GT-001 - ANTI-LEWIS Y ANTIBODY WITH SUPERIOR FINE-SPECIFICITY AND REDUCED OFF-TARGET BINDING

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Background The Lewis Y (CD174) carbohydrate antigen is widely expressed in primary and metastatic epithelial tumors like colon, lung, ovarian, and breast. Targeting Lewis Y for cancer therapy was pursued before, however, other anti-Lewis Y antibodies tested in clinical trials showed cross-reactivity to related carbohydrate structures expressed on blood cells and mostly failed for efficacy and/or safety reasons.¹⁻⁴ We have developed a humanized antibody (GT-001) that shows superior fine-specificity and higher affinity compared to clinically tested anti-Lewis Y antibodies BR96 and h3S193.

Methods The specificity and cross-reactivity of GT-001, BR96 and h3S193 were compared. Cross-reactivity binding to related carbohydrate PAA-conjugates was tested via ELISA and affinity towards Lewis Y-PAA was measured using switch-SENSE® technology (DRX2, Dynamic Biosensors). Functional binding to several tumor cell lines and healthy human leukocytes was analyzed via flow cytometry. Binding of GT-001 to different cancer indications was analyzed by immunohistochemistry. Inhibition of tumor cell proliferation was tested using GT-001 coupled to ProtG-MMAE.

Results GT-001 is strictly specific for Lewis Y and does not cross-react with >90 related carbohydrate structures tested. Our lead candidate shows superior fine-specificity compared to BR96, for which we could confirm the reported cross-reactivity towards Lewis X,⁵ and stronger binding of Lewis Y compared to h3S193 as shown by affinity measurement. Further, GT-001 shows no/weak binding to blood cells whereas BR96 and h3S193 significantly bind to different leukocyte subsets. IHC studies reveal that GT-001 stains tumor tissue of different cancer indications (breast cancer, colorectal cancer, head and neck cancer, (non) small cell lung cancer and ovarian cancer) at a high percentage of cases. In ADC surrogate assays, GT-001 potently inhibits the proliferation of several tumor cell lines indicating effective internalization.

Conclusions Lewis Y is expressed on many epithelial tumor indications of high medical need. However, several approaches of targeting Lewis Y have failed in the past for efficacy and/or safety reasons. We have developed a humanized antibody that shows superior fine-specificity and higher affinity compared to clinically tested anti-Lewis Y antibodies BR96 and h3S193. Due to the superior fine-specificity, GT-001 shows no/reduced binding of healthy leukocytes potentially reducing side effects as observed for BR96 in the clinic. Its strong target binding and internalization properties make GT-001 an ideal candidate for ADC development.

REFERENCES

1. Ajani JA, Kelsen DP, Haller D, Hargraves K, Healey D. A multi-institutional phase II study of BMS-182248-01 (BR96-doxorubicin conjugate) administered every 21 days in patients with advanced gastric adenocarcinoma. *Cancer J* 2000;**6**(2):78–81.
2. Saleh MN, Sugarman S, Murray J, Ostroff JB, Healey D, Jones D, Daniel CR, LeBerherz D, Brewer H, Onetto N, LoBuglio AF. Phase I trial of the anti-Lewis Y drug immunoconjugate BR96-doxorubicin in patients with lewis Y-expressing epithelial tumors. *J Clin Oncol* 2000;**18**(11):2282–92.
3. Scott AM, Tebbutt N, Lee FT, Cavicchiolo T, Liu Z, Gill S, Poon AM, Hopkins W, Smyth FE, Murone C, MacGregor D, Papenfuss AT, Chappell B, Saunderson TH, Brechbiel MW, Davis ID, Murphy R, Chong G, Hoffman EW, Old LJ. A phase I biodistribution and pharmacokinetic trial of humanized monoclonal antibody

Hu3s193 in patients with advanced epithelial cancers that express the Lewis-Y antigen. *Clin Cancer Res* 2007;**13**(11):3286–92.

4. Smaletz O, Diz MD, do Carmo CC, Sabbaga J, Cunha-Junior GF, Azevedo SJ, Maluf FC, Barrios CH, Costa RL, Fontana AG, Madrigal V, Wainstein AJ, Yeda FP, Alves VA, Moro AM, Blasbalg R, Scott AM, Hoffman EW. A phase II trial with anti-Lewis-Y monoclonal antibody (hu3S193) for the treatment of platinum resistant/refractory ovarian, fallopian tube and primary peritoneal carcinoma. *Gynecol Oncol* 2015;**138**(2):272–7.
5. Zhang S, Zhang HS, Cordon-Cardo C, Reuter VE, Singhal AK, Lloyd KO, Livingston PO. Selection of tumor antigens as targets for immune attack using immunohistochemistry. II. Blood group-related antigens. *Int J Cancer* 1997;**73**(1):50–6.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.781>