Longitudinal analysis of TCR repertoire in smouldering multiple myeloma reveals relative expansion of shared-sequence TCR clusters compared to healthy donors

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Introduction: While tumour-reactive T cells have been described in multiple myeloma (MM), their role in maintaining disease quiescence in smouldering multiple myeloma (SMM) and progression to MM remains unclear. We sought to perform longitudinal comparisons of TCR dynamics in SMM relative to normal temporal fluctuation in healthy donor (HD) TCR repertoire to help define T-cell contribution to disease control.

Methods: Longitudinal samples from SMM patients enrolled in the COSMOS study (baseline through progression; progressors (P) n=8, non-progressors (NP) n=7) underwent TCR sequencing. HD repertoires were obtained from COVIDsortium participants (n=13, 12-month interval). No significant differences in TCR count or clonality (Simpson's index) were observed between first and last timepoints across HDs, NP, and P. Thus, TCRs were filtered by edit distance ≤2 and clustered using TCRdist to group sequences with similar CDR3 regions (likely to target the same antigen). Per cluster abundance, defined as the summed abundance of constituent TCRs at each timepoint, was compared between baseline and final timepoints and sequentially filtered based on deviations from null expectations. Significantly expanding and contracting clusters (log2 odds ratio >0 and <0, respectively) were identified by a ClusterScore ($-\log_{10}$FDR derived from Fisher's exact test < 0.05).

Results: In paired samples with sufficient TCR reads (HD=13, NP=4, P=4), the proportion of dynamic clusters retained after filtering was comparable between all groups. However, ratio of expanding to contracting clusters revealed a distinct skew at point of progression toward expanding clusters, not observed in HDs or NP. Linear modelling of median ClusterScore incorporating repertoire size confirmed progression status as a significant predictor of contracting cluster number, with progressors exhibiting markedly fewer contracting clusters, resulting in a relative expansion-dominant repertoire shift. Viral annotation using Tcrictionary (viral database) showed minimal enrichment among expanding clusters (1 viral cluster in each of 2 progressors).

Single-cell analysis of matched samples showed clusters were predominantly CD8+ at baseline, enriched for cytotoxic and effector memory phenotypes. At progression, expanding clusters were characterised by increased early terminal effector memory (TEM) cells and reduced cytotoxic TEM representation, a shift not seen in contracting clusters. This indicates preferential expansion of T cells with a suboptimal cytotoxic phenotype, which may impair effective tumour control and facilitate disease progression.

Conclusion: Together, comparison with HD dynamics identifies a progression-associated shift toward expanding TCR clusters, likely reflecting tumour-driven clonal expansion. The accompanying enrichment of less cytotoxic CD8+ TEM cells suggests impaired functional capacity, providing a potential mechanism for loss of immune control and transition from SMM to MM.