543 CIS-BINDING/BLOCKADE OF CD47 BY CD47XPD1 BSAB HX009 ENHANCED PD1 BLOCKADE INDUCED T-CELL ACTIVATION

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Background Immune-checkpoint inhibitors (ICI) like PD1/PD-L1 blockers have been great successes in cancer immunotherapy. It normalizes anti-tumor immunity by reversing effector T cells (T_{eff}) exhaustion within TME. However, majority of treated patients still responded poorly, even for melanoma. Insufficient SIRPα-expression (CD47 ligand) on melanoma cells was blamed for the poor response, leading to a new theory and subsequently confirmed at pre-clinical setting: simultaneous targeting tumor-infiltrate (TIL)-$CD8^+$ T_{eff} by PD1 mAb and the tumor-expressing SIRPα are both required for long-term reversal of exhaustion.[1] We thus hypothesized that PD1xCD47-dual targeting BsAb (HX009)could potentially enhance T-cell activation over PD1-mAb, seemingly consistent with our earlier observation that HX009 consistently showed higher T-cell activation (~3x per EC_{50} value) in a reporter assay over PD1-mAb (HX008),[2] even with low affinity SIRPa/CD47 binding.

Methods A two-cell reporter system containing $PD\text{-}1^+CD47^+$ Jurkat reporter cell (luciferase) and PD-L1-expressing APC was used, where Jurkat cell carries an IL-2 promoter-guided luciferase gene expression that mimics T-cell activation quantitively. Seriel dilutions of HX009/HX008, in the presence of various competing or combining molecules, were added in the co-culture system to investigate T cell activation.

Results We repeated the same reporter assay with the presence of SIRPa-neutralizing mAb, which reduced T-cell activation of HX009 to that of HX008, confirming that the T-cell activation difference between HX009 and HX008 indeed resulted from the T-cell surface CD47 engagement by SIRPa on HX009. In contrast, the addition of CD47 mAb (as equal molar as the neutralizing SIRPa mAb) had no effect on T-cell activation by HX009, suggesting that free CD47 mAb at the tested concentration could not meaningfully engage T-cell surface CD47. Our interpretation is that although with magnitude lower affinity than PD1 mAb, SIRPa's binding avidity to T-cell CD47 remains high due to '*cis*-binding' driven by the PD1 mAb high affinity binding. This was further confirmed by that soluble SIRPa (SIRPa-Fc) had no enhanced effect when combined with HX008 (PD1 mAb). In another word, HX009 BsAb is superior to the combo of HX008 and SIRPa-Fc. This was also further confirmed with the observatiions that HX009 can compete with high-affinity CD47 mAb binding to $PD1^+CD47^+$ double positive T-cells, but not to the $CD47^+$ single positive cells (no enhanced avidity).

Conclusions HX009 CD47xPD1-BsAb T-cell could have potential superior T-cell activation than PD1-mAb, which could be potentially translated into stronger immunotherapy efficacy.

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