Melphalan, Antimelanoma Immunity, and Inflammation—Response
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Our recent publication described the CD8\(^+\) T-cell–dependent immunogenic effects of melphalan (Mel), with implications for clinical melphalan-based isolated limb-perfusion/infusion (ILP/ILI; ref. 1). We would like to thank Martner and colleagues for corroborating our preclinical findings, establishing the role of CD8\(^+\) T cells as primary effector cells for Mel-ILP responsiveness, in a subset of melanoma patients (2).

However, in our opinion, it is tough to conclude from the results Martner and colleagues present that melphalan alone can induce immunogenic cell death (ICD), and additional analyses are required to validate this clinically (1, 3). For instance, T-cell effector responses intratumorally are known to differ from those in peripheral blood and thus comparison of T-cell infiltration in tumor-invasive margin versus tumor core is more relevant for ICD.

Figure 1.
Publicly available gene-expression profiles for various cytokines/chemokines in two independent cohorts (GSE19293/GSE34599) of melanoma patients (detected intratumorally, before Mel-ILP/ILI) were acquired from GEO database (\(n = 67\) patients). The expression values were derived using the webtool GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) such that the patients were "divided" into two groups, i.e., "responders" (32 patients showing clinical complete/partial responses) and "nonresponders" (35 patients showing stable/progressive disease) after Mel-ILP/ILI. Mean \(\pm SD\), \(^*\) \(P < 0.05\) as per \(t\) test with Welch correction.
(3–5). Secondly, the T-cell populations analyzed by Martner and colleagues are applicable to general antitumor immunity irrespective of ICD (3–5). To gain insight into ICD-relevant T-cell responses, the CD4⁺/CD8⁺ T-cell analysis should be extended to Th1/IL17A-producing γδT cells (5). Lastly, ICD detection can be relatively better achieved by confirming peripheral blood-associated danger signals relevant for ICD (calreticulin/HMGB1) or autoantibodies against them (3, 5) and/or the intratumoral expression of key ICD-associated cytokines/chemokines (IL1B/IL8/IFNG).

Based on various in vitro/in vivo parameters, we had outlined that the immunobiology of melphalan-induced cell death is distinct from ICD and is closer to a necrosis-like immunoprofile (1). To further substantiate this, we did a meta-analysis of publicly available cohorts (consisting of 67 melanoma patients treated with Mel-ILP/ILI) for clinical response prediction based on pretreatment, transcript levels, of cytokines/chemokines—relevant for ICD (IL1B, IL8, IFNG), relevant for immunosuppression (IL10), not relevant for ICD (TNF), and relevant for general anticancer immunity (CCL5, IL12A/B, IL6; refs. 4, 5). As evident from Fig. 1, pre-Mel-ILP/ILI levels of only IFNG, TNF, and CCL5 were predictive of positive responsiveness to Mel-ILP/ILI. Thus, ICD-relevant parameters do not exclusively predict for Mel-ILP/ILI responsiveness, consistent with the notion that melphalan-induced melanoma cell death lacks unique characteristics of ICD and is biased toward inflammation (1).

Lastly, caution is required while comparing the Martner and colleagues results with ours. In the study of Martner and colleagues, Mel-ILP treatment involved hyperthermia (2), whereas our murine setup was melphalan-centric (1). Notably, hyperthermia can autonomously exert immunogenic effects (3), suggesting that its combination with Mel could potentiate Mel-ILP immunogenicity—a hypothesis that needs urgent verification. Nonetheless, both our and Martner and colleagues results advocate to search for combination treatments that could increase melphalan-induced immunogenicity.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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