Background While the prognostic role of tertiary lymphoid structures (TLS) has been studied in cancer, the prevalence and role of immature precursor lymphoid structures aka lymphoid aggregates (LA) remains to be evaluated. In this study we examined characteristics and prognostic correlates of lymphoid aggregate presence in histological samples of patients with melanoma (MEL).

Methods We assessed TCGA-SKCM digital slides and University of Pittsburgh melanoma specimens for presence of histologically defined LA and TLS by H&E staining. LA were defined as (1) clusters, where groups of \( \geq 50 \) lymphocytes were present, but were irregularly shaped; (2) nodules, if presenting as circular/spheroid aggregates of \( > 50 \) lymphocytes with concentric placement, and (3) TLS, if present as nodular lymphoid aggregates containing germinal centers (GCs), (4) absent, if none of the above. LA and TLS localization were categorized as peritumoral if present at the invasive margin of tumor, stromal if separated from the tumor parenchyma, and intratumoral if present within tumor parenchyma.

Results From 600 samples screened we excluded those with lymph node metastases, or obtained through core biopsy, or of poor digital slide quality, which resulted in a total of 246 evaluable samples with primary skin (N=185, 75%) and metastatic MEL in skin/subcutaneous/soft tissue (n=61, 25%). Among these samples, 74% of tumor specimens contained histologically defined LA, which were clusters only (46%), nodules only (17%), and n=37% contained both clusters and nodules. LA were located only in peritumoral (34%), intratumoral (5.6%), and stromal (6.1%) zones with remaining samples exhibiting LA in mixed locations. Overall, 12% of MEL specimens contained TLS with GC. Amongst all lesions evaluated, TLS were located in peritumoral (47%), stromal (33%), intratumoral (17%) and mixed (3%) locations. The presence of LA was significantly associated with negative status of sentinel lymph node (\( \chi^2(1, N=148) = 9.86, p=0.002 \)). In contrast to LA which tend to form in primary samples, TLS with GC predominantly formed metastatic vs. primary MEL (OR=3.70, 95% CI 1.68–8.11). In a univariable analysis, the presence of LA was associated with improved overall survival (HR=0.57, 95% CI 0.35–0.94, p=0.027). In a multivariable analysis, after adjusting for age, sex, sample type, and stage, LA continued to be significantly associated with improved OS (HR=0.50, 95% CI 0.30–0.85, p=0.010).

Conclusions Primary MEL with immature LA exhibit improved OS and SLN-negative status. LA and TLS tend to form in divergent locations in the TME. TLS with GC are found primarily in a small proportion of cases with metastatic MEL.