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POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Expression of Cell Surface Immunotherapy Targets across the Multiple Myeloma Disease Spectrum: Implications for Sequencing and Multi-Target Strategies

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Introduction
Targeting surface proteins with immunotherapy including monoclonal antibodies, bispecific antibodies, antibody drug conjugates and chimeric antigen receptor T-cell therapy (CAR-T) has improved outcomes for patients with multiple myeloma (MM). No strategy has proven curative to date and relapse invariably occurs. There is a paucity of literature describing how these cell surface targets change over the disease course or at relapse post-immunotherapy. We comprehensively profiled a large repository of patient samples across the spectrum of disease, including paired samples taken prior to and post commercial B-cell maturation antigen (BCMA) CAR-T failure (administered in patients with triple class exposed disease after ≥4 prior lines of therapy (PLOT), to better understand how targeted therapies could be optimally sequenced or combined for maximum therapeutic benefit.
Methods
Bulk RNA sequencing (RNAseq) from CD138+ selected cells obtained from patients across the disease course (smoldering (SMM), newly diagnosed (NDMM), early relapse (ERMM=1-3 PLOT) and late relapse (LRMM ≥4PLOT) was interrogated for expression of surface markers of known relevance in MM. Matched samples were also evaluated for expression of targets of interests with multiparameter flow cytometry. Antigen density assessment for each marker of interest was calculated utilizing the median fluorescence intensities (MFI) of CD38, CD138, CD20, TACI, CD70, CD44, GPRC5D and IGTB7 under saturating antibody conditions for antigen density measurements. Quantitative expression in molecules per cell (mol/c) was calculated by calibration with custom Quantibrite beads (BD Biosciences) for BCMA, Ly9, SLAMF7 and FCRL5 after controlling for the fluorophore to antibody ratio. Paired patient samples from baseline and at progression after BCMA directed CAR-T were also evaluated. As abundance of co-regulated proteins is often correlated, we also analyzed expression of key targets in the context of their known regulatory network (Renatino-Canevarolo et al, ASH 2022).
Results
In total, RNAseq data was available from N=731 CD138+ selected samples across disease states, (59 SMM, 187 NDMM, 303 ERMM, 182 LRMM) and flow cytometry was performed on N=192 samples (13 SMM, 65 NDMM, 66 ERMM, 48 LRMM) with

matched RNAseq data. Examining the findings between paired samples demonstrated certain markers have better correlation between gene expression and surface protein expression than others, with highest concordance seen for LY9 (r=0.6, p<0.0001), BCMA (r=0.5, p<0.0001) and IGTB7 (r=0.5, p<0.0001). Weak correlation for CD38 (r=0.3, p=0.0004), SLAMF7 (r=0.3, p<0.0001), CD44 (r=0.3, p=0.0003), FCRL5 (r=0.3, p=0.0004), CD19 (r=0.2, p=0.01), CD138 (r=0.2, p=0.002) and TACI (r=0.2, p=0.008), and no significant correlation for CD70 (r=0.09, p=0.2) and GPRC5D (r=0.05, p=0.5) was observed. Bulk seq analysis also identified that several of the genes encoding targets of interest are regulated by a common geneset, e.g. BCMA/CD138/SLAMF7 and FCRL5/TACI.

Certain markers were noted to have higher protein expression over the disease course, in particular BCMA (p=0.0036, **Figure 1**). In contrast, CD38 (p=0.004), CD19 (p<0.0001), TACI (p=0.008), SLAMF7 (p<0.0001), GPRC5D (p=0.02) had lower expression levels as the disease progressed, while LY9, CD138, FCRL5, ITGB7 were stable/similar across disease states. Median protein expression in mol/c in LRMM samples was **593** (range 179-4,678) for BCMA, **11,869** (range 251 - 50,482) for Ly9, **516** (range 84-1,998) for FCRL5 and **5,821** (range 1779 - 20,752) for SLAMF7. Looking at patients with paired plasma cells pre- and post- CAR-T, changes in protein expression in mol/c were variable, although the majority at baseline and relapse had surface expression levels below expected median values identified in the larger cohort with LRMM.

Conclusion:

This large dataset of paired patient samples across the MM disease spectrum provides a comprehensive description of the differences in expression of key immunotherapy targets with important implications for how agents might be sequenced, and what combinatorial strategies could be employed to optimize response. Expression levels of targets may be one of many factors involved in progression after administration of immunotherapy that may be of variable importance depending on the target, and timing and nature of therapy employed.

KHS & FLL contributed equally

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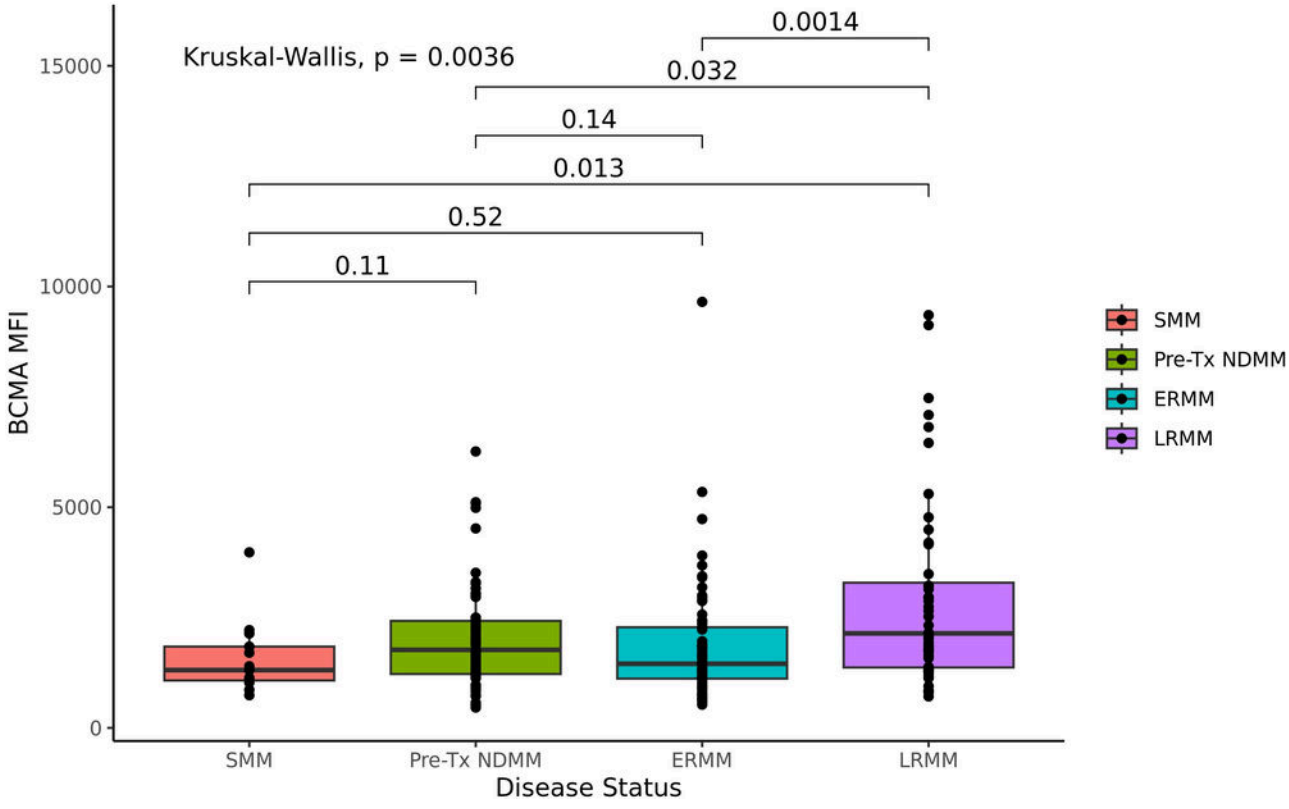

Figure 1

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