DISCOVERY OF NXA01, A NOVEL CD24 ANTIBODY WITH TUMOR SELECTIVITY AND POTENT ANTI-TUMOR ACTIVITY

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Background CD24 is a small glycosylphosphatidylinositol (GPI)-anchored glycoprotein overexpressed on multiple types of cancers including lymphoma, breast cancer, ovarian cancer, where it reportedly regulates cell migration, invasion, proliferation and immune escape. Apart from being viewed as a tumor-associated antigen (TAA), CD24 was recently found to suppress tumor phagocytosis by delivering a ‘don’t-eat-me’ signal via binding to Siglec-10 expressed on tumor-associated macrophages. We hypothesize that selective tumor targeting of the CD24-Siglec-10 pathway by anti-CD24 mAb may represent a novel therapeutic approach to enhance anti-cancer immunity while avoiding potential on-target off-tumor toxicity.

Methods We immunized mice with the extracellular portion of human CD24 purified from both the E. coli and 293T expression systems and screened for hCD24-binding hybridomas by ELISA. We evaluated the affinity of binders by SPR and selective tumor cell binding over normal cells by FACS. We profiled lead antibodies in ADCC and ADCP co-culture studies and in vivo tumor cell efficacy models. We humanized the best clone in silico and affinity matured it by saturation mutagenesis. We determined the epitope of the lead candidate (NXA01) by overlapping peptide blocking and co-crystallography studies.

Results With an affinity of 1.5 nM as determined by SPR, NXA01 bound to hCD24 on tumor cells such as NALM6 and MDA-MB-468 but not to normal human granulocytes or B cells. This tumor selectivity was related to preferential binding of NXA01 to aglycosylated or hypoglycosylated hCD24 as enzymatic deglycosylation of normal cells restored binding. NXA01 blocked hCD24-Siglec10 interaction with an EC50 of 8 nM and as a result enhanced ADCP activity of tafasitamab (anti-CD19). NXA01 could also mediate direct killing of NALM6 and MDA-MB-468 cells through both ADCC and ADCP at EC50s of <10 nM, but it spared normal B cells in a tumor/normal mixed cell culture, further indicating tumor selectivity. Finally, NXA01 treatment significantly inhibited the growth of MC38-hCD24, NALM6 and MDA-MB-468 tumor cells in vivo with maximal inhibition of 104%, 105% and 51%, respectively.

Conclusions NXA01 is a potential best-in-class CD24 blocking antibody that exhibits selective tumor binding and strong anti-tumor activity in vitro and in vivo. The dual mechanism of ADCC and macrophage checkpoint blockade makes NXA01 a good candidate for monotherapy as well as a combination partner with other TAA or immune-oncological drugs. IND-enabling studies are ongoing for NXA01.

REFERENCES

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