Evaluating Efficacy and Immune and Non-Immune Toxicities of Immunotherapy Agents in Humanized Mouse Models of Multiple Myeloma

Translational

Hematology

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ABSTRACT

Chimeric antigen receptor (CAR) T-cell and T-cell engager (TCE) therapies are transforming the treatment landscape of multiple myeloma (MM). Despite their remarkable clinical efficacy, a substantial proportion of patients develop immune- and non-immune–mediated toxicities that limit therapeutic applicability and contribute to disease relapse. A mechanistic understanding of individual undesired adverse events is therefore essential to identify patients at risk and to develop therapeutic strategies to mitigate these side-effects. We previously generated a comprehensive panel of genetically engineered mouse models that faithfully recapitulate the major biological features of human MM (Larrayoz et al., Nat Med 2023). Building on this platform, we selectively replaced endogenous murine genes with their human counterparts encoding key immunotherapy targets, enabling the development of immunocompetent mice that develop human-like MM expressing clinically relevant human antigens. In MICγ1CRBN-I391V mice humanized for BCMA and CD3εδγ, BCMA-directed TCEs induced rapid and durable complete remissions with significantly prolonged survival. These responses were accompanied by T-cell exhaustion and secretion of cytokines associated with cytokine release syndrome (CRS). Notably, combination of the BCMAxCD3 TCE with a CELMoD, or with an anti-Lag3 monoclonal antibody, markedly reduced immune toxicity while preserved antitumor efficacy. In the same model, a murine BCMA CAR T-cell surrogate engineered to express IL-15 elicited potent antimyeloma activity in vivo, but this was accompanied by significant neurotoxicity, manifested as Parkinson-like phenotypes with widespread T-cell infiltration of the brain, marked accumulation of Iba1$^+$ microglial cells, and a reduction in tyrosine hydroxylase (TH)–positive neurons in the substantia nigra. In a second MM model with humanized GPRC5D, we explored therapy-related, non-immune toxicities such as skin rash and dysgeusia, which may limit clinical development of TCEs targeting GPRC5D. Humanized mice selectively recapitulated expression of human GPRC5D in skin hair follicles and filiform papillae of the tongue. In vivo treatment with a GPRC5DxCD3 biosimilar TCE resulted in strong antitumor responses accompanied by non-immune toxicities, characterized by extensive CD4$^+$ and CD8$^+$ T-cell infiltration in skin and tongue tissues surrounding GPRC5D-expressing cells. A third model incorporating humanized CD38 in tumor cells together with human FcγRI, FcγRIIa/b, and FcγRIII enabled the in vivo assessment of anti-CD38 antibody–mediated tumor killing through cellular cytotoxicity (ADCC) and phagocytosis (ADCP). Finally, we developed a multi-humanized immunotherapy platform using murine MM cell lines engineered to express human BCMA, GPRC5D, CD38, and/or FcRH5, engrafted into immunocompetent C57BL/6 mice with humanized CD3, CD28, and CD137 (4-1BB) in T cells. This versatile MM platform enables the evaluation of multiple immunotherapy agents, such as tri-specific antibodies, multi-target CAR T cells, and combination of TCEs and co-stimulatory molecules. Collectively, these humanized multiple myeloma models constitute a robust and clinically predictive platform for the preclinical evaluation of next-generation immunotherapies.

SCIENTIFIC IMPACT

Our humanized multiple myeloma models constitute a major advance in preclinical immuno-oncology by enabling simultaneous, mechanistic evaluation of antitumor efficacy, immune activation, and immune-mediated as well as on-target/off-tumor toxicities in an immunocompetent, disease-relevant setting. By faithfully recapitulating clinically observed therapeutic responses and adverse events across multiple immunotherapy modalities, the platform provides critical insight into the biological determinants of efficacy and toxicity. Its modular design supports biomarker discovery, rational dose and combination optimization, and patient risk stratification, thereby strengthening the translational relevance of preclinical studies and accelerating the development of safer, more effective immunotherapies for multiple myeloma.

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