978 STAGE-APPROPRIATE BIOASSAYS FOR ASSESSING ADCP ACTIVITY OF THERAPEUTIC ANTIBODIES

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Background Antibody-dependent Cellular Phagocytosis (ADCP) is an important mechanism of action (MOA) of therapeutic antibodies designed to deplete tumor cells. ADCP is mediated by effector cells such as monocytes and macrophages, and is induced via simultaneous binding of antibodies to FcγRIIa, FcγRI, or FcγRIIIa on effector cells and a specific antigen on target cells. Traditionally, direct measurement of ADCP relies on ex vivo differentiation of primary macrophages followed by flow cytometry assays. These protocols are laborious and produce highly variable results, due to the diversity of primary cell donors.

Methods We have developed two novel plate-based bioassays for measuring the ADCP activity of therapeutic antibodies, with utility at different stages in the drug development process. For early phase programs, we have created an ADCP assay based on NanoBiT split luciferase technology. Here, primary macrophages are incubated with engineered HiBiT-expressing target cells. At the end of the incubation period, cells are lysed and HiBiT retained in the target cells is released to complement LgBiT in the detection reagent, producing light in proportion to the number of target cells present. Lysosomal degradation of HiBiT following ADCP results in a robust loss of luminescence in the presence of ADCP-inducing antibody. For later phase programs, we have developed an ADCP reporter bioassay in a naturally phagocytic cell background, THP-1, where NanoLuc luciferase reporter activity is driven by signaling through endogenously expressed Fc receptors.

Results The HiBiT ADCP Assay is highly reproducible and easy to execute with little hands-on time, and can be used with many common model tumor cells. It can be used to demonstrate both ADCP activity and specificity of therapeutic antibodies, making it ideal for bridging assays.The THP-1 ADCP reporter bioassay has been prequalified according to ICH guidelines and is suitable for potency and stability studies in quality-controlled settings.

Conclusions Together, these assays expand the toolbox for characterization of ADCP-inducing antibodies, and represent significant advancement in reproducibility, workflow, and biological relevance of ADCP assays for drug development.

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