanoma

## Discussion and Conclusions

- TOX is a transcription factor known to regulate T cell exhaustion in chronic immune environments. In some cancers, high TOX expression has been associated with poorer outcomes.[5]
- In our cohort of cutaneous metastatic melanoma patients, we observed a trend toward longer overall survival in the TOX$^{high}$, though this did not reach statistical significance.
- While much of the literature portrays TOX to be an indicator of poor prognosis, our findings suggests the role that TOX plays in tumor-mediated immunity warrants further study.

## References

1. Kang W, Feng Z, Luo J, He Z, Liu J, Wu J, Rong P. Tertiary Lymphoid Structures in Cancer: The Double-Edged Sword Role in Antitumor Immunity and Potential Therapeutic Induction Strategies. Front Immunol. 2021 Jul 29;12:689270. doi: 10.3389/fimmu.2021.689270. PMID: 34394083; PMCID: PMC8358404.
2. Lynch KT, Young SJ, Meneveau MO, Wages NA, Engelhard VH, Slingluff CL Jr, Mauldin IS. Heterogeneity in tertiary lymphoid structure B-cells correlates with patient survival in metastatic melanoma. J Immunother Cancer. 2021 Jun;9(6):e002273. doi: 10.1136/jitc-2020-002273. PMID: 34103353; PMCID: PMC8190052.
3. Kim, K., Park, S., Park, S.Y. et al. Single-cell transcriptome analysis reveals TOX as a promoting factor for T cell exhaustion and a predictor for anti-PD-1 responses in human cancer. Genome Med 12, 22 (2020). https://doi.org/10.1186/s13073-020-00722-9
4. Ileana Mauldin, Priya Katyal, Nicole Edmonds, Craig Slingluff - 11 Tertiary lymphoid structures in melanomas harbor increased proportions of stem-like effector T-cells compared to tumor-infiltrating lymphocytes: Journal for ImmunoTherapy of Cancer 2022;10:.
5. Li S, Yang S, Hong Y. Higher thymocyte selection-associated high mobility group box (TOX) expression predicts poor prognosis in patients with ovarian cancer. BMC Cancer. 2022 Nov 25;22(1):1216. doi: 10.1186/s12885-022-10336-6. PMID: 36434543; PMCID: PMC9701062.