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C-1027

Antineoplastic Antibiotic

Macromolecular antitumor antibiotic produced by *Streptomyces globisporus* C-1027 with a molecular weight of 15,000 dalton and an isoelectric point of pH 3.5-3.7. It is composed of a noncovalent bound protein moiety and a very labile nonprotein chromophore. The apoprotein is a single-chain polypeptide of 110 amino acids with two disulfide bonds and a molecular weight of 10,500 dalton. The chromophore was identified as a member of the macrocyclic enediyne-type antitumor antibiotics

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C-1027 is a new macromolecular antitumor antibiotic produced by *Streptomyces globisporus* C-1027 (1). This agent has moderate antimicrobial activity against Gram-positive bacteria but is inactive against Gram-negative bacteria except for some strains of *Escherichia coli* and fungi tested (2). A series of studies has also indicated that C-1027 exhibits extremely potent cytotoxicity against various tumor cells *in vitro* and displays inhibitory effects on a panel of transplantable tumors in animals. A comparison of IC_{50} values reveals that C-1027 is much more cytotoxic than other antitumor antibiotics such as adriamycin, mitomycin C and neocarzinostatin, which have been used clinically. C-1027 appears to be the most potent macromolecular peptide antitumor antibiotic ever reported. Because of its highly potent cytotoxic activity, C-1027 is a particularly interesting antitumor candidate for conjugation to monoclonal antibodies (MAbs) for use in MAb-directed tumor chemotherapy.

Source and Chemistry

C-1027 was discovered during antitumor screening of microbial metabolites by using a spermatogonial assay, a new prescreen for detection of antitumor drugs (1), and was isolated from the culture filtrate of a new isolate identified as *Streptomyces globisporus* C-1027 (2, 3). C-1027 is an acidic protein with a molecular weight of 15,000 dalton and isoelectric point of pH 3.5-3.7. This antibiotic is composed of a noncovalent bound protein moiety and a very labile nonprotein chromophore extractable with organic solvents such as ethyl acetate and methanol under alkaline conditions (3). The chemical constitution of C-1027 is similar to that of other macromolecular antitumor antibiotics such as neocarzinostatin, macromomycin and actinoxanthin. The apoprotein of C-1027 has a single polypeptide chain with 110 amino acid residues cross-linked by 2 disulfide bonds. Its molecular weight is 10,500 dalton. The chromophore (Fig. 1) was identified as a

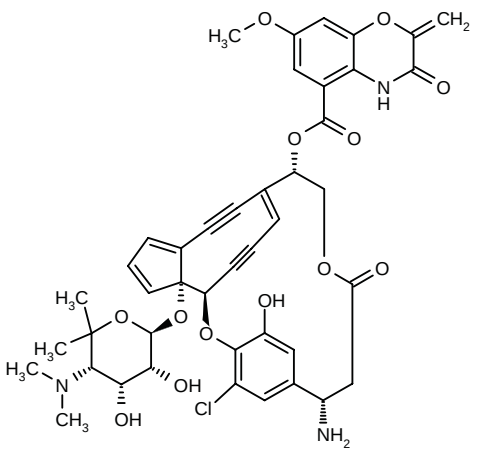

Fig. 1.

member of the potent enediyne family of antitumor antibiotics with a 9-membered 1,5-diyne-3-ene core structure in the 16-membered macrocyclic ring. The isolation procedure and characterization of the C-1027 chromophore fraction have been presented elsewhere (4, 5). In order to investigate the structure-activity relationship of C-1027, 9- and 10-membered ring enediynes of C-1027 chromophore as well as some acyclic enediynes have recently been synthesized in China (Zhu *et al.*, personal communication).

In addition, the C-1027 chromophore was highly unsaturated and the 9-membered enediyne ring was unstable and easily underwent cyclization to lose activity. In order to increase its stability, some prodrugs of C-1027, including epoxides, have been synthesized in China (Li *et al.*, personal communication). The presence of another protein, designated as C-1027-AG, chemically similar to C-1027, was observed in the culture filtrates of *Streptomyces globisporus* (6). Both proteins were similar in molecular weight, isoelectric point and amino acid composition but different in absorption spectra. C-1027-AG

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Table I: Antitumor activity of antibiotics in spermatogonial assay.

Antibiotic	MEC (μg/ml)
Actinomycin D	2.0000
Adriamycin	2.0000
Azaserine	31.0000
Bleomycin	62.0000
Chromomycin A3	8.0000
Daunorubicin	4.0000
Mitomycin C	2.0000
Neocarzinostatin	8.0000
Sibiromycin	8.0000
Streptonigrin	0.5000
C-1027	0.0039

MEC: minimal effective concentration.

had no antimicrobial effect on Gram-positive bacteria and antagonistically suppressed the inhibitory activity of C-1027 against Gram-positive bacteria (7). The cytotoxicity of C-1027 and C-1027-AG was also compared in a proliferating cell inhibition assay against human KB nasopharyngeal carcinoma cells *in vitro*. When KB cells were exposed to the two compounds for 3 days, the IC_{50} values were 0.001 μg/ml and 4 μg/ml for C-1027 and C-1027-AG, respectively. After i.p. administration of 40 mg/kg of C-1027-AG to mice, no tumor growth inhibition was observed (7). Considering that the relationship between C-1027 and C-1027-AG may be similar to that between neocarzinostatin and preneocarzinostatin (8), C-1027-AG may be a precursor protein of C-1027.

Antitumor Activity

Spermatogonial assay was highly sensitive for detection of C-1027. As shown in Table I, C-1027 was the most active of the antitumor antibiotics tested, with a minimal effective concentration (MEC) of 0.0039 μg/ml. The MEC values of the other antibiotics ranged from 2-62 μg/ml, with the exception of streptonigrin (9). As determined by clonogenic assay, C-1027 was very potent against cultured human tumor cells, with IC_{50} values ranging from 0.015-0.31 fmol/l. Among the cell lines tested, lung cancer A-549 cells were the most sensitive to C-1027 (Table II). In addition, C-1027 had potent cytotoxic activity against KB cells *in vitro*. When KB cells were exposed to C-1027 for 3 days in tissue culture, the cytotoxicity exceeded that of adriamycin, with an ED_{50} of 0.001 μg/ml) (2).

C-1027 exhibited inhibitory effects on a panel of transplantable murine tumors, including L-1210 leukemia, P-388, H-22 ascites hepatoma, S-180 sarcoma, Harding-Passdy melanoma and B-16 melanoma in mice at doses of 0.0062-0.1 mg/kg when administered by i.v. or i.p. injection. As shown in Table III, the growth of 3 tumor cell lines was strongly inhibited and there were many long-term survivor mice (9). At the maximum tolerable dose (0.05 mg/kg i.v.), the inhibition of C-1027 on solid S-180 sarcoma reached 53%, which was comparable to that of adriamycin. C-1027 was also highly effective against Harding-Passdy melanoma, with growth inhibition of 81% with a single i.v. injection at tolerable doses. C-1027 had a moderate effect on B-16 melanoma (9). In addition, C-1027 was also effective against C-26 colon cancer in mice with different administration schedules (10) (Table IV). At tolerable doses, C-1027 also exhibited significant inhibition on the growth of human hepatoma and colon cancer xenografts in athymic mice (Table V).

Further studies showed that the chromophore of C-1027 was responsible for the antitumor activity of the compound. As seen in Table VI, C-1027 inhibited the growth of KB cells at very low concentrations. Moreover, the chromophore fraction of C-1027 also possessed

Table II: Cytotoxicity of C-1027 against human tumor cell lines.

Cell line	Antibiotic	IC_{50} (mol/l)
A-549	Adriamycin	1.7×10^{-9}
A-549	Mitomycin C	8.0×10^{-9}
A-549	Neocarzinostatin	1.2×10^{-8}
A-549	C-1027	1.5×10^{-17}
BEL-7402	Adriamycin	1.8×10^{-9}
BEL-7402	Mitomycin C	ND
BEL-7402	Neocarzinostatin	1.6×10^{-8}
BEL-7402	C-1027	3.1×10^{-16}
CNE-2	Adriamycin	1.8×10^{-9}
CNE-2	Mitomycin	1.8×10^{-8}
CNE-2	Neocarzinostatin	1.3×10^{-8}
CNE-2	C-1027	2.2×10^{-16}
MGC-803	Adriamycin	1.5×10^{-9}
MGC-803	Mitomycin	1.6×10^{-8}
MGC-803	Neocarzinostatin	1.3×10^{-8}
MGC-803	C-1027	2.0×10^{-16}

A-549: lung carcinoma, BEL-7402: liver carcinoma, CNE-2: nasopharyngeal carcinoma, MGC-803: gastric carcinoma, ND: not determined.

Table III: Antitumor activity of C-1027 against leukemia and ascites hepatoma in mice.

Tumor	Schedule*	Dose (mg/kg i.p.)	T/C (%)
L-1210	d1	0.0500	359
L-1210	d1	0.0250	382
L-1210	d1	0.0125	357
L-1210	d1	0.0062	315
L-1210	d1,d6	0.0125	382
L-1210	d1,d6,d11	0.0125	382
L-1210	d1-d5	0.0125	317
P-388	d1	0.0500	301
P-388	d1	0.0250	337
P-388	d1	0.0125	341
H-22	d1	0.0250	257
H-22	d1	0.0125	320
H-22	d1	0.0062	147

*Day after tumor inoculation. L-1210 and P-388: mouse leukemia, H-22: mouse ascites hepatoma, antitumor activity expressed as T/C values, where T/C (%) = (mean survival days of C-1027-treated mice)/(mean survival days of control mice) x 100.

Table IV: Antitumor activity of C-1027 (i.v.) against solid tumors in mice.

Tumor	Schedule*	Dose (mg/kg i.v.)	Growth inhibition (%)
S-180	d1,d5,d9,d13	0.0500	53
S-180	d1,d5,d9,d13	0.0250	25
S-180[a]	d1,d5,d9,d13	4.0000	55
S-180[a]	d1,d5,d9,d13	2.0000	36
Harding-Passdy	d1	0.1000	81
Harding-Passdy	d1,d4,d7	0.0250	49
B-16	d1,d4,d7	0.1000	44
B-16	d1,d4,d7	0.0500	36
C -26	d1	0.0125	32
C -26	d1	0.0250	49
C -26	d1	0.0500	70
C -26	d1	0.1500	84
C -26	d1,d4,d7	0.0500	89
C -26	d1,d4,d7	0.1000	94
C -26[b]	d1	5.2000	48
C -26[b]	d1	2.6000	38
C -26[c]	d1	1.2000	42
C -26[c]	d1	0.6000	39

*Day after tumor inoculation. S-180: mouse sarcoma, Harding-Passdy and B-16: mouse melanoma, C-26: mouse colon cancer. Reference compounds: [a]adriamycin, [b]epirubicin, [c]mitomycin.

Table V: Inhibitory effects of C-1027 on the growth of human tumor xenografts in nude mice.

Tumor	Dose (mg/kg i.v.)	Growth inhibition (%)
BEL-7402	0.01 x 3 days	64
BEL-7402	0.05 x 3 days	89
Hce-8693	0.05 x 3 days	40
Hce-8693	0.10 x 3 days	70

Hce-8693: human cecum cancer.

Table VI: Effects of C-1027, its chromophore and protein fractions on the growth of human KB nasopharyngeal cells.

Group	Concentration (ng/ml)	Growth inhibition (%)
C-1027	0.01	-4.5
C-1027	0.10	45.3
C- 1027	1.00	90.5
Chromophore	0.01	62.7
Chromophore	0.10	97.8
Chromophore	1.00	98.0
Chromophore + Protein	0.01	50.8
Chromophore + Protein	0.10	95.7
Chromophore + Protein	1.00	97.7
Protein alone	1000.00	7.3

strong cytotoxicity with an IC_{50} of 0.01 ng/ml. However, the protein component of C-1027 had only weak activity against KB cells at a very high concentration and did not affect the cytotoxic activity of the chromophore. The result suggests that the cytotoxic action of C-1027 is due to its chromophore (11).

Table VII: Cytotoxicity of C-1027, its apoprotein and chromophore against human BEL-7402 hepatoma cells.

Group	IC_{50} (mol/l)
Natural C-1027	$3.1x10^{-16}$
Apoprotein	$7.8x10^{-6}$
Chromophore	$2.0x10^{-16}$
Reconstituted C-1027	$5.0x10^{-16}$
IgG[a]	$> 1.0x10^{-6}$
BSA[b]	$> 1.0x10^{-6}$
C -1027[c]	$4.0x10^{-16}$
Epirubicin[d]	$1.1x10^{-4}$

BSA: bovine serum albumin. Mixed with [a]chromophore, [b]C-1027, [c]BSA, [d]apoprotein and then separated.

In another experiment, the chromophore fraction of C-1027 was separated and rejoined with the apoprotein. The reconstituted C-1027 fully retained its cytotoxic activity, being as potent as natural C-1027. The chromophore-apoprotein association was specific. IgG could not bind to the chromophore and bovine serum albumin could not replace the apoprotein from the C-1027 molecule. When the apoprotein was incubated with epirubicin, reconstitution of C-1027 did not occur. These results further indicate that the chromophore of C-1027 is an active part of the C-1027 molecule (10) (Table VII).

Mechanisms of Action

As determined by tritium-labeled precursor incorporation assay, C-1027 strongly inhibited DNA and RNA synthesis in BEL-7402 cells without affecting protein synthesis. After incubation with BEL-7402 cells for 4 h, IC_{50} values for [^{3}H]-thymidine and [^{3}H]-uridine incorporation were 0.00012 and 0.00032 μmol/l, respectively. A comparison of IC_{50} values revealed that the inhibition on DNA synthesis was approximately 3 times that on RNA synthesis. After a 30-min incubation, C-1027 showed much stronger inhibition on [^{3}H]-thymidine incorporation than adriamycin, mitomycin C and methotrexate, even at a concentration 10,000 times lower (12, 13).

Similar results were obtained in L-1210 cells. IC_{50}s on DNA, RNA and protein synthesis in L-1210 cells after a 3-h treatment was observed at C-1027 concentrations of 3, 10 and 80 ng/ml, respectively, further suggesting that C-1027 preferentially inhibits nucleic acid synthesis, especially DNA synthesis, rather than protein synthesis (11).

Like other enediyne agents, C-1027 was believed to exert its growth inhibition through the induction of cellular DNA damage with subsequent inhibition of DNA and RNA synthesis. It is well known that bleomycin is a DNA-damaging agent (14). BEL-7402 cells were treated with bleomycin A5 and C-1027. After removal of bleomycin A5 and C-1027, the rate of DNA synthesis as pulse-labeled with [^{3}H]-thymidine decreased continuously to about 65% and 23% of control values, respectively, demonstrating that C-1027 exhibits DNA-damaging effects similar to

bleomycin but produces more severe damage to DNA (12). Further studies indicated that C-1027 could cause single- and double-strand scission of DNA. As is well known, bleomycin can cause breaks on DNA strands. Studies showed that the incubation of pBR322 DNA with bleomycin A5 resulted in the conversion of supercoiled plasmid pBR322 DNA (form I) to nicked circular shape (form II) and full-length linear duplex (form III). The accumulation of double-strand degradation products (form III) could conceivably have resulted from clustering of single-strand breaks. Like bleomycin A5, C-1027 was also able to break DNA strands directly. Higher concentrations of C-1027 exerted more marked cleaving effects on the system. Moreover, gel electrophoresis showed that C-1027 cleaved both double- and single-strands of DNA (12, 13). In experiments with L-1210 cells, both C-1027 and its chromophore induced a concentration-dependent decrease in the amount of covalently closed circular (supercoiled) DNA with a concomitant increase in the amount of cleaved DNA forms. Single-strand broken (relaxed) DNA was first detected. At higher concentrations of the two compounds, double-strand broken (linear) DNA was observed and appeared to increase in intensity with an increase in drug concentration. However, C-1027 protein did not cause DNA strand scission even when used at a much higher concentration (13), which corresponded to those in the cytotoxicity experiment. It was concluded that the chromophore of C-1027 plays an important role in causing DNA breakage by the C-1027 molecule. Investigation of the interaction of C-1027 chromophore with various DNA polymers further indicates that the chromophore binds to DNA by more than one mechanism and may prefer A/T base pairs to G/C pairs (15). Since the A/T-rich regions have very different microstructures from G/C-containing sequences, the nature of the specific interaction of C-1027 chromophore with DNA should be of interest for future research.

As determined by flow cytometry, C-1027 delayed the progression of BEL-7402 cells through the S-phase and blocked the cells at the G_2/M phase (13). In mitotic index studies, a severe reduction in the mitotic index was observed within 1 h after the administration of C-1027. An overshot of the mitotic index occurred at 48 h, attaining a value 3 times that obtained for controls. Most of the reappearing mitotic cells exhibited significant changes, including scattering, adhesion and aggregation of chromosomes (13).

Toxicity

The toxicity of C-1027 after a single injection in mice was observed. The LD_{50} values were approximately 0.2 mg/kg i.v. and 0.05 mg/kg i.p. (9). A lethal dose of C-1027 administered i.p. caused peritonitis and subsequently fibrous adhesions of the viscera, and 3-4 days after i.v. injection of a lethal dose of C-1027 in mice, hemopoietic cell numbers were markedly depressed in bone marrow and in spleen. Thymocytes in the cortex of thymus were also decreased in number. No histopathological changes were found in the heart, lungs, liver and kidneys of mice (9).

Therapeutic Use

As mentioned above, C-1027 is the most active macromolecular peptide antitumor antibiotic ever reported. Its potency in tumor cell killing is comparable to that of toxins such as ricin and diphtherial toxin, but its mechanism of action is apparently different from that of the toxins, which inactivate protein synthesis. Studies demonstrated that when one molecule of diphtherial toxin fragment was introduced into a tumor cell, the molecule was able to kill the cell. The IC_{50} of diphtherial toxin against tumor cells was about 3 pmol/l (16). Comparing this value with that of C-1027 suggests that only one molecule of C-1027 might kill a tumor cell.

The use of MAb immunoconjugates is a promising approach to cancer treatment. Compounds that are highly cytotoxic to cancer cells and effective against tumor growth *in vivo* are particularly interesting candidates for conjugation to MAb (17). Because of its very potent cytotoxicity, C-1027 was suggested as an agent that could be linked to MAbs for the purpose of killing tumor cells. A conjugate was made by directly linking C-1027 to MAb. The assembled conjugate, also called enriched conjugate, was prepared by linking apoprotein to MAb and then adding the chromophore to the MAb-apoprotein conjugate. In experiments linking C-1027 to H16, a MAb against hepatoma cells, the IC_{50}s for BEL-7402 hepatoma cells were 42 and 5.5 pmol/l, respectively, for direct conjugate and assembled conjugate. The IC_{50} of the M3-C-1027 assembled conjugate prepared by linking the irrelevant MAb M3 to C-1027 was 1400 pmol/l. These results suggest that the assembled conjugate is much more cytotoxic than the direct conjugate and that the H16-C-1027 assembled conjugate was highly selective for hepatoma cells (18). At a dose of 0.05 mg/kg i.v. for 3 days, the H16-C-1027 conjugate significantly suppressed the growth of human hepatoma xenografts in nude mice by 84% (19).

C-1027 was conjugated to 3A5 (another MAb against hepatoma cells) and its Fab fragment at a molecular ratio of 1:1. In the BEL-7402 cell clonogenic assay, IC_{50}s for MAb-C-1027 and Fab-C-1027 were 42 and 0.86 fmol/l, respectively. Fab-C-1027 was 49-fold more cytotoxic than MAb-C-1027. Moreover, Fab-C-1027 was 160-fold more cytotoxic to BEL-7402 cells than to KB cells, indicating its selective cytotoxicity to hepatoma cells. In experiments with nude mice, the Fab-C-1027 conjugate with different administration schedules inhibited the growth of BEL-7402 hepatoma xenograft (66-85%) without noticeable histopathological changes in vital organs such as heart, lungs, liver, kidneys, bone marrow and gastrointestinal tract (20, 21) (Table VIII). Similar results were obtained with the *in vivo* administration of C-1027 and its MAb (3H11) immunoconjugate in nude mice bearing human

Table VIII: Inhibition of human tumor xenografts by C-1027 and its MAb and Fab conjugates in nude mice.

Tumor	Group	Dose (mg/kg i.v.)	Schedule*	Growth inhibition (%)
Hepatoma	C-1027	0.15	d3,d6,d10	36
Hepatoma	Fab-C-1027	0.15	d3,d6,d10	66
Hepatoma	C-1027	0.10	d3,d7,d11,d15,d19,d23	59
Hepatoma	Fab-C-1027	0.10	d3,d7,d11,d15,d19,d23	85
Gastric cancer	C-1027	0.05	d12-d14	49
Gastric cancer	MAb-C-1027	0.05	d12-d14	78
Gastric cancer	C-1027	0.10	d12-d14	54
Gastric cancer	MAb-C-1027	0.10	d12-d14	83

*Day after tumor inoculation.

BCG-823 gastric cancer (22) (Table VIII). In addition, C-1027 was linked at a 1:1 molecular ratio to MAb directed against colon cancer. The conjugates demonstrated very potent and selective cytotoxicity to colon cancer cells (IC_{50}s = of 0.1-0.01 fmol/l). Moreover, the conjugates showed marked antitumor effects on human colon cancer xenografts in nude mice (22, 23).

Conclusions

C-1027 has very potent cytotoxicity against tumor cells *in vitro* and is effective against a panel of transplantable tumors in animals at tolerable doses. Studies on the mechanism of action of C-1027 indicate that it is an interesting compound with highly potent activity on cellular DNA. The chromophore of C-1027 plays a major role in the expression of C-1027-exerting antitumor activity and may inhibit tumor cell growth by causing DNA damage with subsequent suppression of DNA synthesis. It has also been suggested that the antitumor action of C-1027 is basically similar to that of most macromolecular peptide antitumor antibiotics, *i.e.*, inhibition of nucleic acid synthesis with no significant effect on protein synthesis, direct breakage of supercoiled DNA and blockade of cell progression in the G_2/M phase. However, it should be noted that the effect of C-1027 on DNA is more potent than other antitumor antibiotics.

MAbs in the form of conjugates with toxins, drugs or radioisotopes offer a great opportunity for developing tumor-selective cytotoxic drugs (24). Data from *in vitro* studies and *in vivo* animal models indicate that C-1027 might be an active effector agent for making MAb conjugates and that highly potent conjugates of smaller molecular size can be obtained by linking C-1027 to an antibody fragment at a low molecular ratio through enriched conjugation. At present, clinical trials evaluating the toxicity, pharmacokinetics, immunogenicity and therapeutic efficacy of C-1027-MAb conjugates are being performed in China.

Manufacturer

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences (CN).

References

1. Zhen, Y.S., Xue, Y.C. *Use of murine spermatogonial assay as prescreen for antitumor drugs.* In: Proceedings of the 13th International Congress on Chemotherapy. K.H. Spitzy, K. Karrer (Eds.). Egermann: Vienna 1983, Part 223, No. 7: 38-44.

2. Hu, J., Xue, Y.C., Xie, M.Y., Zhang, R., Otani, T., Minami, Y., Yamada, Y., Marunaka, T. *A new macromolecular antitumor antibiotic, C-1027. I. Discovery, taxonomy of producing organism, fermentation and biological activity.* J Antibiotics 1988, 41: 1575-9.

3. Otani, T., Minami, Y., Marunaka, T., Zhang, R., Xie, M.Y. *A new macromolecular antitumor antibiotic, C-1027. II. Isolation and physico-chemical properties.* J Antibiotics 1988, 41: 1580-5.

4. Otani T., Minami, Y., Sakawa, K., Yoshida, K. *Isolation and characterization of non-protein chromophore and its degradation product from antibiotic C-1027.* J Antibiotics 1991, 44: 564-8.

5. Yoshida, K., Minami, Y., Azuma, R., Saeki, M., Otani, T. *Structure and cycloaromatization of novel enediyne, C-1027 chromophore.* Tetrahedron Lett 1993, 34: 2637-40.

6. Otani, T., Yasuhara, T., Minami, Y., Shimazu, T., Zhang, R., Xie, M.Y. *Purification and primary structure of C-1027-AG, a selective antagonist of antitumor antibiotic C-1027, from Streptomyces globisporus.* Agric Biol Chem 1991, 55: 407-17.

7. Otani, T., Minami, Y., Marunaka, T., Zhang, R., Xie, M.Y. *C-1027-AG, a selective antagonist of the macromolecular antitumor antibiotic, C-1027.* J Antibiotics 1988, 41: 1696-8.

8. Kudo, K., Kikuchi, M., Ishida, N. *Biogenesis of an antitumor antibiotic protein, neocarzinostatin.* Antimicrob Agents Chemother 1972, 1: 289-95.

9. Zhen, Y.S., Ming, X.Y., Yu, B., Otani, T., Saito, H., Yamada, Y. *A new macromolecular antitumor antibiotic, C-1027. III. Antitumor activity.* J Antibiotics 1989, 42: 1294-8.

10. Zhen, Y.S., Xue, Y.C., Shao, R.G. *Antitumor activity of the new enediyne antibiotic C-1027.* Chin J Antibiotics 1994, 19: 164-8.

11. Sugimoto, Y., Otani, T., Oie, S., Wierzba, K., Yamada, Y. *Mechanism of action of a new macromolecular antitumor antibiotic, C-1027.* J Antibiotics 1990, 43: 417-21.

12. Xu, Y.J., Li, D.D., Zhen, Y.S. *Molecular mechanisms of action of C-1027.* Sci Sin 1992, 8: 814-9.

13. Xu, Y.J., Li, D.D., Zhen, Y.S. *Mode of action of C-1027, a new macromolecular antitumor antibiotic with highly potent cytotoxicity, on human hepatoma BEL-7402 cells.* Cancer Chemother Pharmacol 1990, 27: 41-6.

14. Painter, R.B. *Rapid test to detect agents that damage human DNA.* Nature 1977, 265: 650-3.

15. Xu, Y.J. Zhen, Y.S., Goldberg, I.H. *C-1027 chromophore, a potent new enediyne antitumor antibiotic, induces sequence-specific double-strand DNA cleavage.* Biochemistry 1994, 33: 5947-54.

16. Yamaizumi, M., Mekada, E., Uchida, T., Okada, Y. *One molecule of diphtherial toxin fragment A introduced into a cell can kill the cell.* Cell 1978, 15: 245-52.

17. Marx, J.L. *Monoclonal antibodies in cancer.* Science 1982, 216: 283-5.

18. Shao, R.G., Zhen, Y.S. *Antitumor activity of new antibiotic C-1027 and its monoclonal antibody assembled conjugate.* Acta Pharmaceut Sin 1992, 27: 486-91.

19. Zhen, Y.S., Peng, Z., Xu, Y.J., Shao, Y.G. *Antitumor activity of the monoclonal antibody conjugate prepared from a highly potent antitumor antibiotic C-1027.* Proc Am Assoc Cancer Res 1991, 32: 432.

20. Zhen, Y.S., Li, J.Z., Shao, Y.G., Jiang, M. *A highly potent cytotoxic immunoconjugate composed of antitumor antibiotic C-1027 and rat monoclonal antibody 3A5.* Proc Am Assoc Cancer Res 1993, 34: 474.

21. Li, J.Z., Jiang, M., Xue, Y.C., Zhen, Y.S. *Anti-hepatoma activity of C-1027 and its Fab conjugate.* Acta Pharmaceut Sin 1993, 28: 260-5.

22. Zhen, Y.S., Shao, R.G., Jiang, M., Li, J.Z., Wang, N.G., Dong, Z.W. *Antitumor activity of the conjugates composed of C-1027, an antitumor antibiotic, and monoclonal antibody.* Chin J Cancer Biother 1995, 2: 94-8.

23. Zhen, Y.S., Shao, R.G., Li, J.Z., Jiang, M. *The use of C-1027, an antitumor antibiotic with extremely potent cytotoxicity, for making effective immunoconjugates.* Antibody Immunoconj Radiopharm 1994, 7: 89.

24. Morgan, A.C.Jr., Pearson, J.W., Oldham, R.K. *Preclinical evaluation of immunoconjugates.* In: Immunoconjugates. Antibody Conjugates in Radioimaging and Therapy of Cancer. C.W. Vogel (Ed..). Oxford University Press: New York 1987, 241-58.