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Immunotherapy of a Human Small Cell Lung Carcinoma (SCLC) Xenograft Model by the Bispecifie Molecule (BsMol) mAb22xLys^3-Bombesin (M22xL-BN)

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Introduction

Among the approaches being studied in cancer therapy is the application of bispecific monoclonal antibodies (BsAb), which are molecules that link two mAbs, each having different antigenic specificities. These BsAb are able to bridge autologous immune effector cells to tumor cells, thereby enhancing the specific lysis of the target tumor cells [1]. Also being studied for cancer immunotherapy are peptide growth factors linked to mAbs and these are known as bispecific molecules (BsMol) [2].

We have constructed a BsMol by conjugating a bombesin/gastrin releasing peptide (BN/GRP) analog, Lys^3-BN (L-BN), to the FcγRI-specific mAb22 (M22), and call this M22xL-BN [3]. We chose the BN/GRP receptor as our target because: 1) the majority of small cell lung carcinoma (SCLC) cell lines and biopsy specimens from SCLC patients express BN/GRP receptors [4], 2) BN, and its mammalian equivalent, GRP, can act as an autocrine growth factor in the proliferation of SCLC cells [5], and 3) synthetic BN/GRP antagonists and a mAb against GRP have been shown to inhibit SCLC tumor growth *in vitro* as well as *in vivo* [6-8]. On the other hand, FcγRI (CD64) is a potent immune trigger molecule that is expressed on a number of immune effector cells, such as monocytes (Mo), is up-regulated by a variety of cytokines, and binds to M22 without the interference of circulating immunoglobulins [9].

Our earlier studies have shown that M22xL-BN can effectively enhance the *in vitro* cytotoxicity of SCLC cells by cytokine-activated human Mo [3]. In order to further study the effectiveness of this immunotherapeutic approach, a SCLC xenograft model was established by innoculation of human SCLC (DMS273) cells into SCID/NOD mice. Immunotherapy was tested by injection of cytokine-activated Mo and M22xL-BN into these mice after the SCLC cells had been growing for 3 days.

Results and Discussion

The construction of the BsMol, M22xL-BN, was begun by reacting L-BN with SATA (Pierce) and the L-BN-SATA conjugate was purified by C_{18}-HPLC. Deacetylation with NH_2OH gave the free sulfhydryl, L-BN-SH, which was purified by C_{18}-HPLC. M22

Table 1. *Values of two experiments on the left represent tumor weight (mg ± SD), while the two on the right represent the number of peritoneal tumor cells ($1x10^5$ ± SD).*

Group A	490±130	710±400	3.1±2.8	1.9±0.8
Group B	320±170	540±200	2.3±0.2	1.6±1.4
Group C	820±360	630±250	4.4±2.0	1.3±0.7
Group D	25±25	90± 60	0.5±0.1	0.2±0.2

(Medarex, Inc.) was reacted with Sulfo-SMCC (Pierce) to produce a maleimide-containing Ab. The final conjugation of L-BN-SH with the maleimide-containing Ab was effected by mixing equimolar amounts overnight at RT [3].

SCID/NOD mice 6-8 weeks old (Jackson Lab) were maintained in pathogen-free facilities and irradiated with 300 rad immediately before the ip injection of $1x10^6$ DMS273 cells. All mice were checked periodically for evidence of tumor growth and were sacrificed at day 28. Mice (12-16) were divided into four groups and all received DMS273 cells on day 1. Cytokine-activated Mo ($1x10^7$) were mixed with either M22 (Group C) or M22xL-BN (Group D) prior to injecting the cells on day 3 and day 6. Control mice (Group A) received medium only and Group B received cytokine-activated Mo only on days 3 and 6. After the mice were sacrificed the peritoneal cavity was washed with 10 mL of normal saline and peritoneal exudate cells counted, stained and analyzed by flow cytometry. DMS273 cells were identified as positive for human CD15 (mAb PM81) and negative for mouse CD45. The peritoneal cavity was also examined for gross tumor nodules, which were dissected and weighed. The results from two separate experiments are shown in Table 1.

We have established a human SCLC xenograft model for immunotherapy studies in SCID/NOD mice and found that immunotherapy with the BsMol M22xL-BN is effective in this xenograft model and potentially applicable in a clinical setting, after optimal conditions are determined.

References

1. Fanger, M.W., Morganelli, P.M. and Guyre, P.M., *Critical Rev. Immunol.*, 12 (1992) 101.
2. Mokotoff, M., Chen, J., Zhou, J-H. and Ball, E.D., *Curr. Med. Chem.*, 3 (1996) 87.
3. Chen, J., Mokotoff, M., Zhou, J-H., Fanger, M.W. and Ball, E.D., *Clin. Cancer Res.*, 1 (1995) 425.
4. Moody, T.W. and Cuttitta, F., *Life Sci.*, 52 (1993) 1161.
5. Carney, D.N., Cuttitta, F., Moody, T.W. and Minna, J.D., *Cancer Res.*, 47 (1987) 821.
6. Avis, I.L., Kovacs, T.O.G., Kasprzyk, P.G., Treston, A.M., Bartholomew, R., Walsh J.H., Cuttitta, F. and Mulshine, J.L., *J. Natl. Cancer Inst.*, 83 (1991) 1470.
7. Heimbrook, D.C., Saari, W.S., Balishin, N.L., Fisher, T.W., Friedman, A., Kiefer, D.M., Rotberg, N.S., Wallen, J.W. and Oliff, A., *J. Med. Chem.*, 34 (1991) 2102.
8. Staley, J., Coy, D., Taylor, J.E., Kim, S. and Moody, T.W., *Peptides*, 12 (1991) 145.
9. Guyre, P.M., Graziano, R.F., Vance, B.A., Morganelli, P.M. and Fanger, M.W., *J. Immunol.*, 143 (1989) 1650.