An Affinity Threshold for Maximum Efficacy in Anti-PD-1 Cancer Immunotherapy

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Abstract

Monoclonal antibodies (mAbs) targeted to the programmed cell death protein 1 (PD-1) remain the most prevalent cancer immunotherapy both as a monotherapy and in combination with additional therapies. Despite the extensive success of anti-PD-1 mAbs in the clinic, the experimental relationship between binding affinity and functional potency for anti-PD-1 antibodies in vivo has not been reported. Two widely-used FDA-approved anti-PD-1 antibodies, nivolumab and pembrolizumab have similar single digit nanomolar equilibrium binding constants (K_D). Anti-PD-1 antibodies with higher and lower affinity than nivolumab or pembrolizumab are entering the clinic and show varied pre-clinical efficacy. Here, we explore the role of broad-ranging affinity variation on efficacy within a single lineage in a syngeneic immunocompetent mouse model.

Using yeast surface display and affinity maturation, we engineered a panel of murine anti-PD-1 antibodies with varying affinity (ranging from K_D = 20 pM - 15 nM). A combination of equilibrium and kinetic sorting strategies was used to both improve and reduce the affinity of a parental anti-PD-1 clone, 29F.1A12. We characterized the affinity of the antibodies using a number of methods, including ELISA, off-rate bead assays, and bio-layer interferometry, to confirm the wide range of affinity. We also characterized the internalization rate and pharmacokinetic clearance rate of our antibodies to confirm a consistent drug profile aside from mPD-1 affinity. Using these experimentally-determined rates and literature values, we developed a physiologically-based pharmacokinetic (PBPK) model to complement the *in vivo* results and highlight the direct relationship between dose, affinity, and PD-1 target saturation in the tumor.

Through *in vivo* efficacy studies using the MC38 murine adenocarcinoma model, we found that there is an affinity threshold required for maximum efficacy at a given dose of anti-PD-1 immunotherapy. We demonstrate that efficacy can be rescued by increasing the dose of a low affinity clone. Thus alternatively, we show that for a given affinity, there is a dose threshold required to achieve maximum efficacy. Ultimately, we conclude that the anti-PD-1 affinity/dose relationship supports a clear receptor-occupancy mechanism of action.

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