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Advances in Brief

Targeted Therapy with a Novel Enediyene Antibiotic Calicheamicin Θ^I_1 Effectively Suppresses Growth and Dissemination of Liver Metastases in a Syngeneic Model of Murine Neuroblastoma[1]

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Abstract

The suppression of metastases in malignant diseases is one of the major goals in targeted chemotherapy. This was achieved with an antibody drug conjugate between a novel, rationally designed enediyene antibiotic calicheamicin Θ^I_1 of exceptionally high cytotoxic potency and an antiganglioside GD_2 monoclonal antibody 14G2a. Effective suppression of hepatic metastases was demonstrated in a novel syngeneic model of murine neuroblastoma that simulates the situation in patients in terms of antigen heterogeneity and presence of the target antigen on normal tissues. Here, we describe the first successful use of calicheamicin Θ^I_1 for targeted chemotherapy in a clinically relevant syngeneic metastasis model.

Introduction

The direction of therapeutic agents to pathogens such as malignant tumor cells by means of monoclonal antibodies as "magic bullets" or "poisoned arrows" was already envisioned by Paul Ehrlich almost one century ago, when he proposed immunotherapy as a fourth treatment modality of cancer therapy. Preclinical observations with xenografted human tumors in immunodeficient athymic nude mice or severe combined immunodeficient mice and the use of antibody drug conjugates consisting of antihuman mAbs[3] generated impressive data that led to numerous Phase I/II clinical trials. However, in most preclinical animal studies thus far, an antibody drug conjugate was used that consisted of a mAb that specifically recognized a human tumor-associated antigen overexpressed on the human tumor cell line used for the animal experiment (for review see Ref. 1). The favorable xenogeneic setting provided for drug conjugates that are specific for a human tumor-associated antigen in a murine host, which does not have to compete with the expression of this human antigen on normal mouse tissues, overestimates the actual therapeutic efficacy of such a drug conjugate in an entirely syngeneic host, such as a cancer patient. This was demonstrated by the generally disappointing clinical data observed in these clinical Phase I/II trials (1). This disappointment caused investigators to focus on solutions of the major problems observed with antibody drug conjugates. First, the use of immunogeneic murine antibodies as delivery systems was approached by replacing them with chimerized or humanized antibodies, which produced considerably less or no human antimouse immune responses. Second, the conjugation of the cytotoxic agent was optimized to maintain its cytotoxic activity following conjugation, as well as its site-specific toxicity, when cleaved from the linker compound. Third, the search for more potent cytotoxic compounds was intensified to allow for the use small amounts of the tumor-specific mAb for drug delivery and thus limit undesirable nonspecific distribution of the antibody drug conjugate *in vivo*.

These efforts led to the discovery of calicheamicins, which were isolated from a broth extract of a soil microorganism, *Micromonospora echinospora calichensis* (2). These antibiotics were found to exert impressive potencies in a screen for DNA-damaging agents (3) that exceeded the cytotoxic potency of substances, such as cyano morpholinyl anthracyclin (4) and ricin (5), and have been claimed to be some of the most toxic substances known. Within this group of enediyene antibiotics, calicheamicin γ^I_1 was the most potent compound and was found to bind in the minor groove of DNA, and the resulting sequence-specific DNA cleavage has been described (6). Recently, a novel, biologically active and selective enediyene molecule, calicheamicin Θ^I_1, was rationally designed on the basis of the mechanism of action of the naturally occurring calicheamicin γ^I_1. Unlike its natural counterpart, calicheamicin Θ^I_1 contains structural variations that facilitate molecular triggering to form the active compound under mild basic conditions (7, 8). This modification induced the chemical stability of calicheamicin Θ^I_1 under physiological conditions (pH 7.4, 37°C), in contrast to calicheamicin γ^I_1, and a further increase in cytotoxicity over the natural compound when tested against a large variety of different human cancer cell lines, as determined by the decrease in the final molarity, at which 50% of cell viability was observed from 10^{-8}–10^{-9} to $<10^{-12}$ M. The small size of calicheamicins (in the range of M_r 1500), combined with their unique mode of action and the extreme potency of calicheamicin Θ^I_1, suggests that this compound might be a good candidate for targeted chemotherapy.

Here, we establish, for the first time, dosage and efficacy of an antibody drug conjugate between the murine monoclonal antiganglioside GD_2 mAb 14G2a and calicheamicin Θ^I_1 that suppresses growth and dissemination of liver metastases in a novel syngeneic model of murine neuroblastoma. This well-characterized model was deliberately chosen because it features natural and heterogeneous expression of the target antigen GD_2, is syngeneic with a host that also expresses the target antigen on normal tissues, and features metastases at locations similar to the human disease (9). Thus, the antitumor effect observed in this model might be more predictive of future clinical trials with a similar compound consisting of calicheamicin Θ^I_1 and the humanized version of this anti-GD_2 antibody.

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[3] The abbreviation used is: mAb, monoclonal antibody.

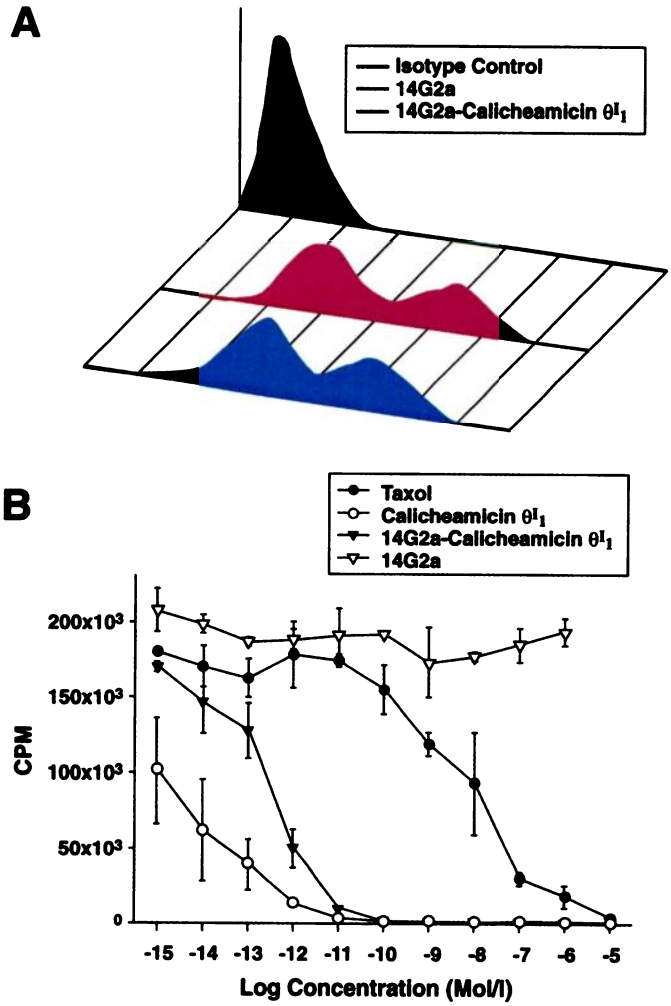

Fig. 1. Binding and *in vitro* cytotoxicity of 14G2a-calicheamicin Θ^I_1 conjugate. *A*, binding of the 14G2a-calicheamicin Θ^I_1 conjugate was tested on NXS2 cells featuring heterogeneous ganglioside GD_2 expression by FACS scan (*X* axis, fluorescence intensity; *Y* axis, cell count). Binding of nonconjugated 14G2a antibody and a nonspecific immunoglobulin of IgG2a isotype are shown as positive and negative control, respectively. *B*, determination of *in vitro* cytotoxicity of 14G2a-calicheamicin Θ^I_1 conjugate on NXS2 cells compared to that of free calicheamicin Θ^I_1 and equivalent amounts of 14G2a used for conjugation. The efficacy of Taxol was determined in view of its use as a control in *in vivo* experiments.

Materials and Methods

Synthesis of Calicheamicin Θ^I_1. Calicheamicin Θ^I_1 was obtained by total synthesis following a variation of the procedure recently described for the preparation of calicheamicin γ^I_1 (8).

Conjugation of Calicheamicin Θ^I_1 to Antiganglioside GD_2 mAb 14G2a. The method used to conjugate calicheamicin Θ^I_1 to 14G2a mAb involved the activation of the amino sugar group with SPDP (Pierce, Rockford, IL), followed by disulfide exchange with iminothiolane (Pierce)-modified 14G2a mAb. Calicheamicin Θ^I_1 (2.0 mg in 100 μl of DMSO) was reacted with 10 μl of SPDP stock (4.26 mg in 100 μl of DMSO) at 4°C for 6 h. Modification of 14G2a mAb (2.0 mg) in sodium carbonate buffer (pH 8.3) containing 1 mM EDTA was achieved with 0.46 mg of iminothiolane in 10 μl of DMSO for 1 h at room temperature. The product was purified by size exclusion chromatography using a NAP-5 column (Pharmacia Biotech, Piscataway, NJ). The conjugation of calicheamicin Θ^I_1 with 14G2a mAb was established following the reaction of the activated calicheamicin Θ^I_1 with the thiolated 14G2a mAb at 4°C under nitrogen for 30 min. Unreacted thiol groups were inactivated by the addition of 10 μl of an iodoacetamide stock solution (2.0 mg/100 μl of DMSO). The 14G2a-calicheamicin Θ^I_1 conjugate was purified by size-exclusion chromatography using a PD10 column (Pharmacia Biotech). Four molar equivalents of calicheamicin Θ^I_1 (M_r 1464) were conjugated to 1 mol of 14G2a mAb (M_r 155,000). The dosage of the 14G2a-calicheamicin Θ^I_1 conjugate is based on its total amount of calicheamicin Θ^I_1. Thus, 1 μg of calicheamicin Θ^I_1 corresponds to 26 μg of 14G2a-calicheamicin Θ^I_1 conjugate, based on the molecular weights and the molar ratio of each individual component.

Cytotoxicity Assay and Flow Cytometry. Tumor target cells (1×10^4 cells/well) were seeded in 96-well plates and incubated for 72 h at 37°C with various concentrations of free calicheamicin Θ^I_1, Taxol, 14G2a mAb, and 14G2a-calicheamicin Θ^I_1 conjugate, each in a total volume of 200 μl. The viability of the cells was determined in a standard assay for [^{3}H]methyl thymidine uptake.

Binding of the antiganglioside GD_2 mAb 14G2a-calicheamicin Θ^I_1 conjugate to GD_2-positive NXS2 cells was determined by flow cytometry and compared to nonconjugated 14G2a mAb and a mouse IgG2a isotype control (PharMingen, La Jolla, CA). Cells were subsequently incubated with 14G2a mAb or 14G2a-calicheamicin Θ^I_1 conjugate and polyclonal goat antimouse IgG FITC (Pharmingen), each at 1 μg per 10^6 cells. Binding of the various compounds to NXS2 cells was analyzed by FACS scan (Becton Dickinson, Franklin Lakes, NJ).

Experimental Liver Metastases Model of Neuroblastoma. The syngeneic model of murine neuroblastoma was used as described previously (9).

Statistics. The statistical significance of differential findings between experimental groups of animals was determined by the parametric Student's *t* test. Arbitrary metastatic scores were analyzed by the nonparametric Mann-Whitney rank sum test. Findings were regarded as significant if two-tailed *P* were ≤0.05.

Results

***In Vitro* Characterization of the Anti-GD_2 Antibody 14G2a-Calicheamicin Θ^I_1 Conjugate.** Binding characteristics and cytotoxic activity of the 14G2a-calicheamicin Θ^I_1 drug conjugate were demonstrated using NXS2 tumor cells (Fig. 1). The binding pattern observed with both the unconjugated 14G2a mAb and the calicheamicin Θ^I_1 drug conjugate revealed a similar, heterogeneous distribution of the target antigen GD_2 (Fig. 1*A*), which is a typical feature of this murine neuroblastoma cell line (9). This indicates that the conjugation of

Table 1 *Effect of an anti-GD_2 antibody-calicheamicin Θ^I_1 conjugate on growth suppression of experimental liver metastases in a syngeneic model of murine neuroblastoma*

Tumor load[a]	Treatment[b]	Liver metastasis[c]	Liver weight (mg)
1×10^5	PBS	>250, >250, >250, >250	2925 ± 455
	14G2a[d]	>250, >250, >250, >250	3051 ± 381
	Taxol (10 mg/kg)[e]	>250, >250, >250, >250	2857 ± 315
	14G2a + Cal. Θ^I_1 (1 μg/kg)	>250, >250, >250, 215, 171, 52	1797 ± 305
	14G2a + Cal. Θ^I_1 (10 μg/kg)	—, —, —, —, —, —[f]	
	14G2a-Cal. Θ^I_1 (1 μg/kg)	>250, >250, >250, 220, 34, 17	2065 ± 873
	14G2a-Cal. Θ^I_1 (10 μg/kg)	9, 32, 42, 83, 115, 123[g]	1657 ± 501[g]
	14G2a-Cal. Θ^I_1 (30 μg/kg)	0, 0, 1, 36[g]	872 ± 186[g]
1×10^4	PBS	4, 4, 4, 3, 3, 1	2020 ± 561
	14G2a + Cal. Θ^I_1 (1 μg/kg)	3, 3, 3, 2, 2, 1	1645 ± 215
	14G2a-Cal. Θ^I_1 (10 μg/kg)	0, 0, 0, 0, 0, 1[g]	991 ± 176[g]

[a] Liver metastases were induced by i.v. injection of either 1×10^5 or 1×10^4 NXS2 cells.

[b] Treatment was initiated 24 h after tumor cell inoculation by i.v. injection of 14.G2a mAb, calicheamicin Θ^I_1, and the 14G2a-calicheamicin Θ^I_1 conjugate on days 1, 3, 5, and 7. Mice receiving 30 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate were injected only twice, on days 1 and 3. Cal. Θ^I_1, calicheamicin Θ^I_1.

[c] Animals were sacrificed at 24 and 28 days after i.v. inoculation with 1×10^5 and 1×10^4 NXS2 tumor cells and analyzed for macroscopic liver metastases. Metastatic foci were counted on livers of mice inoculated with 1×10^5 tumor cells. Fused liver metastases of mice receiving 1×10^4 tumor cells were staged according to the coverage of the liver surface: 0, 0%; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75%.

[d] mAb14G2a was injected at 15.6 μg/injection, equivalent to the highest dose of drug conjugate.

[e] Taxol was administered at the maximal tolerated dose by intraperitoneal injection.

[f] All mice receiving 10 μg/kg free calicheamicin Θ^I_1 died.

[g] The difference between experimental groups and control groups was statistically significant at $P < 0.01$.

calicheamicin Θ^I_1 to 14G2a mAb did not affect binding to the target antigen GD_2. The cytotoxic activity of the 14G2a-calicheamicin Θ^I_1 drug conjugate was compared to that of free calicheamicin Θ^I_1, Taxol, and 14G2a mAb, which were all used as controls for subsequent *in vivo* studies (Fig. 1*B*). The final molarity at which 50% of cell viability was observed (IC_{50}) was about two logarithmic orders higher for the calicheamicin Θ^I_1 drug conjugate (10^{-13} M) than free calicheamicin Θ^I_1 (10^{-15} M), indicating a 100 times reduced cytotoxic activity of conjugated calicheamicin Θ^I_1. The IC_{50} of Taxol was 10^{-8} M, indicating 10^7 and 10^5 times lower cytotoxic activity than free and conjugated calicheamicin Θ^I_1, respectively. The 14G2a antibody, which was used at equivalent concentrations to the 14G2a-calicheamicin Θ^I_1 conjugate, revealed no cytotoxic activity.

Effect of Treatment with 14G2a-Calicheamicin Θ^I_1 Conjugate on Growth Suppression of Liver Metastases of Murine Neuroblastoma. The efficacy of treatment with the 14G2a-calicheamicin Θ^I_1 conjugate was established in two experimental settings using 1×10^5 and 1×10^4 NXS2 cells, respectively (Table 1). Mice were treated with doses of the 14G2a-calicheamicin Θ^I_1 conjugate, ranging from 1 to 30 μg/kg, and compared to controls treated with PBS, 14G2a mAb, Taxol, and a mixture of free calicheamicin Θ^I_1 and 14G2a mAb. Only mice that received 10 or 30 μg/kg of the drug conjugate revealed effective suppression of hepatic metastases. In fact, five of six mice inoculated with 1×10^4 NXS2 cells and treated with 10 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate presented with the complete absence of liver metastases (Table 1 and Fig. 2). Far less efficacy was observed with a low dose of calicheamicin Θ^I_1 conjugate (1 μg/kg). It is important to note that a mixture of 1 μg/kg free calicheamicin Θ^I_1 and 14G2a, which reveal a higher cytotoxic activity *in vitro* than did 10 μg/kg 14G2a-calicheamicin Θ^I_1, is ineffective in this regard *in vivo* (Table 1 and Fig. 2), which indicates effective targeting of the drug conjugate. The use of 10 μg/kg free calicheamicin Θ^I_1, which is the equimolar amount to 10 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate, induces death in all animals injected, demonstrating a dramatic decrease in systemic toxicity following conjugation of calicheamicin Θ^I_1 to 14G2a antibody. Taxol, which was used at the maximal tolerated dose, was entirely ineffective in this model, reflecting its low cytotoxic activity observed against NXS2 cells *in vitro*. The absence of a therapeutic effect with 14G2a mAb alone was demonstrated at concentrations equivalent to the 30 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate. This indicates that the amount of anti-GD_2 antibody used for targeting calicheamicin Θ^I_1 is not efficient in eliciting an antitumor response, *per se*.

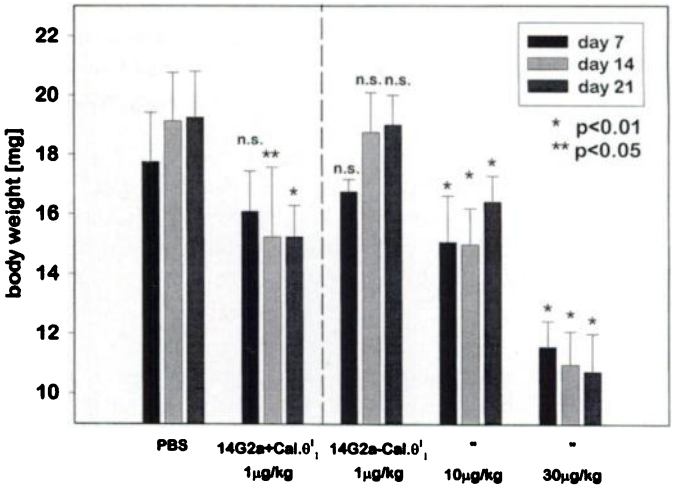

Fig. 3. Toxicity following treatment with 14G2a-calicheamicin Θ^I_1 conjugate. Tumor-bearing mice that received 1×10^5 NXS2 cells i.v. were treated on days 1, 3, 5, and 7 with various doses of 14G2a-calicheamicin Θ^I_1 conjugate, a mixture of equivalent amounts of antibody and 1 μg/kg calicheamicin Θ^I_1 and PBS. Mice receiving 30 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate were injected only twice on days 1 and 3. The average body weight of each experimental group ($n = 6$) was determined after 7, 14, and 21 days, respectively, and compared to mice injected with PBS (*n.s.*, not significant).

Toxicity following Treatment with 14G2a-Calicheamicin Θ^I_1 Conjugate. Systemic toxicity of the 14G2a-calicheamicin Θ^I_1 conjugate was monitored as a function of change in the total body weight of mice in each experimental group (Fig. 3). A dose-dependent decrease in body weight was observed, ranging from no significant difference in mice receiving 1 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate to an almost 50% reduction in body weight in mice injected with 30 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate, as compared to controls injected with PBS. It is important to note that two mice

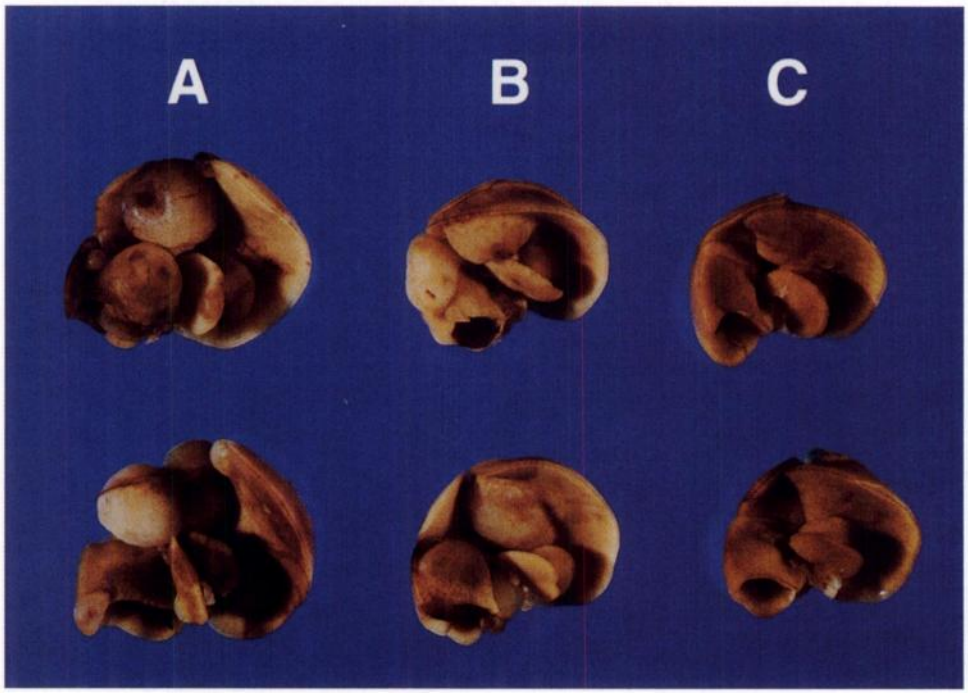

Fig. 2. Effect of anti-GD_2 antibody 14G2a-calicheamicin Θ^I_1 conjugate on suppression of hepatic neuroblastoma metastases. Mice were injected with 1×10^4 NXS2 cells i.v. and sacrificed 28 days after cell inoculation. Treatment was initiated by i.v. injections of 10 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate (*C*), a mixture of an equivalent amount of 14G2a mAb and calicheamicin Θ^I_1 (1 μg/kg) (*B*), or PBS (*A*) on days 1, 3, 5, and 7. Two representative liver specimens per group are depicted.

injected with 30 μg/kg died 2 days after completion of the treatment, indicating that 30 μg/kg is just beyond the maximal tolerated dose. However, mice receiving 10 μg/kg 14G2a-calicheamicin Θ^I_1 conjugate started recovery 2 weeks after the last injection following an initial decrease of ~20% in body weight, which was very well tolerated. This is in contrast to mice that received only 1 μg/kg free calicheamicin Θ^I_1 and 14G2a mAb, which failed to start recovery after a similar drop in body weight at the same time, again indicating a dramatic reduction of systemic toxicity of calicheamicin Θ^I_1, once it was conjugated to the 14G2a mAb.

Discussion

The effective treatment of stage 4 neuroblastoma, one of the most common solid tumors in children (10), remains one of the major challenges in pediatric oncology. The overall survival rate of such patients has not been significantly improved during the last 20 years, despite the introduction of radiotherapy and/or high-dose chemotherapy, followed by allogeneic or autologous bone marrow transplantation (10). Novel therapeutic approaches in adjuvant settings with murine and human/mouse chimeric antibodies directed against ganglioside GD_2 resulted in a response rate of over 50% in Phase I and I/II clinical trials, including several long-term and complete remissions of stage 4 patients (11, 12). The rationale for this approach is provided by the extensive expression of GD_2 on neuroblastoma cells and restricted manifestation on normal tissues, such as the peripheral nervous system and the cerebellum, which is protected from immunoglobulins by the blood brain barrier.

Here, we demonstrate that these promising clinical data with a naked anti-GD_2 antibody could be extended by using it as a conjugate with calicheamicin Θ^I_1, which is currently one of the most potent cytotoxic compounds known (8). This is evident because the use of such an antibody drug conjugate, *i.e.*, 14G2a-calicheamicin Θ^I_1, demonstrated high efficacy in suppression of experimental liver metastases in a clinically relevant model of murine neuroblastoma with heterogeneous expression of the ganglioside GD_2 target antigen. Two lines of evidence make these data even more impressive: first, the lack of any antitumor effect achieved with equivalent doses of 14G2a mAb or a mixture of 14G2a mAb and calicheamicin Θ^I_1; and second, the absence of an antitumor effect with either free calicheamicin Θ^I_1 or Taxol. The ineffectiveness of the latter, which is one of today's well-established drugs for various malignancies, indicates the relative resistance of this mouse tumor model toward conventional drug therapies. It is important to point out that the therapeutic efficacy of the 14G2a-calicheamicin Θ^I_1 conjugate was established in a syngeneic host, which features GD_2 expression on normal tissues mostly of neuroectodermal origin, therefore providing the type of competition for antigen binding that is to be expected in humans. This is in contrast to previously published work in an s.c. mouse xenograft model using conjugates of calicheamicins γ^I_1, α^I_2, and α^I_3 with CT-M-01 mAb, which specifically recognizes human polyepithelial mucin, which is only expressed on the cell surface of human cancerous epithelial cells (13). The absence of competing antigen-binding sites in this model most likely overestimates its therapeutic efficacy while underestimating its toxicity to be expected in humans, in whom this same antigen is expressed on normal epithelial cells. In our syngeneic neuroblastoma model, we demonstrated that, despite antigeneic heterogeneity on the tumor cells and the expression of GD_2 on normal tissues, a most effective suppression of experimental liver metastases was achieved at a safe dosage of 10 μg/kg 14G2a-calicheamicin Θ^I_1, which was found to be well-tolerated by all injected animals.

In summary, we demonstrate, for the first time, that an antibody-drug conjugate of anti-GD_2 mAb 14G2a with a novel enediyene, calicheamicin Θ^I_1 can effectively suppress growth and dissemination of hepatic metastases of neuroblastoma. Considering the effectiveness of passive immunotherapies with naked anti-GD_2 antibodies in human neuroblastoma, an antibody drug conjugate of humanized anti-GD_2 antibody 14G2a with calicheamicin Θ^I_1 may hold promise for effective treatment of neuroblastoma in the minimal residual disease setting.

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References

1. Bodey, B., Siegel, S. E., and Kaiser, H. E. Human cancer detection and immunotherapy with conjugated and non-conjugated monoclonal antibodies. Anticancer Res., *16:* 661–674, 1996.
2. Zein, N., Sinha, A. M., McGahren, W. J., and Ellestad, G. A. Calicheamicin gamma 1I: an antitumor antibiotic that cleaves double-stranded DNA site specifically. Science (Washington DC), *240:* 1198–1201, 1988.
3. Lee, M. D., Manning, J. K., Williams, D. R., Kuck, N. A., Testa, R. T., and Borders, D. B. Calicheamicins, a novel family of antitumor antibiotics. 3. Isolation, purification and characterization of calicheamicins β 1Br, γ 1Br, α 2I, α 3I, β 1I, γ 1I and γ 1I. J. Antibiotics, *42:* 1070–1087, 1989.
4. Acton, E. M., Tong, G. L., Mosher, C. W., and Wolgemuth, R. L. Intensely potent morpholinyl anthracyclines. J. Med. Chem., *27:* 638–645, 1984.
5. Raso, V., Ritz, J., Basala, M., and Schlossman, S. F. Monoclonal antibody-ricin A chain conjugate selectively cytotoxic for cells bearing the common acute lymphoblastic leukemia antigen. Cancer Res., *42:* 457–464, 1982.
6. Zein, N., Poncin, M., Nilakantan, R., and Ellestad, G. A. Calicheamicin γ 1I and DNA: molecular recognition process responsible for site-specificity [published erratum appears in Science (Washington DC), *244:* 1128, 1989. Science (Washington DC), *244:* 697–699, 1989.
7. Nicolaou, K. C., Dai, W. M., Tsay, S. C., Estevez, V. A., and Wrasidlo, W. Designed enediynes: a new class of DNA-Cleaving molecules with potent and selective anticancer activity. Science (Washington DC), *256:* 1172–1178, 1992.
8. Nicolaou, K. C., Li, T., Nakada, M., Hummel, C. W., and Wrasidlo, W. Calicheamicin Θ^I_1: a rationally designed molecule with extremely potent and selective DNA cleaving properties and apoptosis inducing activity. Angew Chem. Int. Ed. Engl., *33:* 183–186, 1994.
9. Lode, H. N., Xiang, R., Varki, N. M., Dolman, C. S., Gillies, S. D., and Reisfeld, R. A. Targeted interleukin-2 therapy of spontaneous neuroblastoma metastases to bone marrow. J. Natl. Cancer. Inst. (Bethesda), *89:* 1586–1594, 1997.
10. Niethammer, D., and Handgretinger, R. Clinical strategies for the treatment of neuroblastoma. Eur. J. Cancer, *31A:* 568–571, 1995.
11. Handgretinger, R., Anderson, K., Lang, P., Dopfer, R., Klingebiel, T., Schrappe, M., Reuland, P., Gillies, S. D., Reisfeld, R. A., and Neithammer, D. A Phase I study of human/mouse chimeric antiganglioside GD2 antibody ch14.18 in patients with neuroblastoma. Eur. J. Cancer, *31A:* 261–267, 1995.
12. Handgretinger, R., Baader, P., Dopfer, R., Klingebiel, T., Reuland, P., Treuner, J., Reisfeld, R. A., and Niethammer, D. A Phase I study of neuroblastoma with the anti-ganglioside GD2 antibody 14.G2a. Cancer Immunol. Immunother., *35:* 199–204, 1992.
13. Hinman, L. M., Hamann, P. R., Wallace, R., Menendez, A. T., Durr, F. E., and Upeslacis, J. Preparation and characterization of monoclonal antibody conjugates of the calicheamicins: a novel and potent family of antitumor antibiotics. Cancer Res., *53:* 3336–3342, 1993.