1377 PRECLINICAL CHARACTERIZATION OF D3L-001, A NOVEL BISPECIFIC ANTIBODY THAT ENHANCE PHAGOCYTOSIS AND ERADICATION OF HER2 POSITIVE SOLID TUMOR VIA HER2 AND CD47 DUAL BLOCKADE

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Background HER2 is overexpressed in different solid tumors, including 15-20% of breast cancer. The advent of HER2-targeted drugs, including antibodies (Ab), TKI and ADC, have revolutionized HER2+ cancer treatment; however, the disease will eventually recur in most patients. Recent studies have suggested efficacy of HER2 target antibodies (Ab) therapy could be further enhanced by antibody-dependent cellular phagocytosis (ADCP) principally regulated by antiphagocytic "don't-eat-me" CD47 signals. CD47 is overexpressed in many HER2+ cancers, which suppresses phagocytosis through binding to SIRPα

In this study, an internally discovered anti-HER2×CD47 bispecific antibody (bsAb), D3L-001, demonstrated synergistic anti-tumor effect by HER2 guided CD47 co-blocking. It enhances macrophage mediated phagocytosis and significantly suppresses *in vivo* tumor growth while sparing hematological toxicities, which are typically induced by anti-CD47 antibodies.

Methods SK-BR-3, HCC1954 and Jurkat cells were used. Cellular binding and *in vitro* blocking of CD47 and SIRPα interaction by Abs were measured by FACS. Monocytes were isolated from PBMC and differentiated into macrophages, which were then used for ADCP assays. Abs *in vivo* efficacy were examined in HER2+ tumor models.

Results D3L-001 was designed to have higher HER2 affinity (K_D < 1 nM) than that of CD47 (K_D >10 nM). With this unique design, D3L-001 showed preferential binding to HER2/CD47 double positive tumor cells as compared to CD47 single positive cells (figure 1). This preferential binding prevents D3L-001 from inducing red blood cell hemagglutination *in vitro*. Moreover, its binding to HER2+ tumor cells wasn't affected by whole blood cell pre-culture treatment, indicating low systemic CD47 antigen sink effect. Intriguingly, we found that D3L-001 can block the interaction between SIRPα and CD47 on HER2+ tumor cells very effectively, probably due to the avidity effect induced by the addition of HER2 binding arm (figure 2). This potent blocking translated well into enhanced *in vitro* phagocytosis ability. D3L-001 showed stronger anti-tumor effect than trastuzumab in a panel of HER2+ tumor models. We observed significant tumor growth inhibition and regression in trastuzumab resistant xenograft models in a dose-dependent manner. D3L-001 demonstrated better efficacy than combination of trastuzumab and magrolimab, indicating synergistic effect of co-blocking HER2 and CD47 in bispecific form. In addition, the combination of D3L-001 with pertuzumab also showed synergistic *in vivo* efficacy.

Conclusions D3L-001 is a novel HER2×CD47 bsAb which demonstrated potent and synergistic anti-tumor effect via HER2 guided CD47 co-blocking in both *in vitro* and *in vivo* models. D3L-001 might provide a novel treatment approach for HER2+ cancers and overcome their resistance to current therapies.

Fig. 1A

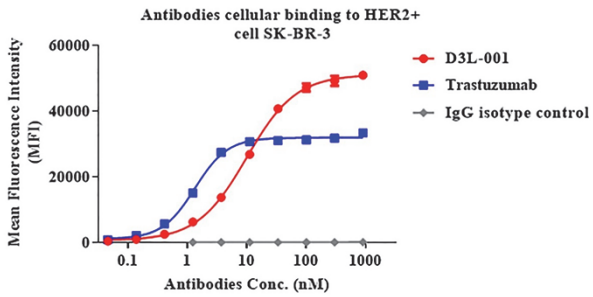
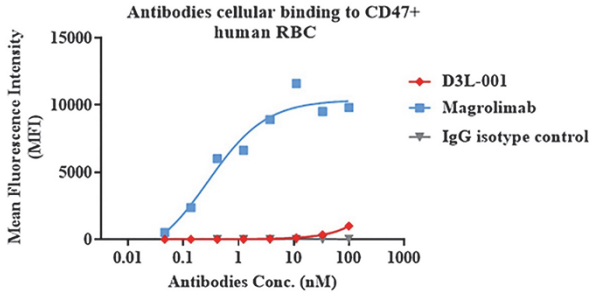
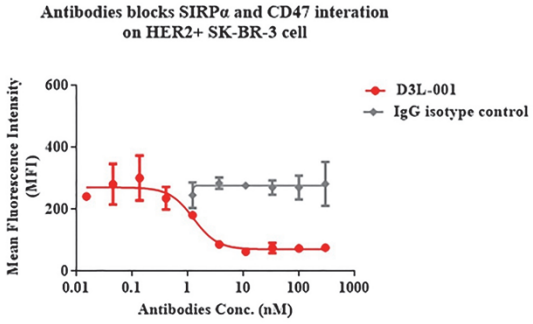


Fig. 1B



Abstract 1377 Figure 1 In vitro cellular binding of D3L-001 to HER2/CD47 double positive SK-BR-3 cell (A) and CD47 single positive red blood cell (B).

Fig. 2



Abstract 1377 Figure 2 In vitro cellular blocking of SIRPα and CD47 interaction by D3L-001

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