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Antitumor Activities in Mouse Xenograft Models of Canine Fibroblastic Tumor by Defucosylated Anti-Epidermal Growth Factor Receptor Monoclonal Antibody

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The epidermal growth factor receptor (EGFR) is involved in tumor malignancy through gene amplification and/or protein overexpression. An anti-human EGFR (hEGFR) monoclonal antibody (clone EMab-134), which explicitly detects hEGFR and dog EGFR (dEGFR), was previously developed. The defucosylated mouse IgG_{2a} version of EMab-134 (134-mG_{2a}-f) exhibits antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) in dEGFR-overexpressed CHO-K1 (CHO/dEGFR) cells and antitumor activities in mouse xenografts of CHO/dEGFR cells. In this study, it was shown that 134-mG_{2a}-f reacts with a canine fibroblastic tumor cell line (A-72) using flow cytometry and immunocytochemistry. Furthermore, 134-mG_{2a}-f exerted ADCC and CDC on A-72 cell line. The administration of 134-mG_{2a}-f significantly inhibited the A-72 xenograft growth. These results suggest that 134-mG_{2a}-f exerts antitumor effects on dEGFR-expressing canine fibroblastic tumors.

Keywords: EGFR, monoclonal antibody, ADCC, CDC, canine fibroblastic tumor, antitumor activity

Introduction

A dog is an animal that spontaneously develops cancers, such as melanomas, breast cancers, and fibroblastic tumors.(1–4) In terms of genetic, histological, and biological features, a dog has strong similarities to humans. Dogs share carcinogenic factors, such as aging, obesity, exposure to carcinogens, and environmental risk factors with humans.(5,6) Dogs grow five to eight times faster than humans, making them suitable models for pathogenesis research and age-related gene profiling.(4) Response to cancer treatment, the acquisition of drug resistance, and cancer metastasis are similarly observed in dogs and humans.(7) Because of its similarity, human cancer research is expected to be applied to dogs.(5,8) The inhibition of the programmed cell death-1 (PD-1) pathway has been reported to be one of the therapeutic strategies in canine metastatic oral malignant melanoma.(9)

Epidermal growth factor receptor (EGFR) is a type I transmembrane protein that forms a dimer by binding its ligand and regulating cell proliferation and survival. Overexpression and mutations of EGFR activate multiple intracellular cascades.(10) EGFR is closely associated with different cancer development and malignancy in humans and dogs.(11–15) EGFR expression also correlates with poor prognosis in canine mammary cancers.(16) Human EGFR (hEGFR) and dog EGFR (dEGFR) have 91% amino acid homology.(17) Therefore, the development of therapeutic methods targeting EGFR is suitable as a canine cancer treatment.

An anti-hEGFR monoclonal antibody (mAb), clone EMab-134 (mouse IgG_1, kappa), was previously developed.(18) The 134-mG_{2a}, an IgG_{2a} type of EMab-134, exerted antitumor activities in a model of human oral squamous cell carcinoma.(19) In addition, the 134-mG_{2a}-f, defucosylated type of 134-mG_{2a}, possesses antitumor effects in mouse xenograft models of dEGFR-expressed cells.(20)

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In this study, we investigated whether 134-mG_{2a}-f possesses antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antitumor activities using a canine fibroblastic tumor xenograft model.

Materials and Methods

Cell lines, antibodies, and animals

A canine fibroblast cell line (A-72) was obtained from the American Type Culture Collection (Manassas, VA). A-72 was cultured in Dulbecco's modified Eagle medium (DMEM; Nacalai Tesque, Inc., Kyoto, Japan), supplemented using 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific Inc., Waltham, MA), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B (Nacalai Tesque, Inc.). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

An anti-hEGFR mAb, EMab-134, was developed as previously described.(18) To generate 134-mG_{2a}, V_H complementary DNA (cDNA) of EMab-134 and C_H mouse IgG_{2a} was subcloned into the pCAG-Neo vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and V_L and C_L cDNAs of EMab-134 were subcloned into the pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation).(19) The vectors of 134-mG_{2a} were transfected into BINDS-09 cells (FUT8-deficient ExpiCHO-S cells) using the ExpiCHO expression system (Thermo Fisher Scientific Inc.).(20) The resulting mAbs (134-mG_{2a}-f) were purified using Protein G-Sepharose (GE Healthcare Biosciences, Pittsburgh, PA).(20) Mouse IgG (cat. no. I8765) and mouse IgG_{2a} (cat. no. M7769) were bought from Sigma-Aldrich (St. Louis, MO).

Female BALB/c nude mice (5 weeks old, weighing 14–17 g) were bought from Charles River Laboratories, Inc. The animal experiments were conducted following relevant guidelines and regulations to minimize animal suffering and distress in the laboratory. The institutional committee for experiments of the Institute of Microbial Chemistry approved animal experiments for ADCC and antitumor activity (permit Nos. 2021-028 for ADCC assays and 2021-021 for antitumor experiments).

Mice were maintained in a specific pathogen-free environment (23°C ± 2°C, 55% ± 5% humidity) on 11 hours light/13 hours dark cycle with food and water supplied *ad libitum* across the experimental period. Mice were monitored for health and weight every 2–5 days during the 3 weeks period of each experiment. The loss of original body weight to a point >25% and/or a maximum tumor size >3000 mm^3 were determined as humane endpoints for euthanasia. Mice were euthanized by cervical dislocation; death was verified through respiratory and cardiac arrest.

Flow cytometry

A-72 cells were harvested by brief exposure to 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (EDTA, Nacalai Tesque, Inc.). After washing with 0.1% bovine serum albumin (BSA) blocking buffer in phosphate-buffered saline (PBS), cells were treated with 1 μg/mL 134-mG_{2a}-f or control blocking buffer for 30 minutes at 4°C. Then cells were incubated in Alexa Fluor 488-conjugated anti-mouse IgG at a dilution of 1:1000 (cat no. 4408S; Cell Signaling Technology, Inc., Danvers, MA) for 30 minutes at 4°C. Fluorescence data were obtained using the EC800 Cell Analyzer (Sony Corp., Tokyo, Japan).

Determination of binding affinity

A-72 cells were suspended in 100 μL of serially diluted 134-mG_{2a}-f (0.006–100 μg/mL) followed by Alexa Fluor 488-conjugated anti-mouse IgG (1:200; Cell Signaling Technology, Inc.). Fluorescence data were obtained using the EC800 Cell Analyzer (Sony Corp.). The dissociation constant (K_D) was calculated by fitting binding isotherms to built-in one-site binding models in GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA).

Immunocytochemical analysis

Subconfluent A-72 cells cultured on acid-wash coverslips were fixed in 4% paraformaldehyde in PBS for 10 minutes at room temperature. After quenching with 50 mM NH_4Cl in PBS containing 0.2 mM Ca^{2+} and 2 mM Mg^{2+} (PBSc/m) for 10 minutes, the cells were blocked using a blocking buffer (PBSc/m containing 0.5% BSA) for 30 minutes and incubated with 10 μg/mL of 134-mG_{2a}-f or control blocking buffer for 1 hour. Then, the cells were labeled using Alexa Fluor 488-conjugated anti-mouse IgG for 45 minutes. The cell nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific Inc.). A fluorescence microscope BZ-X800 (Keyence, Osaka, Japan) was used to obtain fluorescence images.

ADCC

ADCC assay was previously reported.(20–36) In brief, spleen cells from five mice were used as the source of mononuclear cells to evaluate ADCC. After euthanasia by cervical dislocation, the spleens were eliminated aseptically and a syringe was used to force spleen tissue through a sterile cell strainer (352360; BD Falcon, Corning, New York, NY) and obtain single-cell suspensions. Erythrocytes were lysed by 10 seconds exposure to ice-cold distilled water. Splenocytes were washed using DMEM and resuspended in DMEM with 10% FBS to be used as effector cells.

A-72 cells were labeled with 10 μg/mL of Calcein AM (Thermo Fisher Scientific, Inc.) and resuspended in the same medium. A-72 cells (2×10^4 cells/well) were plated in 96-well plates and mixed with splenocytes (effector/target cells ratio, 50), 100 μg/mL of 134-mG_{2a}-f or control mouse IgG_{2a}. After 4.5 hours incubation at 37°C, the amount of calcein released into the supernatant was measured in each well. The fluorescence intensity was determined using a microplate reader (Power Scan HT; BioTek Instruments, Inc., Winooski, VT) with an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

Cytolytic activity (% lysis) was calculated as follows: % lysis = $(E - S)/(M - S) \times 100$, where ''E'' is the fluorescence measured in combined cultures of target and effector cells, ''S'' is the spontaneous fluorescence of target cells only, and ''M'' is the maximum fluorescence measured after the lysis of all cells with a buffer containing 0.5% Triton X-100, 10 mM Tris-HCl (pH 7.4), and 10 mM EDTA.

CDC

CDC assay was previously reported.(20–36) In brief, A-72 cells were labeled with 10 μg/mL of Calcein AM (Thermo Fisher Scientific, Inc.) and resuspended in the same medium. A-72 cells were then plated in 96-well plates at 2×10^4

cells/well using rabbit complement (final dilution 1:10; Low-Tox-M Rabbit Complement; Cedarlane Laboratories, Hornby, Ontario, Canada) and 100 μg/mL of 134-mG$_{2a}$-f or control mouse IgG$_{2a}$. After 4 hours of incubation at 37°C, the amount of calcein released into the supernatant for each well was measured.

Cytolytic activity (% lysis) was calculated as follows: % lysis = (E − S)/(M − S) × 100, where ''E'' is the fluorescence measured in combined cultures of target and effector cells, ''S'' is the spontaneous fluorescence of target cells only, and ''M'' is the maximum fluorescence measured after the lysis of all cells with a buffer containing 0.5% Triton X-100, 10 mM Tris-HCl (pH 7.4), and 10 mM EDTA.

Antitumor activity of 134-mG$_{2a}$-f in xenografts of A-72 cells

Sixteen female BALB/c nude mice were used in experiments once they reached 7 weeks of age. A-72 cells (0.3 mL 1.33×10^8 cells/mL in DMEM) were mixed with 0.5 mL of BD Matrigel Matrix Growth Factor Reduced (BD Biosciences, San Jose, CA); 100 μL of this suspension (5×10^6 cells) was injected subcutaneously into the left flanks of the mice.

On day 7 postinoculation, 100 μg of 134-mG$_{2a}$-f ($n = 8$), or control mouse IgG ($n = 8$) in 100 μL of PBS was injected intraperitoneally. Additional antibody injections were administered on days 14 and 21. On day 25 after cell implantation, all mice were euthanized through cervical dislocation. Tumor diameters and volumes were determined as previously described.(20)

Statistical analyses

All data are expressed as mean ± standard error of the mean. Statistical analysis was conducted using Tukey's test for ADCC and CDC and Welch's *t*-test for tumor weight. Analysis of variance and Sidak's multiple comparison tests were performed for tumor volume and mouse weight. All calculations were conducted using GraphPad Prism 8 (GraphPad Software, Inc.). A p-value of <0.05 was considered statistically significant.

Results

Flow cytometry analysis against canine fibroblastic tumor cell line, A-72 cells using 134-mG$_{2a}$-f

In our previous study, an anti-hEGFR mAb, EMab-134, recognized CHO/dEGFR cells, showing that EMab-134 cross-reacts with dEGFR.(20) In this study, the defucosylated mouse IgG$_{2a}$ type of EMab-134 (134-mG$_{2a}$-f) detected A-72 cells (Fig. 1A), showing that 134-mG$_{2a}$-f detects endogenous dEGFR expressed on A-72 cells.

Determination of binding affinity

A kinetic analysis of the interactions of 134-mG$_{2a}$-f with A-72 cells was conducted using flow cytometry. As shown in Figure 1B, the K_D for the interaction of 134-mG$_{2a}$-f with A-72 cells was 1.1×10^{-9} M, suggesting that 134-mG$_{2a}$-f shows a high affinity for A-72 cells.

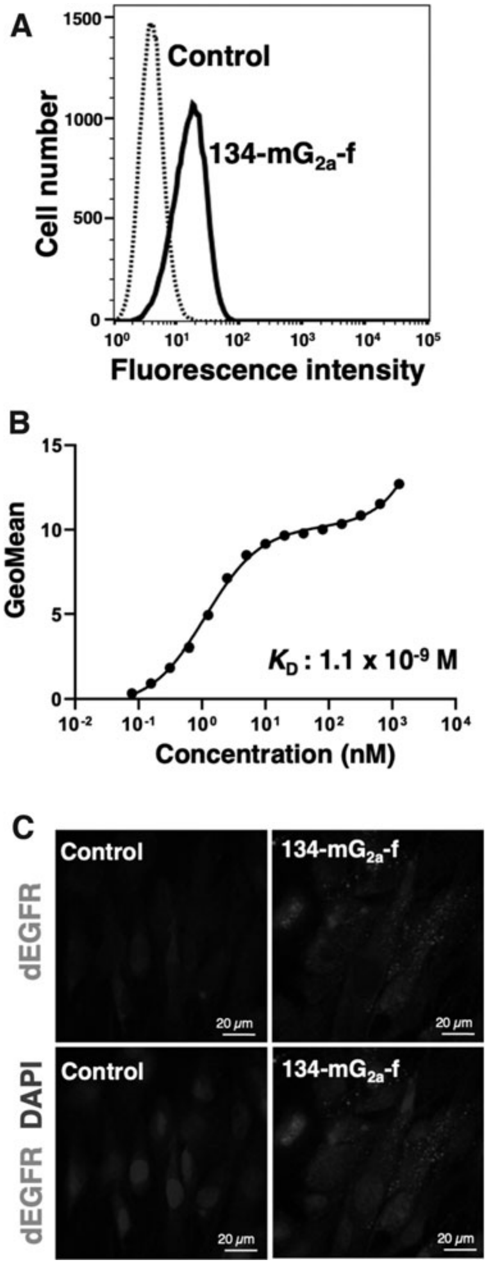

FIG. 1. Flow cytometry and immunocytochemistry using 134-mG$_{2a}$-f. **(A)** Flow cytometry using A-72 cells and 134-mG$_{2a}$-f. **(B)** Determination of the binding affinity of 134-mG$_{2a}$-f for A-72 cells using flow cytometry. **(C)** Immunocytochemical analyses using A-72 cells and 134-mG$_{2a}$-f. DAPI, 4',6-diamidino-2-phenylindole; dEGFR, dog epidermal growth factor receptor.

Immunocytochemical analysis against A-72 cells using 134-mG$_{2a}$-f

The 134-mG$_{2a}$-f was applied to immunocytochemistry in A-72 cells. As shown in Figure 1C, 134-mG$_{2a}$-f, but not buffer control, visualized dEGFR in A-72 cells, suggesting that 134-mG$_{2a}$-f recognizes endogenous dEGFR in A-72 cells.

134-mG$_{2a}$-f-mediated ADCC and CDC in A-72 cells

Next, it was investigated whether 134-mG$_{2a}$-f has ADCC against A-72 cells. As indicated in Figure 2A, 134-mG$_{2a}$-f showed ADCC (15.8% cytotoxicity) against A-72 cells, which is more potent than mouse IgG$_{2a}$ (5.6% cytotoxicity) and PBS (4.0% cytotoxicity).

It was then investigated whether 134-mG$_{2a}$-f has CDC against A-72 cells. As shown in Figure 2B, 134-mG$_{2a}$-f elicited a higher degree of CDC (43.2% cytotoxicity) in A-72 cells than that elicited by mouse IgG$_{2a}$ (36.1% cytotoxicity) and PBS (31.8% cytotoxicity). These results showed that 134-mG$_{2a}$-f exerts ADCC and CDC against dEGFR-expressing A-72 cells.

Antitumor activities of 134-mG$_{2a}$-f in the mouse xenografts of A-72 cells

In the A-72 xenograft models, 134-mG$_{2a}$-f (100 μg) and control mouse IgG (100 μg) were injected intraperitoneally into mice on days 7, 14, and 21 after the injection of A-72 cells. The tumor volume was measured on days 7, 11, 14, 18, 21, and 25 postinoculation. The administration of 134-mG$_{2a}$-f caused a significant reduction in tumor development on days 11 ($p < 0.01$), 14 ($p < 0.01$), 18 ($p < 0.01$), 21 ($p < 0.01$), and 25 ($p < 0.01$) compared with that of the control mouse IgG (Fig. 3A).

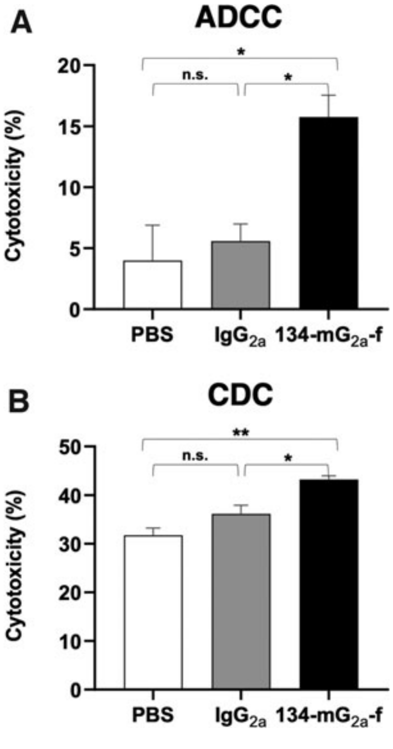

FIG. 2. Evaluation of ADCC and CDC elicited by 134-mG$_{2a}$-f. **(A)** ADCC elicited by 134-mG$_{2a}$-f, mouse IgG$_{2a}$, or PBS targeting A-72 cells. Asterisks indicate statistical significance (*$p < 0.05$, n.s.; Tukey's test). **(B)** CDC elicited by 134-mG$_{2a}$-f, mouse IgG$_{2a}$, or PBS targeting A-72 cells. Values are means ± SEM. Asterisks indicate statistical significance (**$p < 0.01$, *$p < 0.05$, n.s.; Tukey's test). ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; n.s., not significant; PBS, phosphate-buffered saline; SEM, standard error of the mean.

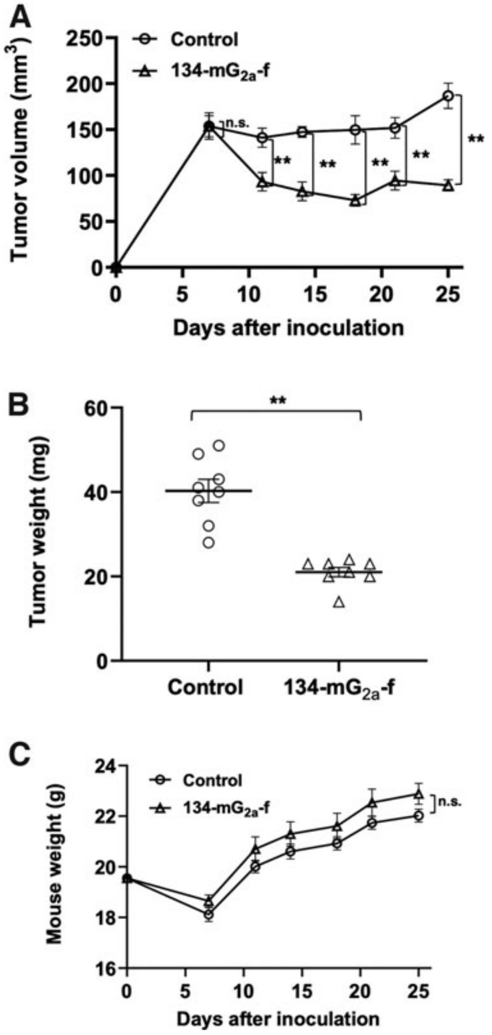

FIG. 3. Evaluation of antitumor activity of 134-mG$_{2a}$-f in A-72 xenografts. **(A)** A-72 cells were subcutaneously injected into the left flank. On day 7, 100 μg 134-mG$_{2a}$-f or mouse IgG in 100 μL PBS was injected intraperitoneally into mice; additional antibodies were then injected on days 14 and 21. The tumor volume was measured on days 7, 11, 14, 18, 21, and 25 after the injection. Values are means ± SEM. Asterisks indicate statistical significance (**$p < 0.01$, n.s.; ANOVA and Sidak's multiple comparisons test). **(B)** A-72 xenografts were resected from 134-mG$_{2a}$-f and mouse IgG groups. Tumor weight on day 25 was measured. Values are means ± SEM. Asterisk indicates statistical significance (**$p < 0.01$, Welch's *t*-test). **(C)** Body weights of mice implanted with A-72 xenografts were recorded on days 7, 11, 14, 18, 21, and 25 (n.s.). ANOVA, analysis of variance; n.s., not significant.

The administration of 134-mG$_{2a}$-f caused 52% reduction in tumor volume compared with that of the control mouse IgG on day 25 postinjection. Furthermore, the tumor weight of the 134-mG$_{2a}$-f-treated mice was significantly lower than that of IgG-treated mice (48% reduction; $p < 0.01$, Fig. 3B). The total body weights of the two groups did not significantly change (Fig. 3C). Altogether, these results indicate that the administration of 134-mG$_{2a}$-f effectively suppresses tumor growth of A-72 xenografts.

Discussion

EGFR is an essential oncoprotein, which promotes tumor development.(10) Therefore, therapeutic agents targeting EGFR have been developed.(37,38) The EGFR-targeted antibody drugs, including panitumumab and cetuximab,(39,40) have similar binding affinities to EGFR but different epitopes on the EGFR.(41–43) These mAbs can inhibit the EGFR signaling pathways and induce apoptosis in EGFR-expressing cancer cells and exhibit therapeutic potential for patients with advanced colorectal cancer.(44,45)

Approved drugs for humans are often used for canine cancer treatment due to their similarities.(5,46) Because EGFR is involved in canine cancer malignancy and poor prognosis, EGFR-targeting drugs are considered an important therapeutic strategy. It has been previously shown that the 134-mG_{2a}-f has an antitumor effect against a dEGFR-overexpressed CHO-K1 model.(20) This study also demonstrated antitumor activities of a defucosylated IgG_{2a} type of anti-hEGFR mAb (134-mG_{2a}-f) *in vitro* and *in vivo* on canine fibroblastic tumor cells expressing endogenous dEGFR (Figs. 2 and 3).

The combination therapy of cetuximab and pembrolizumab is successful for human head and neck squamous cell carcinomas.(47) Pembrolizumab is an immune checkpoint inhibitor that recognizes immunosuppressive molecule PD-1 expressed on T cells and has been expanded to various human cancers recently.(48) Immune checkpoint inhibitors and chimeric antigen receptor-T cell therapy are also used to treat canine cancers.(49) Therefore, the combination of anti-EGFR mAb therapy and immune checkpoint inhibitors is expected to be more effective in canine cancer treatment. Further studies are needed to investigate the antitumor activity of 134-mG_{2a}-f to spontaneously develop canine cancers.

Author Disclosure Statement

No competing financial interests exist.

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References

1. Gordon I, Paoloni M, Mazcko C, and Khanna C: The comparative oncology trials consortium: Using spontaneously occurring cancers in dogs to inform the cancer drug development pathway. PLoS Med 2009;6:e1000161.
2. Graim K, Gorenshteyn D, Robinson DG, Carriero NJ, Cahill JA, Chakrabarti R, Goldschmidt MH, Durham AC, Funk J, Storey JD, Kristensen VN, Theesfeld CL, Sorenmo KU, and Troyanskaya OG: Modeling molecular development of breast cancer in canine mammary tumors. Genome Res 2020;31:337–347.
3. Kabir FML, DeInnocentes P, Agarwal P, Mill CP, Riese Nd DJ, and Bird RC: Estrogen receptor-α, progesterone receptor, and c-erbB/HER-family receptor mRNA detection and phenotype analysis in spontaneous canine models of breast cancer. J Vet Sci 2017;18:149–158.
4. Rowell JL, McCarthy DO, and Alvarez CE: Dog models of naturally occurring cancer. Trends Mol Med 2011;17:380–388.
5. Paoloni M, and Khanna C: Translation of new cancer treatments from pet dogs to humans. Nat Rev Cancer 2008;8:147–156.
6. Sorenmo KU, Kristiansen VM, Cofone MA, Shofer FS, Breen AM, Langeland M, Mongil CM, Grondahl AM, Teige J, and Goldschmidt MH: Canine mammary gland tumours; a histological continuum from benign to malignant; clinical and histopathological evidence. Vet Comp Oncol 2009;7:162–172.
7. Schiffman JD, and Breen M: Comparative oncology: What dogs and other species can teach us about humans with cancer. Philos Trans R Soc Lond B Biol Sci 2015;370:20140231.
8. Khanna C, London C, Vail D, Mazcko C, and Hirschfeld S: Guiding the optimal translation of new cancer treatments from canine to human cancer patients. Clin Cancer Res 2009;15:5671–5677.
9. Maekawa N, Konnai S, Nishimura M, Kagawa Y, Takagi S, Hosoya K, Ohta H, Kim S, Okagawa T, Izumi Y, Deguchi T, Kato Y, Yamamoto S, Yamamoto K, Toda M, Nakajima C, Suzuki Y, Murata S, and Ohashi K: PD-L1 immunohistochemistry for canine cancers and clinical benefit of anti-PD-L1 antibody in dogs with pulmonary metastatic oral malignant melanoma. NPJ Precis Oncol 2021;5:10.
10. Uribe ML, Marrocco I, and Yarden Y: EGFR in cancer: Signaling mechanisms, drugs, and acquired resistance. Cancers (Basel) 2021;13:2748.
11. Yewale C, Baradia D, Vhora I, Patil S, and Misra A: Epidermal growth factor receptor targeting in cancer: A review of trends and strategies. Biomaterials 2013;34:8690–8707.
12. Hanazono K, Fukumoto S, Kawamura Y, Endo Y, Kadosawa T, Iwano H, and Uchide T: Epidermal growth factor receptor expression in canine transitional cell carcinoma. J Vet Med Sci 2015;77:1–6.
13. Bertagnolli AC, Ferreira E, Dias EJ, and Cassali GD: Canine mammary mixed tumours: Immunohistochemical expressions of EGFR and HER-2. Aust Vet J 2011;89:312–317.
14. Araújo MR, Campos LC, Damasceno KA, Gamba CO, Ferreira E, and Cassali GD: HER-2, EGFR, Cox-2 and Ki67 expression in lymph node metastasis of canine mammary carcinomas: Association with clinical-pathological parameters and overall survival. Res Vet Sci 2016;106:121–130.
15. Carvalho MI, Guimarães MJ, Pires I, Prada J, Silva-Carvalho R, Lopes C, and Queiroga FL: EGFR and microvessel density in canine malignant mammary tumours. Res Vet Sci 2013;95:1094–1099.
16. Gama A, Gärtner F, Alves A, and Schmitt F: Immunohistochemical expression of Epidermal Growth Factor Receptor (EGFR) in canine mammary tissues. Res Vet Sci 2009;87:432–437.
17. Singer J, Weichselbaumer M, Stockner T, Mechtcheriakova D, Sobanov Y, Bajna E, Wrba F, Horvat R, Thalhammer JG, Willmann M, and Jensen-Jarolim E: Comparative oncology: ErbB-1 and ErbB-2 homologues in canine cancer are susceptible to cetuximab and trastuzumab targeting. Mol Immunol 2012;50:200–209.
18. Itai S, Yamada S, Kaneko MK, Chang YW, Harada H, and Kato Y: Establishment of EMab-134, a sensitive and specific anti-epidermal growth factor receptor monoclonal antibody for detecting squamous cell carcinoma cells of the oral cavity. Monoclon Antib Immunodiagn Immunother 2017;36:272–281.

19. Hosono H, Takei J, Ohishi T, Sano M, Asano T, Sayama Y, Nakamura T, Yanaka M, Kawada M, Harada H, Kaneko MK, and Kato Y: Anti-EGFR monoclonal antibody 134-mG2a exerts antitumor effects in mouse xenograft models of oral squamous cell carcinoma. Int J Mol Med 2020;46:1443–1452.
20. Tateyama N, Nanamiya R, Ohishi T, Takei J, Nakamura T, Yanaka M, Hosono H, Saito M, Asano T, Tanaka T, Sano M, Kawada M, Kaneko MK, and Kato Y: Defucosylated anti-epidermal growth factor receptor monoclonal antibody 134-mG(2a)-f exerts antitumor activities in mouse xenograft models of dog epidermal growth factor receptor-overexpressed cells. Monoclon Antib Immunodiagn Immunother 2021;40:177–183.
21. Tateyama N, Asano T, Ohishi T, Takei J, Hosono H, Nanamiya R, Tanaka T, Sano M, Saito M, Kawada M, Kaneko MK, and Kato Y: An anti-HER2 monoclonal antibody H(2)Mab-41 exerts antitumor activities in mouse xenograft model using dog HER2-overexpressed cells. Monoclon Antib Immunodiagn Immunother 2021;40:184–190.
22. Asano T, Ohishi T, Takei J, Nakamura T, Nanamiya R, Hosono H, Tanaka T, Sano M, Harada H, Kawada M, Kaneko MK, and Kato Y: AntiHER3 monoclonal antibody exerts antitumor activity in a mouse model of colorectal adenocarcinoma. Oncol Rep 2021;46:173.
23. Tanaka T, Ohishi T, Asano T, Takei J, Nanamiya R, Hosono H, Sano M, Harada H, Kawada M, Kaneko MK, and Kato Y: An antiTROP2 monoclonal antibody TrMab6 exerts antitumor activity in breast cancer mouse xenograft models. Oncol Rep 2021;46:132.
24. Hosono H, Ohishi T, Takei J, Asano T, Sayama Y, Kawada M, Kaneko MK, and Kato Y: The anti-epithelial cell adhesion molecule (EpCAM) monoclonal antibody EpMab-16 exerts antitumor activity in a mouse model of colorectal adenocarcinoma. Oncol Lett 2020;20:383.
25. Kaneko MK, Ohishi T, Takei J, Sano M, Nakamura T, Hosono H, Yanaka M, Asano T, Sayama Y, Harada H, Kawada M, and Kato Y: AntiEpCAM monoclonal antibody exerts antitumor activity against oral squamous cell carcinomas. Oncol Rep 2020;44:2517–2526.
26. Kaneko MK, Ohishi T, Kawada M, and Kato Y: A cancer-specific anti-podocalyxin monoclonal antibody (60-mG2a-f) exerts antitumor effects in mouse xenograft models of pancreatic carcinoma. Biochem Biophys Rep 2020;24:100826.
27. Kaneko MK, Ohishi T, Nakamura T, Inoue H, Takei J, Sano M, Asano T, Sayama Y, Hosono H, Suzuki H, Kawada M, and Kato Y: Development of core-fucose-deficient humanized and chimeric anti-human podoplanin antibodies. Monoclon Antib Immunodiagn Immunother 2020;39:167–174.
28. Takei J, Kaneko MK, Ohishi T, Hosono H, Nakamura T, Yanaka M, Sano M, Asano T, Sayama Y, Kawada M, Harada H, and Kato Y: A defucosylated antiCD44 monoclonal antibody 5mG2af exerts antitumor effects in mouse xenograft models of oral squamous cell carcinoma. Oncol Rep 2020;44:1949–1960.
29. Takei J, Ohishi T, Kaneko MK, Harada H, Kawada M, and Kato Y: A defucosylated anti-PD-L1 monoclonal antibody 13-mG2a-f exerts antitumor effects in mouse xenograft models of oral squamous cell carcinoma. Biochem Biophys Rep 2020;24:100801.
30. Ohishi T, Kato Y, Kaneko MK, Ohba SI, Inoue H, Harakawa A, and Kawada M: Anti-metastatic activity of an anti-EGFR monoclonal antibody against metastatic colorectal cancer with KRAS p.G13D mutation. Int J Mol Sci 2020;21:6037.
31. Takei J, Kaneko MK, Ohishi T, Kawada M, Harada H, and Kato Y: H2Mab-19, an anti-human epidermal growth factor receptor 2 monoclonal antibody exerts antitumor activity in mouse oral cancer xenografts. Exp Ther Med 2020;20:846–853.
32. Kato Y, Ohishi T, Takei J, Nakamura T, Sano M, Asano T, Sayama Y, Hosono H, Kawada M, and Kaneko MK: An anti-human epidermal growth factor receptor 2 monoclonal antibody H2Mab-19 exerts antitumor activity in mouse colon cancer xenografts. Monoclon Antib Immunodiagn Immunother 2020;39:123–128.
33. Takei J, Kaneko MK, Ohishi T, Kawada M, Harada H, and Kato Y: A novel anti-EGFR monoclonal antibody (EMab-17) exerts antitumor activity against oral squamous cell carcinomas via antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Oncol Lett 2020; 19:2809–2816.
34. Kato Y, Ito Y, Ohishi T, Kawada M, Nakamura T, Sayama Y, Sano M, Asano T, Yanaka M, Okamoto S, Handa S, Komatsu Y, Takei J, and Kaneko MK: Antibody-drug conjugates using mouse-canine chimeric anti-dog podoplanin antibody exerts antitumor activity in a mouse xenograft model. Monoclon Antib Immunodiagn Immunother 2020;39:37–44.
35. Kato Y, Ohishi T, Kawada M, Maekawa N, Konnai S, Itai S, Yamada S, and Kaneko MK: The mouse-canine chimeric anti-dog podoplanin antibody P38B exerts antitumor activity in mouse xenograft models. Biochem Biophys Rep 2019;17:23–26.
36. Itai S, Ohishi T, Kaneko MK, Yamada S, Abe S, Nakamura T, Yanaka M, Chang YW, Ohba SI, Nishioka Y, Kawada M, Harada H, and Kato Y: Anti-podocalyxin antibody exerts antitumor effects via antibody-dependent cellular cytotoxicity in mouse xenograft models of oral squamous cell carcinoma. Oncotarget 2018;9:22480–22497.
37. Kuwano M, Sonoda K, Murakami Y, Watari K, and Ono M: Overcoming drug resistance to receptor tyrosine kinase inhibitors: Learning from lung cancer. Pharmacol Ther 2016;161:97–110.
38. Guardiola S, Varese M, Sánchez-Navarro M, and Giralt E: A third shot at EGFR: New opportunities in cancer therapy. Trends Pharmacol Sci 2019;40:941–955.
39. Van Cutsem E, Lenz HJ, Köhne CH, Heinemann V, Tejpar S, Melezínek I, Beier F, Stroh C, Rougier P, van Krieken JH, and Ciardiello F: Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol 2015;33:692–700.
40. Peeters M, Douillard JY, Van Cutsem E, Siena S, Zhang K, Williams R, and Wiezorek J: Mutant KRAS codon 12 and 13 alleles in patients with metastatic colorectal cancer: Assessment as prognostic and predictive biomarkers of response to panitumumab. J Clin Oncol 2013;31:759–765.
41. López-Albaitero A, and Ferris RL: Immune activation by epidermal growth factor receptor specific monoclonal antibody therapy for head and neck cancer. Arch Otolaryngol Head Neck Surg 2007;133:1277–1281.
42. Dienstmann R, Markman B, and Tabernero J: Application of monoclonal antibodies as cancer therapy in solid tumors. Curr Clin Pharmacol 2012;7:137–145.
43. García-Foncillas J, Sunakawa Y, Aderka D, Wainberg Z, Ronga P, Witzler P, and Stintzing S: Distinguishing features of cetuximab and panitumumab in colorectal cancer and other solid tumors. Front Oncol 2019;9:849.

44. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, McAllister PK, Morton RF, and Schilsky RL: American Society of Clinical Oncology provisional clinical opinion: Testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009;27:2091–2096.
45. El Guerrab A, Bamdad M, Kwiatkowski F, Bignon YJ, Penault-Llorca F, and Aubel C: Anti-EGFR monoclonal antibodies and EGFR tyrosine kinase inhibitors as combination therapy for triple-negative breast cancer. Oncotarget 2016;7:73618–73637.
46. Simon D, Schoenrock D, Baumgärtner W, and Nolte I: Postoperative adjuvant treatment of invasive malignant mammary gland tumors in dogs with doxorubicin and docetaxel. J Vet Intern Med 2006;20:1184–1190.
47. Sacco AG, Chen R, Worden FP, Wong DJL, Adkins D, Swiecicki P, Chai-Ho W, Oppelt P, Ghosh D, Bykowski J, Molinolo A, Pittman E, Estrada MV, Gold K, Daniels G, Lippman SM, Natsuhara A, Messer K, and Cohen EEW: Pembrolizumab plus cetuximab in patients with recurrent or metastatic head and neck squamous cell carcinoma: An open-label, multi-arm, non-randomised, multicentre, phase 2 trial. Lancet Oncol 2021;22:883–892.
48. Doroshow DB, Bhalla S, Beasley MB, Sholl LM, Kerr KM, Gnjatic S, Wistuba, II, Rimm DL, Tsao MS, and Hirsch FR: PD-L1 as a biomarker of response to immune-checkpoint inhibitors. Nat Rev Clin Oncol 2021;18:345–362.
49. Panjwani MK, Smith JB, Schutsky K, Gnanandarajah J, O’Connor CM, Powell DJ, Jr., and Mason NJ: Feasibility and safety of RNA-transfected CD20-specific chimeric antigen receptor T cells in dogs with spontaneous B cell lymphoma. Mol Ther 2016;24:1602–1614.

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