Evaluation of Efficacy and Toxicity of CD137 Immunotherapy with Urelumab-mlgG1 Chimeric Antibody in CD137 HuGEMM™

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INTRODUCTION
CD137 (4-1BB) is a powerful T cell co-stimulatory molecule which belongs to the TNF receptor superfamily, promoting cytotoxic T cell survival and memory formation upon CD137L ligation. CD137 has become an attractive immuno-oncology therapeutic target with multiple agonistic antibodies in clinical testing, including urelumab and umolubumab, with promising responses in combination with anti-PD-1 immunotherapies such as nivolumab. However, clinical application of CD137 agonistic antibodies is hampered by dose-limiting off-tumor liver toxicity (urelumab) or lower efficacy (umolubumab). The cause of liver toxicity is reported primarily due to Fcγ receptor mediated cross linking4). CD137 agonistic antibodies may also trigger hepatotoxicity through activation of IL-17 secreting liver Kupffer cells and monocytes2). The remaining challenge to decouple the efficacy from liver toxicity is the lack of preclinical mouse models which can be used to assess both efficacy and immune-related adverse events (irAE) of human CD137 agonistic antibodies.

METHODS
To mimic the clinical outcome of urelumab, we utilized a humanized CD137 knock-in mouse (CD137 HuGEMM) to evaluate its efficacy against CT26.WT syngeneic tumors. Tumor growth inhibition (TGI) was calculated as: TGI% = (1-T1/T0)*100; T0 as the mean tumor volume of the treatment group on the measurement day; T1 as the mean tumor volume of control group at the measurement day. Liver toxicity was analyzed by monitoring the fasting serum ALT/AST levels at different time points after the final dose. Liver histology and immune cell infiltration were analyzed by H&E and IHC.

RESULTS
Urelumab showed significant anti-tumor response at the dose level of 5mg/kg (TGI>77.2%), while serum ALT/AST levels showed no difference compared to isotype control, suggesting that due to the different binding capacity of human IgG4 Fc domain to mouse FcγR, the human version of the agonistic antibody cannot fully recapitulate its effect on HuGEMM mice. Therefore, a chimeric antibody with mouse IgG1 Fc domain (urelumab-mlgG1) was created to disentangle the potential role of FcγR mediated cross linking in both efficacy and liver toxicity. An urelumab-mlgG1-DANA variant with D265A/N297A mutation to abolish Fc effector function was also included as a dominant negative control. We found that urelumab-mlgG1 showed further enhanced efficacy (TGI=92%) compared to urelumab through FcγR mediated cross linking, while urelumab-mlgG1-DANA showed compromised anti-tumor response. In addition, urelumab-mlgG1 caused chronic liver inflammation and hepatocyte damage indicated by immune cell infiltration in the liver and significantly elevated serum ALT levels, which was also abolished by the urelumab-mlgG1-DANA variant. The study also compared the treatment of urelumab in CD137 HuGEMM head-to-head with a mouse surrogate agonistic antibody (3H3) in wild-type BALB/c mice. 3H3 showed robust tumor growth inhibition as well as dramatic ALT elevation, F4/80 macrophage and CD8 T cell infiltration to liver tissue.

REFERENCES

CONCLUSION
The clinically observed liver toxicity associated with tumor growth inhibition by urelumab was recapitulated using a chimeric version of urelumab in CD137 HuGEMM indicating the importance of both the mouse model and antibody version in evaluation of efficacy and irAE.