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RESEARCH PAPER

The Airways, a Novel Route for Delivering Monoclonal Antibodies to Treat Lung Tumors

Agnès Maillet • Laurent Guilleminault • Etienne Lemarié • Stéphanie Lerondel • Nicolas Azzopardi • Jérôme Montharu • Nicolas Congy-Jolivet • Pascale Reverdiau • Brigitte Legrain • Christelle Parent • Dominique-Henri Douvin • José Hureaux • Yves Courty • Michèle De Monte • Patrice Diot • Gilles Paintaud • Alain Le Pape • Hervé Watier • Nathalie Heuzé-Vourc'h

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ABSTRACT

Purpose Lung cancer is the leading cause of cancer-related death worldwide. The efficacy of current systemic treatments is limited, with major side effects and only modest survival improvements. Aerosols routinely used to deliver drugs into the lung for treating infectious and inflammatory lung diseases have never been used to deliver monoclonal antibodies to treat lung cancer. We have shown that cetuximab, a chimeric anticancer anti-EGFR mAb, is suitable for airway delivery as it resists the physical constraints of aerosolization, and have evaluated the aerosol delivery of cetuximab *in vivo*.

Methods We developed an animal model of lung tumor sensitive to cetuximab by injecting Balb/c Nude mice intratracheally with A431 cells plus 10 mM EDTA and analyzed the distribution, pharmacokinetics and antitumor efficacy of cetuximab aerosolized into the respiratory tract.

Results Aerosolized IgG accumulated durably in the lungs and the tumor, but passed poorly and slowly into the systemic circulation. Aerosolized cetuximab also limited the growth of the mouse tumor. Thus, administering anticancer mAbs via the airways is effective and may limit systemic side effects.

Conclusion Delivery of aerosolized-mAbs via the airways deserves further evaluation for treating lung cancers.

KEY WORDS aerosols · antibodies · carcinoma · monoclonal · non-small-cell lung

INTRODUCTION

The lungs are the organs most commonly affected by primary and metastatic tumors. Non-small-cell lung cancer

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A. Maillet • L. Guilleminault • E. Lemarié • P. Reverdiau • C. Parent • Y. Courty • M. De Monte • P. Diot • A. Le Pape • N. Heuzé-Vourc'h
INSERM U618, Université François Rabelais de Tours
Tours, France

E. Lemarié • P. Diot
CHRU de Tours
Tours, France

S. Lerondel • A. Le Pape
TAAM–CIPA UPS44
Orléans, France

N. Azzopardi • N. Congy-Jolivet • G. Paintaud • H. Watier
UMR6239 GICC, CHRU de Tours
Tours, France

J. Montharu
PPF « Animaleries », Faculté de Médecine
Tours, France

B. Legrain • D.-H. Douvin
Laboratoires Novaxia
Saint Laurent Nouan, France

J. Hureaux
CHU d'Angers Service de Pneumologie
Angers, France

N. Heuzé-Vourc'h (✉)
Faculty of Medicine, INSERM U618
10 Blvd Tonnellé
37032 Tours, France
e-mail: Nathalie.vourch@med.univ-tours.fr

(NSCLC) is the major form of primary lung cancer, accounting for 85% of cases (1,2). Stages I and II NSCLC are primarily treated by pulmonary resection. However, NSCLC is often diagnosed at an advanced stage and has a poor prognosis. Several therapeutic regimens are presently available, including targeted therapies combined with conventional approaches and double/triple chemotherapy. Although platinum-double chemotherapy has become a standard, the median survival with palliative chemotherapy does not exceed one year. Other conventional cytotoxic agents and targeted therapies have been added to doublet chemotherapy, but treatment outcomes of NSCLC remain unsatisfactory (3). In general, systemic mono- or multimodal therapies may provide improved quality of life but do little to prolong survival (4).

The airways are an attractive alternative to the systemic route for treating lung affections. They are routinely used in clinical practice to treat inflammatory and infectious lung diseases like asthma, cystic fibrosis and pneumonia with aerosolized drugs. Delivering an aerosolized anticancer drug directly to the lungs could provide higher drug concentrations at the target site and lower drug diffusion to other organs, thereby increasing the therapeutic index. Preclinical studies and clinical trials have demonstrated the safety, pharmacokinetic advantage and antitumor efficiency of several chemotherapeutic agents (doxorubicin, gemcitabine 9-nitrocamptothcin, liposome-encapsulated paclitaxel, etc.) delivered through the airways (5). Interleukin-2 has also been delivered via the respiratory tract as an alternative way of treating patients with pulmonary metastases of renal cell carcinoma, and clinical studies have shown that IL-2 aerosols are both efficient and well tolerated (6). Despite those promises of aerosol therapy, delivery of anticancer agent via the airways has not yet been approved for clinical use (5,7).

Monoclonal antibodies (mAbs) are large molecules, around 150 kDa and a well established class of therapeutics; many FDA-approved mAbs are now available for treating a wide range of disorders (8,9). Twelve antitumor mAbs have been approved since 1995, and over a hundred are currently in clinical development. Bevacizumab, a mAb that targets vascular endothelial growth factor (VEGF), is now approved as a first line treatment in combination with chemotherapy for patients with advanced NSCLC. However, its infusion is restricted to cancers without a squamous histology because patients with squamous-cell carcinoma suffered from frequent, serious pulmonary bleedings (3). The promising results of anti-EGFR mAbs in combination with cytotoxic therapies in animals have lead to various clinical trials. Adding cetuximab to chemotherapy or radiation therapy extends the survival of patients with metastatic colorectal and locally advanced head and neck cancers. Phase 3 studies have shown that cetuximab slightly improved the overall survival and response rates of patients with NSCLC over those of patients on chemotherapy alone, but it has not yet received FDA approval (10). Several clinical trials are presently evaluating cetuximab plus radiochemotherapy in lung cancer (ClinicalTrials.gov). Cetuximab exerts its antitumor effect by preventing the binding of endogenous EGFR ligands to tumor cells and consequently inhibiting the activation of EGFR and its underlying pathways leading to cell growth and survival. Cetuximab triggers an ADCC (antibody-dependent cytotoxicity) immune response (11). Anticancer mAbs themselves are rarely curative in solid tumors, and their administration by systemic infusion is associated with a variety of adverse effects. These limitations have spurred the development of strategies designed to improve their efficacy, reduce their systemic toxicity, and eventually increase their therapeutic benefits. The airways may be a route for overcoming the drawbacks of intravenous administration of mAbs for treating lung cancer.

We have previously used cetuximab to show that anticancer mAbs resist the physical constraints of aerosolization only with certain devices (12). In particular, the mesh nebulizer AeronebPro™ maintained the immunological and pharmacological properties of cetuximab while avoiding the formation of aggregates during the process of aerosolization. We have also demonstrated that this device can produce an aerosol with the aerodynamic characteristics compatible with mAb delivery to a tumor site within the respiratory tract. We have now evaluated the distribution, pharmacokinetics and antitumor efficacy of cetuximab delivered via the airways in an animal model of ectopic lung tumors. Our results show that administering anticancer mAbs via the airways is effective and may limit systemic side effects.

MATERIAL AND METHODS

Antibodies

Cetuximab (2 mg/mL in sodium phosphate buffer) was purchased from Merck KGaA (Germany). Polyclonal anti-total EGFR (SC-03) antibody was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), and polyclonal anti-phospho EGFR (pY1068) was from Merck KGaA. Anti-human IgG was purchased from Jackson ImmunoResearch (UK). Xenofluor750™ was purchased from Caliper (USA). FITC-conjugated cetuximab was a kind gift from Anthony Courtois (France). A431 human epidermoid carcinoma cells were obtained from the ATCC (American Type Culture Collection) and grown as recommended by the supplier.

Mice and Intrabronchial Tumor Implantation

Female Balb/c nude mice (5 weeks old) were purchased from Charles River Laboratories (France). Animals were handled in accordance with the guide for the care and use of laboratory animals (National Research Council 1996) and European directives EEC 86/809. All studies used protocols approved by the Ethics Committee (CREEA, France).

A431 cells (2.5×10^6) in medium with or without 10 mM EDTA were implanted intrabronchially in mouse lungs as previously described (13). The deposition of cells labeled with 99mTc into the lungs was monitored by scintigraphy as previously described (14).

Preparation and Aerosolization of Cetuximab

Cetuximab was concentrated passively using a membrane with a 7.5 KDa cut-off (Vivascience, Germany). Cetuximab was labeled with Xenofluor750™ using the Fluorescent Dye Kit for *In Vivo* Imaging (Caliper Life Sciences, France) following the conditions recommended by the manufacturer. The quality of Xenofluor750™ conjugation to cetuximab was analyzed by size exclusion HPLC using a TSK 2,000 column (Tosohas) and NaCl 0.9% (1 mL/min) as eluent. Protein and Xenofluor750 were detected using absorbance and fluorescence detections (750/810 nm). Mixture with 5% or less of free fluorophore contamination was accepted for the *in vivo* assay. Samples of cetuximab or cetuximab-Xenofluor750™ were filtered, aerosolized using a Microsprayer™ (Penn-Century Inc., USA) attached to a high-pressure syringe (Penn-Century Inc.) and deposited in the trachea of mice. The maximum delivery volume was 50 µL containing cetuximab plus a 99mTc-labelled tin colloid solution to trace deposition by scintigraphy. Immediately after nebulisation delivery, the mice were submitted to a planar scintigraphic acquisition for 1 min on anterior face using a small animal dedicated gamma camera equipped with a high resolution parallel collimator (Biospace measures). From the 2D images, regions of interest were delineated surrounding the lungs, and oropharyngeal active areas and corresponding activity were determined as compared to fantoms with calibrated 99mTc samples.

Integrity of Cetuximab Following Aerosolization or Labeling

The impact of Microsprayer™-aerosolization on the pharmacological properties of cetuximab was assessed using A431 cells as previously described (12). The affinity of cetuximab conjugated to Xenofluor750™ for EGFR was assessed by a competitive assay using A431 cells and flow cytometry analysis as previously described (12).

Tumor Treatment Study

Tumors were implanted in mice, and 3 weeks later, the mice were assigned to one of two groups. One group was given cetuximab (2 mg) via the airways once a week for 3 weeks (group 1, $n=13$). The other, positive control group, was untreated and used to assess tumor growth (group 2, $n=14$). All the mice were killed at the end of the 3-week period, and their lungs were removed.

Histopathological Study

Samples of fixed tissue were embedded in paraffin, and serial sections were cut and stained with haematoxylin and eosin (H&E). Tumor nodules were identified in the whole lung, and the tumor growth determined by a pathologist measuring the tumor size (tumor diameter) of each nodule with an ocular micrometer.

Distribution

Mice that had harbored tumors for 6–7 weeks were given cetuximab-Xenofluor750™ (60–80 µg) intravenously (i.v.) (group 3, $n=12$) or via the airways (group 4, $n=11$). The distribution of cetuximab was monitored for the next 72 h. The mice were then killed, and the lungs were removed. The drug distribution within them was determined by reflectance near-infrared fluorescence imaging (NIRF) with an IVIS Lumina (Caliper, USA) device using 30 nm bandpass filters (745 nm excitation; 800 nm emission).

Western Blotting of Cetuximab

Treated and control mice were killed. Their lungs were removed, frozen, cryo-dissected and lysed with RIPA buffer. The proteins lysates (20 µg) from whole lung tissues were separated by SDS-PAGE and transferred to a PVDF membrane. Any cetuximab in the lysates was detected by Western blotting using a HRP-conjugated anti-human IgG (Jackson Immunoresearch).

Pharmacokinetics

Mice that had harbored a tumor for 6–7 weeks were given cetuximab-Xenofluor750™ (60–80 µg) i.v ($n=7$ belonging to group 3) or via the airways ($n=6$ belonging to group 4). Blood samples (100–200 µL) were collected submandibularly, and the concentrations of cetuximab in the plasma were measured by ELISA (15). The pharmacokinetics of cetuximab were studied by compartmental and

non-compartmental approaches. The compartmental approach consisted of population pharmacokinetic modeling, using WinNonMix Professional 2.0.1 (Pharsight, Mountain View, USA). This method describes the concentrations measured after both routes of administration with the same model, optimizes the estimation of common parameters, and allows calculation of the fraction absorbed after airways delivery (F). Among the tested models, a two-compartment model with a first-order absorption rate (ka) for the pulmonary route gave the best description of the data. The secondary parameters maximum concentration (Cmax), time of Cmax (Tmax) and area under the concentration *versus* time curve (AUC) were obtained using the compartmental approach. Non-compartmental methods were used to estimate mean residence time (MRT), mean absorption time (MAT) and absorption half-life (T½abs).

Statistical Analysis

All the results are expressed as medians and their dispersion as interquartile ranges. The differences between groups in the growth inhibition assays were compared using the Kruskal-Wallis non-parametric test. For antitumor activity, the difference between groups was compared using the Mann-Withney non-parametric test. A p value of 0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

Establishment of Animal Murine Model with Cetuximab-Sensitive Lung Tumors and Validation of Cetuximab Aerosolization in Animals

The high specificity of mAbs for their target antigens means that there is no ideal animal model for testing the concept of airways delivery of aerosolized cetuximab for treating lung tumors (16). The non-pulmonary cells, A431, which bear high concentrations of EGFR and are very sensitive to cetuximab *in vitro* and after subcutaneous inoculation, have been commonly used for the preclinical evaluation of cetuximab (12,17,18). Therefore, we set up a xenogeneic proof-of-concept model by introducing A431 into the lungs of immunodeficient mice using X-ray and scintigraphic imaging to locate the catheter (Fig. 1a). EDTA was added to the cell suspensions to disrupt the pulmonary epithelium and surfactant layer, thereby improving cell implantation (19) (see Supplementary Figure S1). The tumor nodules produced 6 to 8 weeks later were usually single, relatively small carcinomas (1–10 mm) located in the lung alveolae (Fig. 1b). Some nodules were detectable as early as 3 weeks after inoculation.

The method of aerosol administration is a key factor in the design of animal studies for delivering drugs to the

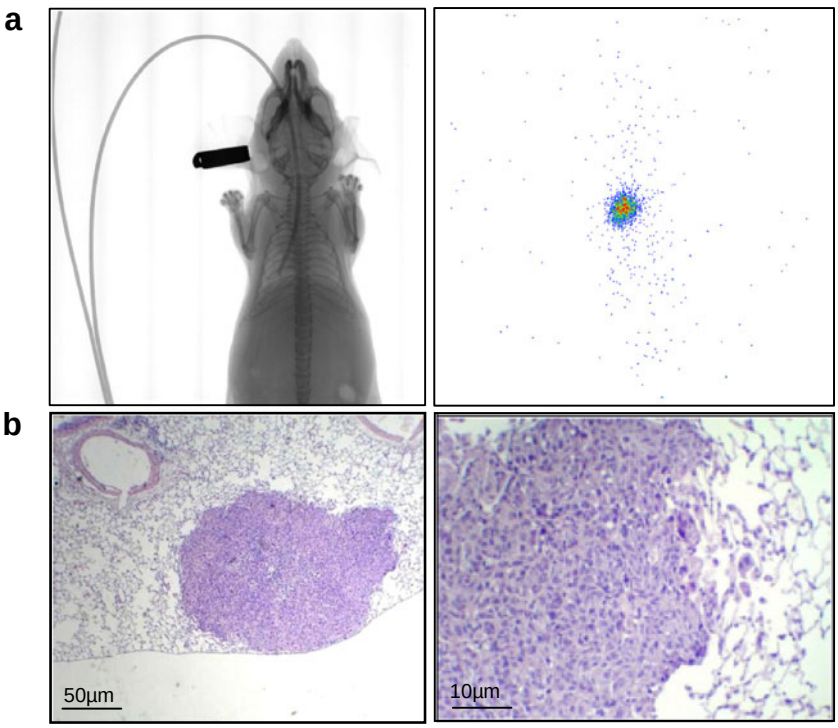


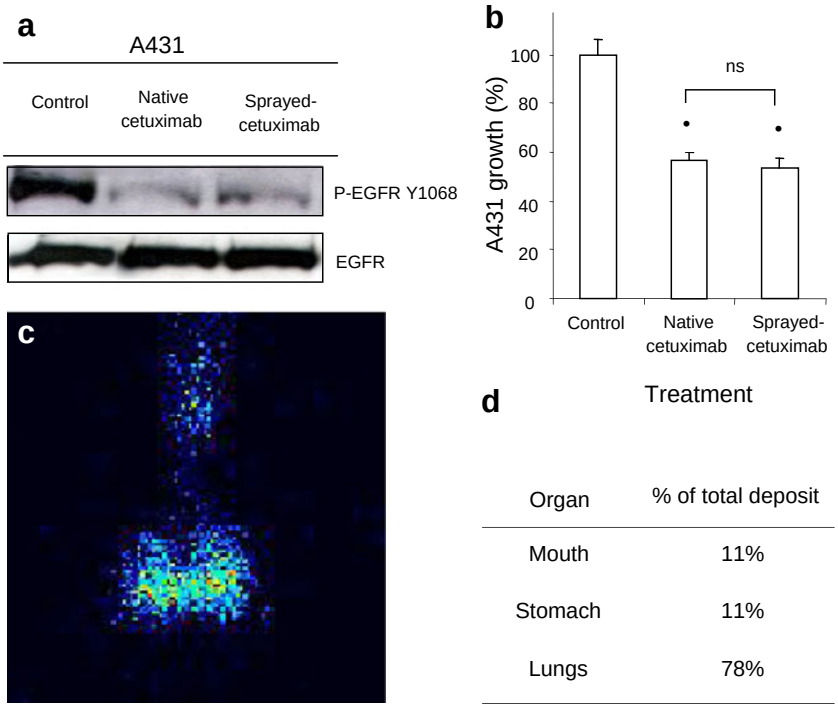
Fig. 1 An animal model of ectopic lung tumor sensitive to cetuximab. A431 cells were gently infused into the main bronchus via a catheter. The position of the catheter was monitored by X-ray imaging (**a**. *left*). Technetium^{99}m was added to the solution of cells to check the quality of the cell deposits in the lungs (**a**. *right*). Tumor nodules in lungs (**b**).

lungs, as it has a major influence on the accuracy of the results obtained (20–22). We ensured reproducible dosing and controlled the lung-regional distribution of the drug in nose-breathing animals by delivering the cetuximab as a spray directly into the respiratory tract of the mice via tracheal instillation using a Microsprayer™. We first checked its integrity after aerosolization. Sprayed cetuximab strongly reduced EGFR phosphorylation following induction with EGF, in the same way as native cetuximab (Fig. 2a). Native and sprayed cetuximab both inhibited the growth of A431 cells by 45% (Fig. 2b). These results indicate that spraying did not affect the capacity of cetuximab to interfere *in vitro* with the EGFR signalling pathway or to inhibit tumor cell growth. The affinity of cetuximab was not altered after spraying (IC_{50} of sprayed cetuximab = $2{,}95.10^{-3}$ µg/mL and IC_{50} of native cetuximab = $2{,}87.10^{-3}$ µg/mL) as shown by a competitive assay (Supplementary Figure S2) (12). The sprayed cetuximab was also uniformly distributed throughout the lungs (Fig. 2c), while only a limited amount of the drug was coughed up or swallowed (Fig. 2d).

Subsequently, we checked that neither the spray itself nor the spraying of an immunoreactive chimeric IgG1 (that binds efficiently to murine Fc receptors and FcRn) produced a toxic response (Supplementary Figure S3). However, further studies will be needed to determine the toxicity of cetuximab administered through airways as cetuximab does not bind to murine EGFR.

Distribution of Airways-Administered Cetuximab

The distribution of a mAb delivered by systemic infusion may lead to limited response and relapse, with tumors taking up only a fraction of the injected dose and the mAb distribution in the tumor nodule being non-uniform. Delivering a drug via the airways might enhance its concentration at the target lung cancer site, thereby improving the antitumor effect. We compared the distributions and lung uptakes of cetuximab given i.v. and via the airways. The antibody was first conjugated to Xenofluor750™, which did not alter its affinity for EGFR (Supplementary Figure S4). The kinetics of Xenofluor™-



d

Organ	% of total deposit
Mouth	11%
Stomach	11%
Lungs	78%

Fig. 2 Validation *in vitro* and *in vivo* of the aerosolization of cetuximab with the Microsprayer™. (**a**) A431 cells (4.10^5) were seeded in 6-well plates and treated with native or sprayed cetuximab (50 µg/mL) for 24 h. The cells were then incubated with 10 ng/mL recombinant EGF for 10 min and lysed. Immunoblots of protein lysates were analyzed for phosphorylated EGFR and total EGFR. (**b**) Cells (5.000 cells per well in a 96-well plate) were treated for 48 h with native or sprayed cetuximab (50 µg/mL) and then counted using a Malassez haematometer. Results are expressed as medians of the percent of relative growth and are representative of three independent experiments. Circles $p < 0.025$ *vs.* control. Sprayed cetuximab: cetuximab aerosolized with the Microsprayer™. (**c**) Dynamic 60 s scintigraphic imaging acquired immediately after orotracheal delivery of cetuximab plus a Tc99m-colloid with a Microsprayer™ in a mouse (anteroposterior view). The images show deposition of the aerosol in the lungs area after the Microsprayer™ was correctly introduced into the trachea. (**d**) Deposits of sprayed cetuximab were analyzed by scintigraphy in 30 mice and quantitated. Deposits were expressed as percent of the nebulized dose for each mouse and results are expressed as medians of the % deposit in each region for all the 30 mice.

cetuximab distribution after i.v. injection or pulmonary spraying in tumor-bearing mice was then studied by near infrared fluorescence (NIRF) imaging. Control free Xenofluor750™ administered either through i.v or the airways is shown in Supplementary Figure S5. Antibody injected i.v. resulted in fluorescence signals in several tissues (Fig. 3a). Xenofluor750™-cetuximab rapidly accumulated in the liver, where it persisted for 72 h, suggesting that its clearance was hepatic (23–25). No Xenofluor750™-cetuximab was ever detected in the lungs. However, NIRF imaging of autopsied lungs revealed them to be weak; focused fluorescent and histological studies confirmed its location on tumor nodules (Fig. 3b). Thus, the tumors in the lungs specifically took up systemically injected cetuximab. In contrast, the lungs of mice treated with aerosolized Xenofluor750™-cetuximab showed intense, persistent fluorescence (Fig. 3a). Reflectance fluorescence was the only method available, but it is not suitable for quantitative measurements in deep tissues. The signal collected is influenced by the curvature of the thorax, which meant that only qualitative patterns of distribution were obtained. Fluorescence was also detected in the liver, which is not a target for the free dissociated fluorochrome (Supplementary Figure S5) as previously reported (26), indicating that the lungs can release the antibody into the circulation. Examination of excised lungs showed that the labelled drug was taken up by the A431-tumor nodules (Fig. 3b). This suggests that the airways barrier is

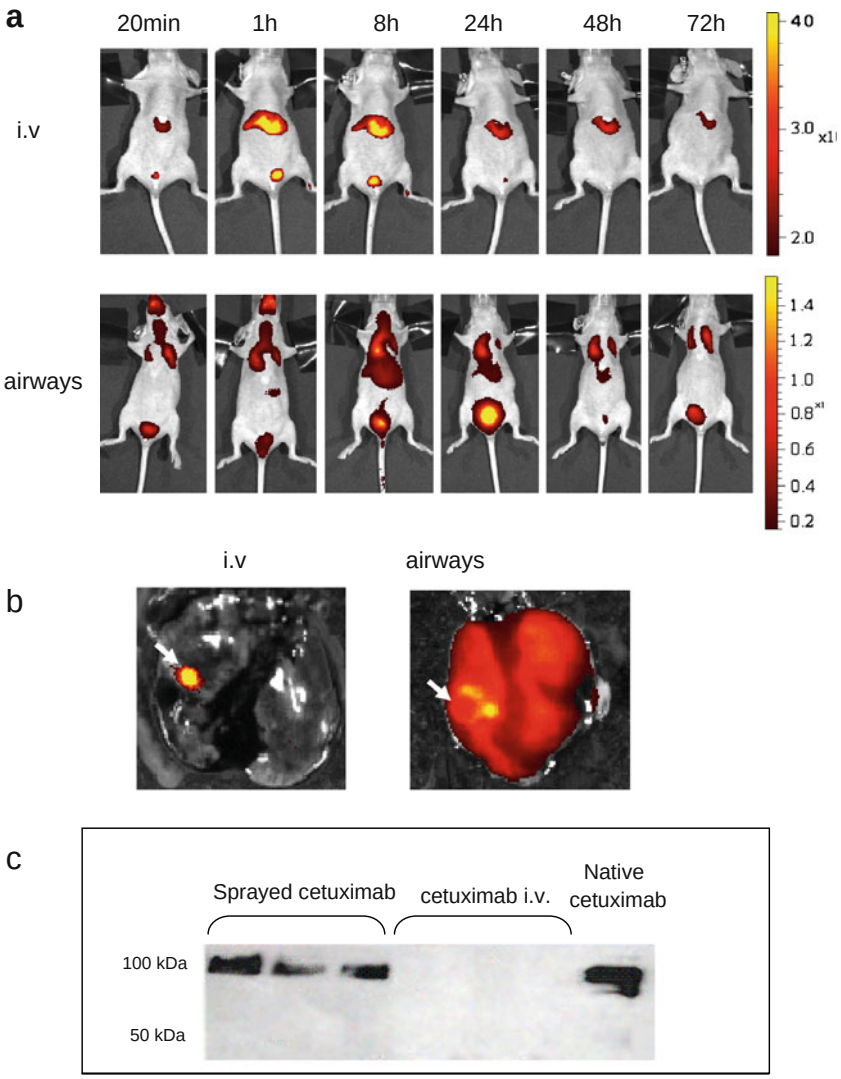
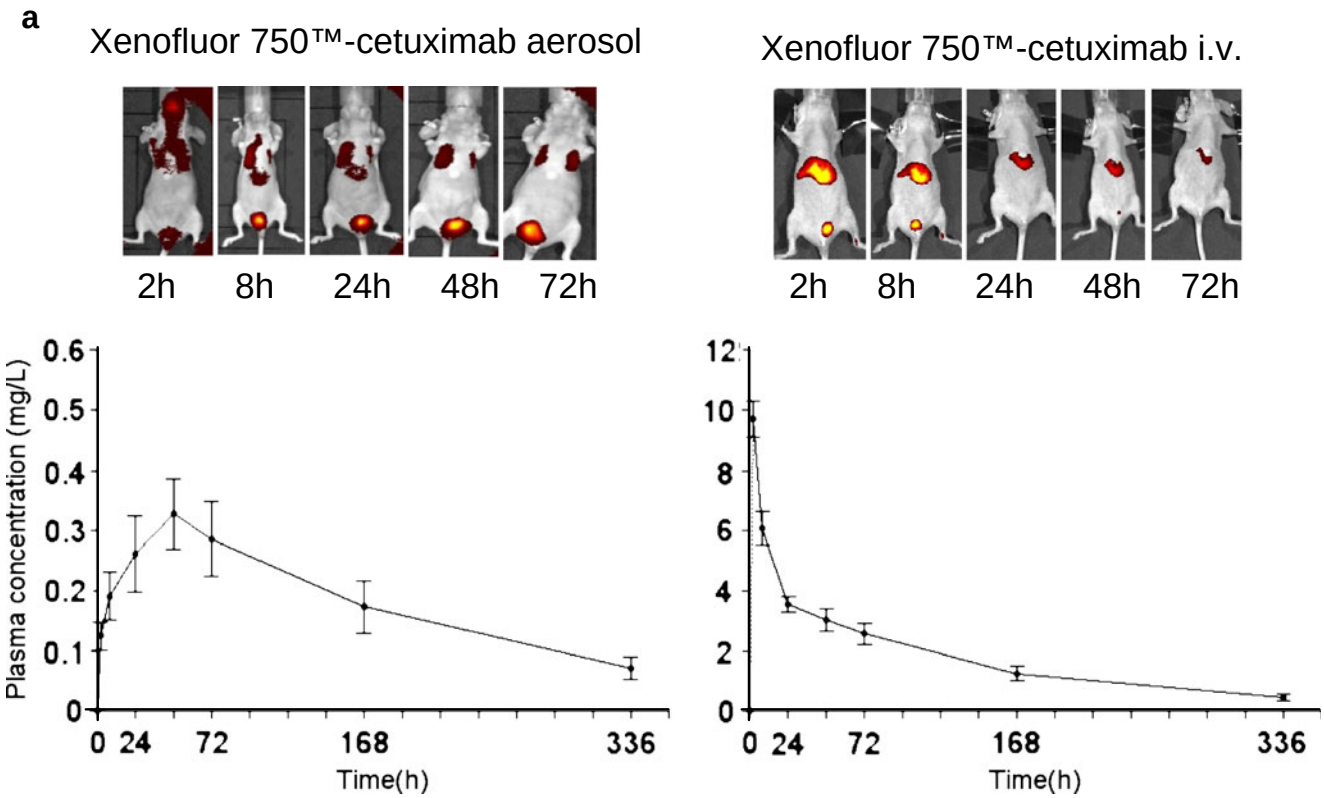


Fig. 3 Distribution of Xenofluor750™-cetuximab administered via different routes in mice bearing A431 lung tumors. (**a**) NIRF images at indicated times after i.v. injection (*upper panel*) or spray delivery (*lower panel*) of Xenofluor 750™-cetuximab to a A431 tumor-bearing mouse. (**b**) NIRF images of autopsied lungs after Xenofluor750TM-cetuximab given by i.v injection (*left*) or sprayed into the airways (*right*). The white arrows indicate the positions of lung tumors determined histologically. (**c**) Immunodetection of cetuximab-Xenofluor750™ in protein lysates from autopsied lungs with an anti-human IgG. The images show the results for 3 mice of each group (out of 11 for sprayed cetuximab group and 12 for cetuximab i.v. group) showing similar profiles.

permeable to mAbs, despite the fact that they are large proteins.

We also found marked fluorescence signals in the bladder soon after mAb administration by i.v. injection and by spraying. This was probably due to the traces of free Xenofluor750™ in the preparation, which passively cross the glomerular barrier of the kidneys to be excreted in the urine (Supplementary Figure S5). While the overall fluorescence gradually decreased over the 24 h after i.v injection, it persisted for 72 h after airway delivery. The molecular profile of the Xenofluor750™-cetuximab in the lungs was analyzed by western blotting to determine whether the persistent bladder signal was due to metabolic clearance of the antibody from the lungs. Two high molecular weight bands, similar to those of native antibody, were detected in the protein lysates from whole lung tissues of mice given sprayed Xenofluor750™-cetuximab (Fig. 3c). Moreover, ascending instant thin-layer chromatography of urine samples collected at 48 or 72 h revealed significant amount of free Xenofluor750™, whereas no peptides conjugated to the fluorophore were detected (not shown). This indicates that the persistent bladder signal is due to the release of free Xenofluor750™ from cetuximab in the airways. Altogether, our results suggest that the aerosolized cetuximab accumulated in the airways was mainly intact. No such bands were detected in the lung extracts of systemically injected mice (Fig. 3c), corroborating the signal intensity observed in NIRF

a



b

	aerosol	i.v.
AUC (µg.h/mL)	73 (+/- 31)	654 (+/- 194)
Mean F (%)	11,2	
C_{max} (µg/mL)	0.33 (+/- 0.12)	
T_{max} (h)	48	
MRT* (h)	148 (+/- 17)	114 (+/- 19)
$T_{½}$ abs.* (h)	19.8 (+/- 7.9)	
Mean MAT* (h)	34	

Fig. 4 Plasma concentrations (**a**) and pharmacokinetic parameters (**b**) of Xenofluor750™-cetuximab administered via the airways or i.v. to a mouse bearing an A431 lung tumor. Parameters estimated by compartmental or non-compartmental (*) analyses. Data are expressed as means. Standard deviations are indicated in parenthesis. (MRT) mean residence time, (F) bioavailability, (t½ abs) absorption half-life. The images show 1 mouse out of 6 and 7 mice, for sprayed cetuximab group and cetuximab i.v. group respectively, showing similar profiles.

images. Thus, very little of the i.v. injected mAb reached the target organ, as previously shown for other anticancer agents (27). The delivery of anticancer mAbs via the airways therefore results in a higher concentration in the lungs than does systemic injection.

Pharmacokinetic of Cetuximab Delivered Via the Airways

The pharmacokinetic of an aerosolized mAb delivered directly into the lung will be governed by various factors, such as the site of deposition, drug formulation, local drug metabolism and mechanisms of absorption into the systemic circulation. Unlike small molecules that usually rapidly cross the plasma membrane by passive diffusion, specific transporters or via tight junctions, the way in which peptides and proteins penetrate into the lungs is not very clear (21,28). Recent evidence indicates that the IgG transcytosis receptor FcRn, which is borne by epithelial cells in the human upper respiratory tract, is implicated in the passage of Fc-conjugated proteins across the lung epithelium into the bloodstream (27,29–31).

We studied the lung deposition and local clearance of aerosolized cetuximab so as to compare its pharmacokinetics with those of systemically administered cetuximab. Xenofluor™-cetuximab was injected i.v. or sprayed into the lungs of mice bearing A431 lung tumors. The cetuximab concentration in plasma samples was measured for the next 15 days (14) (Fig. 4a). Drug distribution was also evaluated by NIRF imaging (Fig. 4a). We detected Xenofluor™-cetuximab in the circulation after airways delivery, in agreement with NIRF images, indicating that the lungs absorbed the antibody. However, little cetuximab left the lungs because its plasma concentrations were nine times lower after aerosol delivery than after i.v injection. The area under the concentration *versus* time curves (AUC) estimated by the compartmental model was 73 µg.h/mL after aerosol delivery and 654 µg.h/mL after i.v. injection (Fig. 4b). Xenofluor™-cetuximab also entered the bloodstream slowly after airways administration, with a mean absorption time (MAT) of 34 h and a peak plasma concentration (Tmax) at around 48 h (Fig. 4b). These results indicate that anticancer mAbs delivered via the airways remain at the target site for some time.

Pharmacokinetic analysis also showed that the bioavailability of airways-delivered cetuximab was low, around 11%. It remains to be determined now whether transcytosis of aerosolized cetuximab from the deep lung

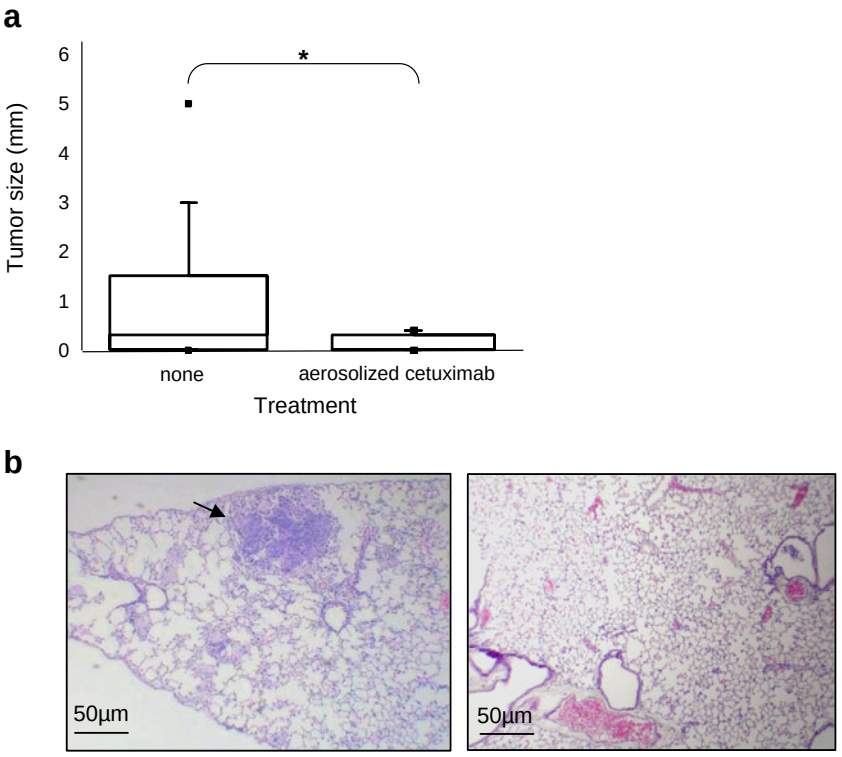


Fig. 5 Antitumor response to cetuximab delivered via the airways as an aerosol to a mouse bearing an A431-lung tumor. Mice were treated with sprayed cetuximab or untreated. They were killed, and serial lung sections were examined to determine the presence and extent of lung tumors. (**a**) Box plot representing the median, quartiles, deciles (*bars*), minimum and maximum (*squares*) of lung tumor size. Asterisk means $p < 0.05$. The tumor size corresponds to the largest diameter of the nodule or the sum of the largest diameters of each nodule for mouse having multiple tumors. The results are presented as medians and correspond to tumor measures of 14 untreated mice and 13 sprayed cetuximab mice. (**b**) Photographs of serial lung sections from animals treated with sprayed cetuximab (*left*) or untreated (*right*). The *arrow* indicates the position of the tumor nodule.

into the systemic circulation is as active in humans as it is in mice. The distribution of FcRn in the respiratory tract differs from one species to another, with possibly fewer receptors in the alveolar region of human and NHP lungs than in those of small rodents (29). Further experiments in NHP should clarify this point. Because cetuximab does not cross-react with murine EGFR, such a primate model would also take into account the antigen uptake and how it may influence mAb pharmacokinetics and biodistribution after airway delivery. Until then, our results should be extrapolated to humans with great care. Nevertheless, we have shown that airways delivery can reduce the possible side effects associated with systemic infusion of anticancer mAbs, while demonstrating a clear therapeutic benefit.

Antitumor Activity of Cetuximab Delivered Via the Airways

Molecular imaging data showed that aerosolized cetuximab delivered via the airways was taken up by the tumor. We evaluated the antitumor efficacy of sprayed cetuximab in the xenogeneic murine model of lung tumor to determine whether cetuximab is pharmacologically active after airways delivery.

Previous experiments have shown that the greatest regression of subcutaneous A431 xenografts was obtained following early treatment with twice weekly intraperitoneal injections of 1 mg cetuximab (17,32,33). We randomly assigned our mice to one of two groups 3 weeks after implanting the A431 cells. The control group (group 1) was untreated, and the treated mice (group 2) were given aerosolized cetuximab with the Microsprayer™ for 3 weeks. The A431 lung tumors in the animals treated with sprayed cetuximab were significantly smaller than those in the control group (Fig. 5a and b), indicating that cetuximab delivered via the airways inhibited tumor growth. Thus, anticancer mAb delivered locally via the airways produces an antitumor response.

CONCLUSION

This proof-of-concept study demonstrates that the airways can be suitable for delivering aerosolized mAbs to treat lung cancer. We have used aerosolized cetuximab in a xenogeneic murine model to show that the airway barriers are permeable to mAbs, the pulmonary route enhances mAb concentration at the target while it limits its passage into the bloodstream, and aerosolized-mAb produces an antitumor response. As the lungs are the most common sites of primary and metastatic tumors and as primary lung cancer is the leading cause of cancer-related deaths in both men and women worldwide, our findings open up new promising therapeutic perspectives. Further studies are required to validate this concept and determine whether the pulmonary route really does increase the therapeutic benefit of mAbs delivered to patients with lung cancer.

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