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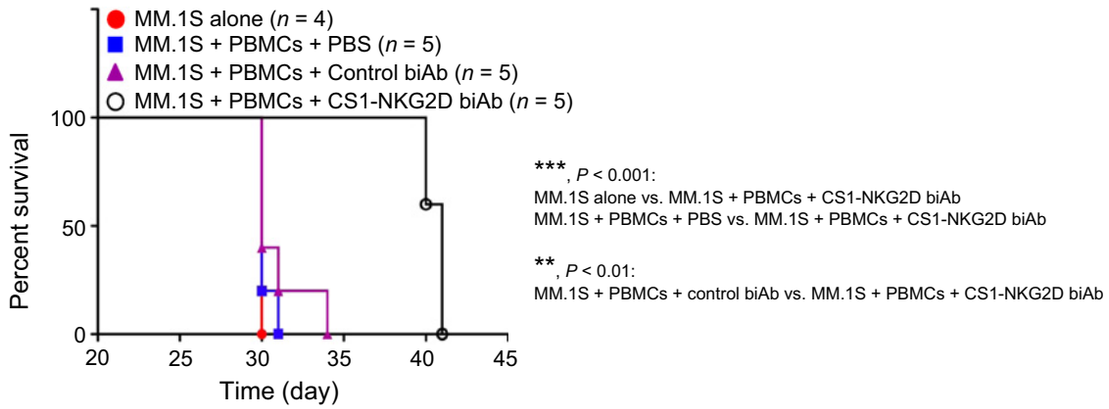

Figure 7.

Prolonged survival in MM bearing NSG mice received human effector cells and CS1-NKG2D biAb. **A,** Kaplan–Meier curve for NSG mice that received MM.1S MM cells alone ($n = 4$), MM.1S MM cells + human PBMCs + PBS ($n = 5$), MM.1S MM cells + PBMCs + control biAb ($n = 5$), or MM.1S MM cells + PBMCs + CS1-NKG2D biAb ($n = 5$). A total of 5×10^6 MM.1S MM cells and 5×10^6 PBMCs were used. Dose of control biAb or CS1-NKG2D biAb was 200 μg/kg. Data from experiments using human PBMCs of two healthy donors. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ using log-rank test.

anti-NKG2D component of the CS1-NKG2D biAb had a binding affinity in the nanomolar range, thus potentially leading to a more potent activation of $NKG2D^+$ cytolytic effector cells (21). The possibility exists that CS1-NKG2D–activated effector cells could have bystander killing effects on the $CS1^-$ MM cells. However, in our experiment using MM patient samples as target cells, we found a reduction of the $CS1^+CD38^{bright/+}CD138^+$ subset, but not of the $CS1^-CD38^{dim/+}$ subset. More experiments are needed to confirm the possible bystander effects from the CS1-NKG2D–activated effector cells.

The ligands for NKG2D are often expressed by tumor cells, both as membrane bound and in secreted forms, with the latter proposed as a mechanism of tumor escape (33). Zhang and colleagues proposed a biAb protein fusing an anti-CD3 scFv and the extracellular domain of NKG2D (30). This scFv–NKG2D fusion protein made use of the ligand binding ability of NKG2D to probe for NKG2D ligand–expressing tumor cells. However, there would be concern that this strategy, or others using similar NKG2D ligands as a binding domain (34), may be limited in efficacy because of the massive amounts of NKG2D ligands that can be shed from the tumor cell surface into both the microenvironment and the circulation, thereby chronically desensitizing $NKG2D^+$ effector cells (35, 36). Thus, triggering NKG2D via the CS1-NKG2D biAb described herein may circumvent this potential limitation of effector cell desensitization. Previous reports suggest the NKG2D ligand MICA can shed from MM cell surfaces, which can desensitize NK cells (29). A low concentration of circulating soluble CS1 (no more than 10 ng/mL) is also detectable in serum of MM patients (28). These soluble targets may potentiate the effect of CS1-NKG2D biAb. However, our experiments show no significant difference in the E:T synapse formation and effectors' cytotoxicity against MM1.S cells in the presence of biAb with or without a soluble NKG2D-Fc chimera or a CS1 protein.

We demonstrated that both $CD3^+$ T cells and $CD56^+$ NK cells contributed to the *in vitro* cytotoxicity against $CS1^{high}$ MM cells. This corroborated our hypothesis that the activation of different $NKG2D^+$ immune effector cells provided improved cytotoxicity when compared with either effector population alone. We also observed that IL2 is required for the functional activation of NKG2D induced by the CS1-NKG2D biAb. Our group and others have previously proposed using IL2 combined with targeted therapy for the treatment of non-Hodgkin lymphoma (37, 38). IL2 combined with zoledronate was demonstrated to be a feasible maintenance therapy for multiple myeloma in a phase II clinical trial (39). We foresee that the combination of IL2 and the CS1-NKG2D biAb deserves a future clinical exploration.

In our preclinical model of MM using NSG mice, those engrafted with $CS1^{high}$ MM.1S MM cells showed significantly prolonged survival, whereas those engrafted with $CS1^{int}$ MM NCI-H929 cells showed a less significant effect. This finding corroborates with the *in vitro* results, where the EC_{50} for MM.1S MM cells was one log lower than that for NCI-H929 MM cells. These data suggest that the efficacy of our CS1-NKG2D biAb *in vivo* is likely to be dependent not only on the fraction of cytolytic immune effector cells, but also on surface density expression of CS1 by MM. However, approximately 95% of MM patients have high CS1 expression, and the expression remains stable after common MM drug treatment such as VDTPACE or bortezomib (40). Thus, although the preclinical data appear promising in providing improved activity over the naked anti-CS1 mAb in MM, clinical efficacy might prove to be restricted to the $CS1^{high}$ subset of MM patients and might be enhanced by improved immune modulation of $NKG2D^+$, effector populations *in vivo*.

Disclosure of Potential Conflicts of Interest

W.K. Chan, M.A. Caligiuri, and J. Yu have submitted a patent through The Ohio State University for the CS1-NKG2D bispecific antibody described in this report. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.K. Chan, S. Kang, Y. Youssef, E.N. Glankler, E.R. Barrett, A.M. Carter, E.H. Ahmed, A. Prasad, L. Chen, D.M. Benson Jr
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W.K. Chan, S. Kang, Y. Youssef, J. Zhang, J. Yu
Writing, review, and/or revision of the manuscript: W.K. Chan, S. Kang, Y. Youssef, E.R. Barrett, A. Prasad, L. Chen, D.M. Benson Jr, M.A. Caligiuri, J. Yu

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Study supervision: W.K. Chan, S. Kang, M.A. Caligiuri, J. Yu

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