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POSTER ABSTRACTS

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

The Impact of Soluble BCMA and BCMA Gain on Anti-BCMA Immunotherapies in Multiple Myeloma

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B cell maturation antigen (BCMA) targeting chimeric antigen receptor T cells (CAR T) and bispecific T cell engagers (TCE) have remarkable efficacy in multiple myeloma (MM). While BCMA antigen escape, driven by *TNFRSF17* deletions or mutations, is a mediator of anti-BCMA CAR T/ TCE resistance, the majority of patients retain BCMA surface expression at progression. Furthermore, patients with high disease burden and extramedullary disease associated with poorer response to anti-BCMA therapies, have high levels of soluble BCMA (sBCMA). The mechanisms mediating this resistance, and in particular the contribution of sBCMA, have not been fully elucidated.

In order to assess the effect of sBCMA on anti-BCMA CAR T function, we measured CAR T cells activation with an in-house manufactured anti-BCMA CAR transduced with an NFAT-GFP reporter gene after co-culture with increasing concentrations of sBCMA (0-2500 ng/mL). sBCMA bound to CAR scFv in a dose dependent manner, effectively competing with the fluorophore tagged BCMA peptide binding. Despite their stable engagement, sBCMA failed to induce the expression of GFP, CD69, CD25, or 41BB on CAR T cells. To further examine the functional effect of sBCMA on CAR T cytotoxicity, we generated isogenic OPM2 cell lines with distinct BCMA expression profiles: OPM2 over-expressing BCMA (OPM2_BCMA ^{high}) generated by lentiviral transduction of *TNFRSF17*, and Cas9 BCMA knockout OPM2 (OPM2_BCMA $^{-/-}$). OPM2_BCMA ^{high} cells exhibited one-log-fold increase in surface BCMA expression and 10-fold increase in sBCMA and were more resistant to CAR T mediated lysis. In addition, in co-culture assays, elevated sBCMA impaired OPM2 target cell lysis by anti-BCMA CAR T in a dose dependent-manner. To gain insight into the effect of chronic sBCMA exposure on CAR T, we exposed anti-BCMA CAR T to OPM2 cells secretome in the absence of direct cell to cell contact in a trans-well co-culture system. On day 24, CAR T in co-culture with OPM2_BCMA ^{high} (sBCMA levels of 2343 ng/mL) significantly contracted with no viable cells, while CAR T in culture with parental OPM2 (sBCMA level of 267 ng/mL) did persist with up-regulated TIM3 and TIGIT. In contrast CAR T cultured with OPM2_BCMA $^{-/-}$ remained viable with no expression of exhaustion markers.

In examining the impact of sBCMA on TCE activity, sBCMA level as low as 50 ng/mL *in vitro* impaired anti-BCMA TCE binding to K562 cells transduced to stably express BCMA (K562_wtBCMA) . Consistent results were found in cytotoxicity assays wherein the addition of 2 ng/mL of sBCMA was sufficient to induce a reduction in anti-BCMA TCE mediated cytotoxicity of K562_wtBCMA cells by healthy donor peripheral blood mononuclear cells.

To identify genomic events that lead to elevated sBCMA expression and subsequent resistance to CAR T/TCE, we conducted bulk whole genome sequencing (100X) on CD138+ bone marrow MM cells obtained from 40 MM patients treated with anti-BCMA CAR T/ TCE. Structural variants (SVs) at or adjacent to *TNFRSF17* locus were identified in four patients. These included i) focal copy number gain of *TNFRSF17* (3 copies) in a patient who progressed within 3 months post anti-BCMA TCE, ii) subclonal duplication of *TNFRSF17* locus in another patient (n=1), as well as iii) focal copy number losses 5′ or 3′ to the *TNFRSF17* gene locus (n=2). In addition to SVs affecting *TNFRSF17* locus, we also identified mutations involving *TNFRSF17* transcriptional regulators. Notably, MM clones at progression 4 months post anti-BCMA TCE (n=1) harboured copy number gains (3 copies) of *POU2AF1* gene on chr11q23. *POU2AF1* encodes a key transcriptional regulator of *TNFRSF17* expression. Indeed, sBCMA level from this patient was significantly elevated at relapse at a level (638 ng/mL) correlated with resistance to anti-BCMA TCE. In addition, we identified translocation involving *MAP3K14* encoding the NF-kB regulator NIK in a patient with short remission post anti-BCMA CAR T. Of interest, analysis of the CoMMpass dataset revealed significantly higher BCMA transcripts in patients with translocations involving *MAP3K14* locus.

Our findings highlight the impact of sBCMA levels and its chronicity of exposure on anti-BCMA TCE and CAR T activities in MM. We further identified structural genomic events driving *TNFRSF17* over-expression and their correlation to sBCMA levels facilitating tumoral immunotherapeutic escape.

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