(DNAM1 Axis Expression in MM)

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The Expression of the PVRIG/TIGIT Pathway is Dominant in the Bone Marrow of Patients with Multiple Myeloma

Blocking inhibitory immune receptors has shown limited clinical benefit in patients with multiple myeloma (MM), warranting the discovery of alternative immune inhibitory pathways. Blockade of the immune checkpoint TIGIT was shown to enhance anti-tumor immunity in MM pre-clinical models. In addition to TIGIT, the DNAM1 axis includes the novel inhibitory receptor PVRIG. Both TIGIT and PVRIG deliver inhibitory signals to T and NK cells and compete with the co-activating receptor DNAM1 for binding to PVR and PVRL2, respectively. Accordingly, TIGIT and PVRIG co-blockade was shown to synergize in enhancing anti-tumor immunity in preclinical models. PVRL2 and PVR were shown to be expressed on MM plasma cells in the bone marrow (BM). In this study, we evaluated DNAM1 axis receptor expression in the BM of patients with MM.

BM mononuclear cells derived from 21 patients with MM were analyzed for the expression of PD1 and DNAM1 axis molecules by flow cytometry. Patients classified with progressed disease (PD), or complete response (CR) were treated with multiple lines of therapies including targeted therapies, chemotherapies, proteasome inhibitors.

PVRIG demonstrated the highest expression among all evaluated receptors on NK (88%), NKT (81%) and $CD8^+$ T cells (79%), significantly higher than PD1 ($p<0.0001$) and TIGIT ($P<0.001$). TIGIT showed substantially increased expression on NK (64%, $P<0.0001$) and $CD8^+$ T cells (58%, $p<0.05$) compared to PD1 (expressed on 12% of NK cells and 42% of $CD8^+$ T cells). Importantly, 50% and 60% of $CD8^+$ and NK cells, respectively, co-expressed TIGIT and PVRIG, and all examined cell populations showed increased levels of DNAM1 (>50%). Additionally, the expression of PVRIG ligand, PVRL2, on BM plasma cells from 6 patients with MM was demonstrated by immuno-histochemistry and flow cytometry.

A fraction of $CD8^+$ T cells were $DNAM1^-$ and positive for PVRIG/TIGIT/PD1 (22%), suggesting the accumulation of an exhausted $CD8^+$ T cell population in the MM tumor microenvironment. PVRIG showed significantly higher ($p<0.0001$) expression on $DNAM^+$ $CD8^+$ T cells (81%), compared to TIGIT (43%) and PD1 (34%), supporting the potential of PVRIG blockade to enhance DNAM1 signaling and subsequent $CD8^+$ T cell activation. Finally, patients with CR had a trend towards higher DNAM1 expression on $CD8^+$ T cells (75%) compared to PD patients (54%, $p=0.057$).

To conclude, DNAM1 axis receptors are dominantly expressed on MM BM lymphocytes, with PVRIG exhibiting the most prominent expression. The reduced expression of DNAM1 in patients with PD

suggests a link between DNAM1 axis and clinical outcome. Our findings highlight for the first time the dominant expression of PVRIG, as well as TIGIT and suggest that combined blockade of these checkpoints may potentially benefit patients with MM, placing the DNAM1 axis as a promising therapeutic pathway in MM therapy.