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1 **Exploring the fate of inhaled monoclonal antibody in the lung parenchyma by**
2 **microdialysis**

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42

43

44

45 **Abstract**

46 Therapeutic antibodies (Abs) are emerging as major drugs to treat respiratory diseases, and
47 inhalation may provide substantial benefits for their delivery. Understanding the behavior of
48 Abs after pulmonary deposition is critical for their development. We investigated the
49 pharmacokinetics of a nebulized Ab by continuous sampling in lung parenchyma using
50 microdialysis in non-human primates. We defined the optimal conditions for microdialysis of
51 Ab and demonstrated that lung microdialysis of Ab is feasible over a period of several days.
52 The concentration-profile indicated a two-phase non-linear elimination and/or distribution of
53 inhaled mAbX. Lung exposition was higher than the systemic one over a period of 33 hours
54 and above MabX affinity for its target. The microdialysis results were supported by an
55 excellent relationship with dosages from lung extracts.

56

57 **Introduction**

58 Since the market release of the first therapeutic antibody (Ab) in 1986, Ab-based
59 therapeutics have experienced a remarkable expansion and demonstrated therapeutic success
60 in various diseases. The overall clinical pipeline was estimated to include 470 Abs in 2016,¹
61 mostly full-length IgGs (Mw \approx 150 kDa). Respiratory diseases continue to represent major
62 unmet medical needs and are a growing area of use for therapeutic Abs, with >40 molecules
63 (excluding those used in lung cancer) studied in clinical trials. While Abs for cancer treatment
64 are mainly delivered through the intravenous (i.v.) route, other routes may be beneficial for
65 respiratory diseases because Abs do not passively diffuse through the different compartments
66 of the body. Among these other routes, inhalation is a promising non-invasive method of
67 delivery for Abs to treat respiratory diseases, as it has allowed for therapeutic responses in

68 various models and leads to high Ab concentration in the lungs, while limiting Ab passage
69 into the systemic circulation.²⁻⁵

70 Prior to clinical trials, preclinical studies are mandatory to explore the
71 pharmacodynamics, pharmacokinetics (PK) and toxicity profiles of Abs. More precisely, PK
72 studies characterize the distribution and local concentrations of compounds and thereby guide
73 toxicity studies; both study types generate critical data used to select the accurate doses and
74 regimens for clinical trials. Classically, PK parameters are estimated by monitoring drug
75 concentrations in the systemic circulation, from which the behavior of the drug at the target
76 site is predicted after computation by mathematical compartmental models. However, this
77 indirect estimation of tissue concentrations requires knowledge of intrinsic and extrinsic
78 factors influencing Ab behavior, which should be previously determined experimentally.⁶⁻¹⁰
79 Additionally, scaling across animal species is questionable for Abs because differences in
80 antigen-Ab and FcRn-Ab binding exist, and may drive significant differences in Ab
81 distribution and clearance. Simplified allometric scaling across species, in particular from
82 monkey data, is only predictive (in humans) when the Ab kinetic profile is linear. In other
83 cases, interpretations and translation of the PK results remain speculative. In the case of
84 inhalation as a delivery route, the limited understanding of the processes and molecular
85 drivers involved in absorption, as well as considerable species differences, makes interpreting
86 PK results even more challenging.

87 To follow the behavior of inhaled Abs in relevant tissues, *i.e.*, the lungs, few methods
88 are available, and each has specific strength and weakness. Iterative tissue sampling uses
89 many animals and is ethically questionable. Broncho-alveolar lavage (BAL) cannot be
90 repeated on the same animal and reflects only the airway compartment. Real time-imaging
91 techniques rely on conjugation of Abs to isotopes/dyes that may modify Ab binding to their
92 target antigen or Fc receptors. Alternatively, *in vivo* microdialysis appears to be an interesting

93 semi-invasive PK sampling technique to measure unmodified drugs in the interstitial spaces
94 of various tissues, including the lungs.^{11,12} Microdialysis enables continuous repetitive
95 sampling of drugs in a single animal using a semi-permeable membrane inserted in the tissue
96 and perfused with physiological buffer at constant flow rate. Although mainly described for
97 small molecules, microdialysis of proteins and Abs has recently become possible due to the
98 availability of large molecular weight cutoff membranes and the push-pull perfusion
99 technique to avoid fluid loss.^{13,14} Herein, we attempted to use microdialysis for the dynamic
100 quantification of an Ab administered by inhalation in non-human primates (NHP); the Ab
101 targets a soluble antigen in the interstitial lung space (optimized mAb provided by Sanofi
102 Genzyme, target undisclosed for confidential reasons)..

103

104 **Results**

105 We first optimized the microdialysis flow to achieve a reliable and optimal diffusion
106 rate of the antibody of interest, mAbX, by using the experimental setup illustrated in
107 supplemental Figure S1. As previously reported, we observed an inverse relationship between
108 the perfusion flow rate and the mAbX diffusion rate.^{13,14} The recovery rate, corresponding to
109 the percentage of mAbX diffusing into the perfusion fluid (Figure 1A), ranged from 15.5% (1
110 $\mu\text{L}/\text{min}$) to 40.25% (0.2 $\mu\text{L}/\text{min}$). A flow rate of 0.3 $\mu\text{L}/\text{min}$ was selected based on an
111 acceptable duration of time to collect 50 μL of microdialysate and achieve a median recovery
112 rate value of 28.9% [4.5%], (3 probes, 30 h of observation). It is noteworthy that the recovery
113 rate variability was very low over a two-day period (data not shown), which encompassed the
114 duration of the PK study. In a second step, we qualified the reversibility of the probe *in vitro*
115 using an irrelevant murine IgG1 and mAbX by retrodialysis. As shown in Figure 1B, the
116 murine IgG1 (35.67% [15.33%]) displayed similar reverse diffusion to mAbX (33.40%
117 [3.95%]), and may be considered as standard tracer for *in vivo* retrodialysis. The recovery rate

118 of the murine IgG1 was investigated *in vivo* by retrodialysis and was equal to 27.1% [16.9%]
119 (median value of 34 measurements performed in two animals). Overall, the recovery rate
120 obtained *in vitro* with mAbX was considered accurate for correcting calculation of mAbX
121 concentration *in vivo*.

122 On the basis of the *in vitro* results, we designed the experimental setup (Figure 2A) to
123 carry out microdialysis sampling of mAbX *in vivo*. Non-human primates (macaques) were
124 chosen because they are highly similar to humans in aspects relevant to aerosol therapy (lung
125 anatomy, delivery method) and are recommended for preclinical studies of Ab development.³
126 Immediately after delivery of mAbX by inhalation [3 mL at 10 mg/mL for 15-20 min to
127 obtain on average 2.5% of the dose, *i.e.*, 0.75 mg deposited in the lungs (Figure 2B)] in
128 spontaneously breathing animals, animals were anesthetized, and the microdialysis probe was
129 implanted in the lung parenchyma by thoracic surgery. Animals were thereafter maintained
130 under prolonged anesthesia and mechanical ventilation for up to 2 days (Figure 2A). The
131 follow-up measurements of clinical parameters showed that all physiological parameters of
132 the animals were maintained within normal levels throughout the experiments (Table 1).
133 Next, the circulation of perfusion fluid was started, and microdialysate and blood were
134 sampled on a preset schedule to quantify mAbX and endogenous/control markers. The
135 stability of the blood/microdialysate urea ratio throughout the study (median [IQR]=1.1 [0.3],
136 n=9 for each animal) underlined the correct permeability of the microdialysis probe (Figures
137 2C). Figure 3A showed the concentration-time profiles of “unbound” mAbX in the
138 microdialysate (corrected by the *in vitro* recovery rate) and in the blood, which represent the
139 lung interstitial fluid concentration and transfer of inhaled mAbX from the airways into the
140 bloodstream, respectively. Overall, we observed: 1) a two-phase disappearance of mAbX in
141 the microdialysate, with a linear/continuous decrease for 33 hours followed by a pseudo-
142 plateau thereafter ; and 2) low and slow passage of mAbX into the bloodstream (Figure 3A)

143 as previously described.^{3,5} It is noteworthy that the disappearance profiles showed similar
144 trends in each animal. Although heterogeneous between animals, the lung was more exposed
145 than the systemic circulation to mAbX (Figure 3B) during the experiment, and this was more
146 remarkable during the first 33 hours (ratio $AUC_{\text{microdialysate}}$ over $AUC_{\text{serum}} = 22.37, 1.58$ and
147 57.55 for each animal). As shown in Figure 3A, the residual amount of “unbound” mAbX
148 corresponded to 2.4% [1.3%] of the initial amount measured in the microdialysate. This value
149 is consistent with the residual amounts of mAbX detected in the lungs after animal sacrifices
150 (median [IQR]=4.9% [2.6%]) and normalized to the deposited mass estimated from the
151 scintigraphy imaging (Figure 2B). Accordingly, mAbX was immunodetected in lung sections
152 from animals after sacrifice up to 54 hours after the inhalation, and was mainly observed
153 within airway epithelial cells and alveolar macrophages (Figure 4).

154

155 **Discussion**

156 Therapeutic Abs are emerging therapies for pulmonary diseases, including cancer and rare
157 diseases like fibrosis. These pathologies would be better addressed with focused distribution
158 of Ab, especially in diseases associated with marked rarefaction of blood vessels,^{15,16} which
159 impedes the diffusion of systemic drugs into the lungs. Indeed, Abs are large macromolecules
160 that exhibit limited distribution to the lungs after i.v. injection.^{3,17,18} Because inhaled Ab
161 passes slowly and poorly from the airways to the systemic circulation after pulmonary
162 delivery,^{2,3} the lung interstitial concentration of inhaled mAbX is expected to be higher than
163 that in the blood compartment. Consequently, the concentration-profile in the blood
164 compartment may not reflect the true kinetics of inhaled mAbX in the lungs. Determining the
165 concentration of the Ab in the lung interstitium is crucial for obtaining a true representation of
166 the inhaled mAbX exposure in the target organ and for helping in the development of more
167 predictive PK models. In this study, we reported for the first time the dynamic quantification

168 of an inhaled Ab (mAbX) that targets a soluble antigen in the lung interstitium by continuous
169 lung microdialysis in a large animal model.

170 *In vivo* microdialysis is a semi-invasive method and a powerful technique to
171 continuously measure soluble molecules from the interstitial fluid of one animal. Although it
172 is a widely used and well-established technique for small molecules, only a few successful
173 examples of the technique has involved Ab microdialysis.^{13,14} Indeed, a major limitation of
174 Ab microdialysis is the risk of low Ab recovery, mainly due to the high mass transport
175 resistance and small aqueous diffusion coefficients typical of Abs. Herein, we used a large
176 pore probe to favor Ab diffusion. After optimization, the selected parameters allowed a stable
177 recovery rate of 28.9% during 30 hours of *in vitro* monitoring, which was remarkable for a
178 high molecular weight protein.^{13,14} Another issue is the calibration of the microdialysis probe
179 membrane, which should maintain its efficiency throughout the experiment to ensure the
180 consistent appropriate measurement of the mAbX diffusion rate. As previously reported,¹¹⁻
181 ^{14,19} this can be achieved through different methods. Herein, we showed that measuring the
182 disappearance of a murine IgG1, which was used as an internal standard with similar physico-
183 chemical properties as mAbX, *in vivo* was in the same range of the recovery rate obtained *in*
184 *vitro*, thus validating the use of *in vitro* results to calculate the accurate concentration of the
185 molecule of interest in animals.¹⁹ Besides, the measurement of an endogenous molecule, such
186 as urea in different compartments (circulation and lung) may act as a surrogate for
187 qualification of probe permeability during the *in vivo* experiment.

188 The dynamic quantification of inhaled mAbX in the lung interstitium by microdialysis
189 was conducted in NHP. Inhalation therapy is probably one of the most challenging delivery
190 routes in animal models because of the disparities between human and animal model
191 anatomy, respiratory patterns and physiology.²⁰ Macaques are appropriate models for Ab
192 inhalation because: 1) they allow accurate prediction of aerosol deposition in the lung and are

193 comparable to human infants, since they are in the weight range of a full-term newborn
194 human and display similar ventilation parameters;²¹⁻²³ 2) they can breathe spontaneously
195 through a face mask connected to an aerosol device, although they are mainly nose breathers
196 like infants; and 3) they are the species recommended by regulatory authorities for preclinical
197 development of Abs. Herein, the average lung deposition of mAbX, as measured by
198 scintigraphy, was low, since the device is not intended for infants. The dose predicted to
199 deposit in the lungs by cascade impaction (inhalable fraction (expressed as the % of loaded
200 mAbX): 47.1% \pm 3.4%; respirable fraction of mAbX > 0.5 μ m and < 3 μ m: 71.8% \pm 1.8%) is
201 higher than the deposited dose in macaques and is compatible with aerosol therapy in humans.

202 The PK of inhaled mAbX was not linear. The concentration-profile indicated a two-
203 phase absorption/elimination. This result may be explained by an alpha-phase distribution of
204 mAbX from the lung to the systemic compartment, followed by pseudo-equilibrium of mAbX
205 between the different compartments. The human antibody mAbX binds to a soluble protein: it
206 cross-reacts with both the NHP antigen and endogenous neonatal Fc receptor (FcRn)
207 recycling system. Thus, the first distribution and/or clearance phase may also be attributed to
208 target mediated drug disposition (TMDD)²⁴ or active IgG intracellular catabolism due to
209 saturation of FcRn. Of note, FcRn is restricted to alveolar macrophages and epithelial cells of
210 the upper airways in NHP airways.²⁵ Additional experiments (e.g., with different doses)
211 would be required to decipher further the mechanisms underlying non-linear PK profile of
212 inhaled mAbX. After 33 hours, mAbX elimination was slower or null, and “soluble” mAbX
213 was maintained at low but detectable concentrations in the lung interstitial fluid. We
214 hypothesize that the low but sustained presence of mAbX in the interstitial fluid may be
215 attributed to the continuous release of intracellular mAbX through FcRn recycling. This
216 hypothesis is supported by the immunohistochemistry profile showing some staining within
217 cells in the lungs at the end of the experiment. Of major importance from a therapeutic point

218 of view, lung concentrations achieved during the first 24 h hours after inhalation were above
219 the affinity constant of mAbX for its target. As anticipated,^{3,5} the amount of mAbX reaching
220 the blood circulation was low and characterized by a slow passage. Based on the literature
221 showing poor diffusion from the circulation to the lung compartment,^{17,18} it would have been
222 necessary to administer a huge amount of mAbX via the i.v. route to achieve the equivalent
223 lung exposition obtained by inhalation, thereby possibly raising toxicity concerns.

224 Overall, compared to classical PK studies, long-term lung microdialysis of Ab appears
225 suitable to better understand the interstitial tissue distribution and behavior *in situ* of inhaled
226 Abs targeting soluble antigens. Importantly, from an ethical point of view, this individual
227 dynamic sampling and monitoring can reduce animal use by using each animal as its own
228 control and follow-up. Nevertheless, even though microdialysis is considered a minimally
229 invasive technique, complicated logistics are required, and a notably “human-like” surgical
230 procedure is necessary to access the lungs and implant the probes without promoting tissue
231 trauma and inflammation. In fact, the continuous lung microdialysis and the long half-life of
232 Ab in the biological fluids necessitate expert anesthesiologist skills and dedicated animal
233 facilities to maintain animals under adequate analgesia and sedation for prolonged mechanical
234 ventilation. Altogether, these factors restrict the use of this promising technology to
235 specialized teams with dedicated animal facilities.

236 To conclude, we achieved the dynamic quantification of an Ab in the interstitial lung
237 space by microdialysis. By overcoming the technical, including zootechnical, challenges of
238 microdialysis, we established, for the first time, the conditions for lung microdialysis in the
239 lung parenchyma of NHP, thereby simultaneously evaluating the kinetics of an inhaled Ab in
240 the lung compartment and in the bloodstream. Overall, we were able to quantify the local
241 concentrations of a nebulized mAb and observed a complex non-linear
242 elimination/distribution. Our dynamic results were highly supported by the excellent

243 correspondence between the residual amounts of mAbX measured in the dialysate and the
244 lungs after animal sacrifices more than 48 h after inhalation delivery. Altogether, our data
245 open avenues for tests aiming to strengthen the local delivery, the residual/recycling
246 concentration and the efficacy of therapeutic Abs.

247

248 **Materials and Methods**

249 *In vitro* microdialysis

250 The mAbX was an optimized mAb provided by Sanofi Genzyme (target undisclosed for
251 confidential reasons). A 1 MDa cut-off semipermeable membrane (CMA 20) was incubated
252 with the antibody solution (mAbX 3.3 mg/mL) and was perfused continuously with ringer
253 acetate buffer at different flow rates using a push-pull system (KDS Legato 272 push/pull
254 pump) while maintained at 37°C. The recovery rate corresponds to the percent of mAbX
255 diffusing in the perfusion fluid and was calculated as follows: $(C_{\text{microdialysate}}/C_{\text{reservoir}}) \times 100$,
256 where C corresponds to the concentration of mAbX in the dialysate ($C_{\text{microdialysate}}$) or reservoir
257 ($C_{\text{reservoir}}$) (Supplemental Figure S1). Results are representative of the recovery rate obtained
258 with 3 probes and are expressed as median [IQR]. The recovery rate of an irrelevant murine
259 IgG1 isotype was assessed for probe calibration in the *in vivo* experiments and measured by
260 retrodialysis. In this case, the microdialysis probe was perfused with ringer acetate buffer
261 containing the murine IgG1 (1 µg/mL, $C_{\text{perfusate}}$). To ensure that the murine IgG1 mimicked
262 mAbX, mAbX was added in the perfusion fluid and the probe was incubated in the buffer
263 only in some experiments. The residual murine IgG1 or mAbX (C_{residual}) concentrations were
264 measured in the dialysate, and the recovery rates corresponded to the percentage of murine
265 IgG1 or mAbX lost from the perfusion fluid (retrodialysis). The retrodialysis rate was

266 calculated as follows: $((C_{\text{perfusate}} - C_{\text{residual}})/C_{\text{perfusate}}) \times 100$, where C corresponds to the
267 concentration of the murine IgG1 or mAbX.

268

269 Animals and housing

270 Three healthy female cynomolgus macaques, weighing 3.6 to 4.5 kg and obtained from
271 Bioprim®, were housed in accordance with the most recent European legislation (Directive
272 2010/63/UE). Animals were acclimatized to laboratory conditions and were trained to receive
273 an aerosol before the first set of experiments. The experimental protocol was conducted
274 according to NIH guidelines for the care and use of laboratory animals and was approved by
275 the ethics committee of Val de Loire (n°04372.01).

276

277 Aerosol administration

278 Formulation and nebulization conditions as well post nebulization integrity of mAbX was
279 previously characterized (data not shown). Macaques were placed on a restraint chair after
280 being sedated via intramuscular (i.m.) injection of ketamine (10 mg/kg). Inhalation of mAbX
281 (3 mL of 10 mg/mL solution) was done in conscious animals with a customized vibrating
282 membrane eFlow® Technology nebulizer connected to the eFlow® PULSE controller at
283 50ms/50ms of ON/OFF associated with a baby face mask (Smartmask Baby), all from PARI
284 Pharma GmbH. The volume mean diameter (VMD) was $3.3 \pm 0.1 \mu\text{m}$ as measured by laser
285 diffraction, and the respirable fraction ($0.5 \mu\text{m} < \text{particles} < 3 \mu\text{m}$) was $34.2\% \pm 6.5\%$ with
286 0.9% NaCl solution. Aerosol administration lasted no longer than 15 min. In specific studies
287 designed to evaluate pulmonary deposition in each animal with the device, a placebo solution
288 traced with DTPA-Tc^{99m} (3 mL) was delivered in the same conditions, and animals were
289 imaged with a gamma camera (Ecam, Siemens) immediately after the aerosol session, as

290 previously described.² Aerosol deposition in the lungs, stomach, ears, nose and throat was
291 calculated taking into account the background, coefficient of tissue attenuation and
292 radioactivity decay.

293

294 Implantation of the microdialysis probe and *in vivo* microdialysis

295 Immediately after aerosol delivery, animals were sedated with ketamine (10 mg/kg i.m.) then
296 anesthetized with isoflurane. Animals were orotracheally intubated (tube internal diameter of
297 3.5 mm) and mechanically ventilated in a supine position with Datex Flexima ventilator.
298 Ventilator settings were: tidal volume 70 ml, respiratory rate: 30/min, air and oxygen flows
299 between 0.5 and 1L/min, oxygen inspiratory fraction: 50%, Pi Max: 25 cmH₂O, FeCO₂: 35-40
300 mmHg. Equipment for anesthesia monitoring was set up (pulse oximetry, end-tidal carbon
301 dioxide, non-invasive blood pressure, and temperature monitoring), as was a urinary catheter
302 with an hourly diuresis unit, an electric warming blanket and pressure ulcer-reducing devices.
303 After inserting two peripheral venous catheters, isoflurane was relayed by continuous
304 injection of midazolam (0.9 to 5 mg.kg⁻¹.h⁻¹), ketamine (9 to 40 mg.kg⁻¹.h⁻¹) and
305 buprenorphine (6 to 25 µg.kg⁻¹.h⁻¹) for up to 54 hours. Animals were supplemented with
306 dextrose in normal saline solution (Glucidion G10%, injected at 2 mL/h).

307 The microdialysis probe was inserted in the lung parenchyma through right mini-thoracotomy
308 under direct vision and was sutured on the lung. An intercostal pleural drain was inserted
309 before closing the surgical wound. Arterial catheterization of the femoral artery was then
310 performed to facilitate blood sampling and for continuous hemodynamic monitoring. The
311 probe was continuously perfused with ringer acetate buffer at 0.3 µL/min. According to *in*
312 *vitro* results, in some experiments a murine IgG1 (1µg/mL, Mouse IgG1 Isotype Control
313 #MAB002, R&D System) was added to the perfusate to evaluate the *in vivo* recovery rate.
314 Blood and microdialysate were collected on a predefined schedule (every 6 and 3 hours,

315 respectively) to analyze murine IgG1 (retrodialysis), mAbX and urea (used as an additional
316 control of probe permeability). The clinical parameters (body temperature, mean arterial
317 pressure, arterial blood oxygen saturation, heart rate and respiratory rate) of the cynomolgus
318 macaques were monitored continuously during this period (n=54 for each animal). At the end
319 of the experiment, animals were sacrificed by exsanguination, lungs were excised, and a part
320 of each lobe was kept frozen at -80°C to measure mAbX in protein lysates.

321

322 Protein extraction and quantitative measurements of biological markers and Abs

323 Briefly, tissues (~30 to 60 mg) were dissociated in RIPA buffer (1% Triton X-100, 1%
324 sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 25 mM Tri-HCl pH 7.6) with a
325 gentleMACS Dissociator (Miltenyi M tubes, program Protein_01), using 1 mL RIPA/20 mg
326 tissue. After 5 minutes of centrifugation at 4,000xg, supernatants were collected, and proteins
327 were quantitated using a BCA Protein Assay Kit (Thermo Fisher Scientific). Urea was
328 quantified by a quantitative colorimetric assay (Bioassay System) according to the
329 manufacturer's instructions. Murine IgG1 and mAbX were measured in the serum and/or the
330 microdialysate by ELISA. The murine IgG1 was measured with the Mouse IgG1 Ready-SET-
331 Go!® (affymetrix eBioscience) kit according to the manufacturer's instructions. For mAbX
332 detection, the home-made ELISA allowed a range of quantification from 100 ng/mL to 410
333 pg/mL, with intra-assay CV% (1.13 [1.10]%) and inter-assay CV% (5.81 [6.10]%).

334

335 Immunohistochemistry of mAbX in the lung tissue

336 For the detection of residual mAbX in the lung tissue (at the end of the experiment), the lungs
337 were sampled and fixed in 10% neutral formalin for 24 hours. Tissue from an animal that did
338 not receive Ab with Fc-human was used as a control. Inhaled mAbX was detected on lung

339 sections (5 μm) with a secondary anti-human IgG (horseradish peroxidase-conjugated anti-
340 human IgG , #2049-05, Southern Biotech) , which do not cross-react with macaque IgGs (as
341 confirmed by dot blot, data shown). The antigen was recovered by boiling the sections in a
342 citrate-based buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0). Endogen peroxidase
343 activity was quenched by incubating the slides for 15 minutes in 3% H_2O_2 in methanol. For
344 blocking, sections were incubated in diluted normal blocking serum (0.3% normal goat serum
345 (#G9023, Sigma-Aldrich)) and 2.5% bovine serum albumin (#A7906, Sigma-Aldrich) in
346 phosphate-buffered saline (PBS) 1X for 1 hour and washed twice. Next, sections were
347 incubated with the diluted secondary peroxidase-conjugated anti-human IgG at 10 $\mu\text{g}/\text{mL}$ in
348 PBS-T (PBS 1X, 0.1% Tween 20) overnight at 4°C. They were then washed twice, and
349 mAbX was then detected by incubation with DAB-peroxidase solution substrate (Dako, Les
350 Ulis, France) until a satisfactory signal was obtained. The sections were counterstained with
351 hematoxylin solution (Gill N°1, Sigma-Aldrich), cleared and mounted.

352

353 Statistical analysis

354 Results are expressed as median [IQR] for the indicated number of experiments performed
355 independently. For the time-concentration profiles, results are expressed as mean +/- SEM.
356 Statistical significance between the different values was analyzed by the Kruskal-Wallis test
357 for multi-group comparisons or by the Friedman test for non-parametric repeated measures.
358 Statistical analyses were performed using GraphPad Prism5 software. A p value of <0.05 was
359 considered to be significant.

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377

378 **Abbreviations:**

379 Abs: antibodies

380 BAL: broncho-alveolar lavage

381 CV: coefficients of variability

382 Da: dalton

383 DTPA: diethylenetriamine penta-acetic acid

384 ELISA: enzyme-linked immunosorbent assay

385 FcRn: neonatal Fc receptor
386 FeCO₂: fraction of expired of CO₂
387 IQR: interquartile range
388 i.v.: intravenous
389 i.m.: intramuscular
390 NHP: non-human primates
391 Pi Max: maximal inspiratory pressure
392 PBS: phosphate-buffered saline
393 PK: pharmacokinetic
394 RIPA buffer: radioimmunoprecipitation assay buffer
395 SDS: sodium dodecyl sulfate
396 SEM: mean standard error
397 Tc^{99m}: technetium-99m
398 TMDD: target-mediated drug disposition
399 VMD: volume mean diameter
400

401 **Figure Legends**

402 **Figure 1: Setup for mAbX antibody microdialysis.** **A)** The recovery rate of mAbX using a
403 microdialysis probe, corresponding to its diffusion through a 1 MDa cut-off semi-permeable
404 membrane (CMA 20), was assayed *in vitro*. The probe was incubated with antibody (mAbX
405 3.3 mg/mL) solution and was perfused continuously with ringer acetate buffer at different
406 flow rates and maintained at 37°C. For each flow rate, the recovery rate is represented by
407 histograms with error bars (left Y axis), and the sampling duration is indicated by an empty
408 circle (right Y axis). The recovery rate corresponds to the percent of mAbX diffusing in the
409 perfusion fluid. It was calculated as follows: $(C_{\text{microdialysate}}/C_{\text{reservoir}}) \times 100$, where C
410 corresponds to the concentration of mAbX in the dialysate or reservoir. The optimal flow rate
411 of 0.3 $\mu\text{l}/\text{min}$ was chosen based on the recovery rate and the sampling duration (180 min)
412 required to obtain 50 μL microdialysate and was used for the following experiments. **B)** The
413 recovery rate of the internal control, an irrelevant murine IgG1 isotype used later for probe
414 calibration in the *in vivo* experiments, was assessed by retrodialysis. The microdialysis probe
415 was perfused with ringer acetate buffer containing the murine IgG1 (1 $\mu\text{g}/\text{mL}$, $C_{\text{perfusate}}$). To
416 ensure that the murine IgG1 mimicked mAbX, mAbX was added in the perfusion fluid and
417 the probe was incubated in the buffer only in some experiments. The residual murine IgG1 or
418 mAbX (C_{residual}) concentrations were measured, and the recovery rate corresponded to the
419 percentage of murine IgG1 or mAbX lost from the perfusion fluid (retrodialysis). The
420 retrodialysis rate was calculated as follows: $((C_{\text{perfusate}} - C_{\text{residual}})/C_{\text{perfusate}}) \times 100$, where C
421 corresponds to the concentration of the murine IgG1 or mAbX in the perfusate. Results are
422 expressed as the median [IQR] from three independent experiments. n.s. non-significant
423 (Kruskal-Wallis test).

424

425 **Figure 2: *In vivo* lung microdialysis of inhaled mAbX.** A) **Scheme of the experimental**
426 **setup.** Prior to microdialysis, pulmonary deposition in each animal was determined by
427 scintigraphy imaging. Inhalation of mAbX (3 mL of 10 mg/mL solution) was done in
428 conscious animals, and the microdialysis probe was then immediately inserted in the lung
429 parenchyma by thoracic surgery. Animals were maintained under mechanical ventilation and
430 prolonged anesthesia for up to 54 hours and were monitored continuously during this period.
431 Blood and microdialysate were collected on a predefined schedule (every 6 and 3 hours,
432 respectively) to analyze mAbX and urea (as a control of probe permeability). At the end of the
433 experiment, animals were sacrificed by exsanguination and lungs were excised. **B) Lung**
434 **deposition with the customized mesh nebulizer in conscious macaques.** Scintigraphic
435 imaging of a representative animal that received the formulation without the antibody spiked
436 with ^{99m}Tc -DTPA as a tracer and the dose (in %, right) deposited in the different regions of
437 interest (ear/nose and throat (ENT), lungs, stomach) determined from the digitalized images
438 using lung tissue attenuation coefficients. All results are expressed as the percentage of the
439 activity loaded in the nebulizer and correspond to the median [IQR] (n=3 animals). **C) Urea**
440 **ratio to assess the probe permeability *in vivo*.** Urea was measured in the microdialysate and
441 in the blood throughout the experiment. Results are expressed as the ratio of urea in the
442 microdialysate to blood urea and correspond to the median [IQR], n=3 animals. n.s. non-
443 significant (Friedman test).

444

445 **Figure 3: Fate of mAbX after inhalation in macaques.** (A) Time-concentration of mAbX
446 after inhalation in the lung interstitium (microdialysate, blue line) and serum (red line) in
447 macaques. Results are expressed as the mean \pm SEM and representative of three animals.
448 (B) AUC values ($\mu\text{g}\cdot\text{h}\cdot\text{L}^{-1}$) for microdialysate, serum and microdialysate to serum ratio. AUC,
449 area under the curve.

450

451 **Figure 4: Immunodetection of residual mAbX in the lung tissue.** Animals were sacrificed
452 by exsanguination at the end of the microdialysis experiment, up to 54 hours after mAbX
453 inhalation. High-magnification ($\times 40$, black scale=20 μm) of hematoxylin staining and mAbX
454 immunodetection in the lungs of an animal after inhalation of mAbX are represented and
455 compared with control lung tissue from an animal that did not receive mAbX. AM: alveolar
456 macrophages; BEC: bronchial epithelial cells.

457

458 **Supplemental Figure S1: Scheme of experimental setup for *in vitro***
459 **microdialysis/retrodialysis.**

460

461 **Table 1: Clinical parameters of the macaques during the microdialysis experiment.**
462 Parameters were measured each hour. Results are expressed as the median [IQR] for each
463 macaque over a period of up to 54 hours.

464

465 **References**

- 466 1. Reichert JM. Antibodies to watch in 2016. *MAbs* 2016; 8:197–204.
- 467 2. Hervé V, Rabbe N, Guilleminault L, Paul F, Schlick L, Azzopardi N, Duruisseaux M,
468 Fouquenot D, Montharu J, Redini F, et al. VEGF neutralizing aerosol therapy in primary
469 pulmonary adenocarcinoma with K-ras activating-mutations. *MAbs* 2014; 6:1638–48.
- 470 3. Guilleminault L, Azzopardi N, Arnoult C, Sobilo J, Hervé V, Montharu J, Guillon A,
471 Andres C, Herault O, Le Pape A, et al. Fate of inhaled monoclonal antibodies after the
472 deposition of aerosolized particles in the respiratory system. *J Control Release* 2014;
473 196:344–54.
- 474 4. Respaud R, Marchand D, Pelat T, Tchou-Wong K-M, Roy CJ, Parent C, Cabrera M,
475 Guillemain J, Mac Loughlin R, Levacher E, et al. Development of a drug delivery system
476 for efficient alveolar delivery of a neutralizing monoclonal antibody to treat pulmonary
477 intoxication to ricin. *J Control Release* 2016; 234:21–32.
- 478 5. Respaud R, Vecellio L, Diot P, Heuzé-Vourc’h N. Nebulization as a delivery method for
479 mAbs in respiratory diseases. *Expert Opin Drug Deliv* 2015; 12:1027–39.
- 480 6. Kamath AV. Translational pharmacokinetics and pharmacodynamics of monoclonal
481 antibodies. *Drug Discov Today Technol* 2016; 21–22:75–83.
- 482 7. Liu L. Pharmacokinetics of monoclonal antibodies and Fc-fusion proteins. *Protein Cell*
483 2018; 9:15–32.
- 484 8. Shah DK, Betts AM. Towards a platform PBPK model to characterize the plasma and
485 tissue disposition of monoclonal antibodies in preclinical species and human. *J*
486 *Pharmacokinet Pharmacodyn* 2012; 39:67–86.
- 487 9. Wang J, Iyer S, Fielder PJ, Davis JD, Deng R. Projecting human pharmacokinetics of
488 monoclonal antibodies from nonclinical data: comparative evaluation of prediction
489 approaches in early drug development. *Biopharm Drug Dispos* 2016; 37:51–65.
- 490 10. Ferl GZ, Theil F-P, Wong H. Physiologically based pharmacokinetic models of small
491 molecules and therapeutic antibodies: a mini-review on fundamental concepts and
492 applications. *Biopharm Drug Dispos* 2016; 37:75–92.
- 493 11. Elmquist WF, Sawchuk RJ. Application of microdialysis in pharmacokinetic studies.
494 *Pharm Res* 1997; 14:267–88.
- 495 12. Brunner M, Langer O. Microdialysis versus other techniques for the clinical assessment
496 of in vivo tissue drug distribution. *AAPS J* 2006; 8:E263-271.
- 497 13. Jadhav SB, Khaowroongrueng V, Derendorf H. Microdialysis of Large Molecules. *J*
498 *Pharm Sci* 2016; 105:3233–42.
- 499 14. Jadhav SB, Khaowroongrueng V, Fueth M, Otteneder MB, Richter W, Derendorf H.
500 Tissue Distribution of a Therapeutic Monoclonal Antibody Determined by Large Pore
501 Microdialysis. *J Pharm Sci* 2017; 106:2853–9.

- 502 15. Colombat M, Mal H, Groussard O, Capron F, Thabut G, Jebrak G, Brugière O, Dauriat G,
503 Castier Y, Lesèche G, et al. Pulmonary vascular lesions in end-stage idiopathic pulmonary
504 fibrosis: Histopathologic study on lung explant specimens and correlations with
505 pulmonary hemodynamics. *Hum Pathol* 2007; 38:60–5.
- 506 16. Hanumegowda C, Farkas L, Kolb M. Angiogenesis in pulmonary fibrosis: too much or
507 not enough? *Chest* 2012; 142:200–7.
- 508 17. Hart TK, Cook RM, Zia-Amirhosseini P, Minthorn E, Sellers TS, Maleeff BE, Eustis S,
509 Schwartz LW, Tsui P, Appelbaum ER, et al. Preclinical efficacy and safety of
510 mepolizumab (SB-240563), a humanized monoclonal antibody to IL-5, in cynomolgus
511 monkeys. *J Allergy Clin Immunol* 2001; 108:250–7.
- 512 18. Dall'Acqua WF, Kiener PA, Wu H. Properties of human IgG1s engineered for enhanced
513 binding to the neonatal Fc receptor (FcRn). *J Biol Chem* 2006; 281:23514–24.
- 514 19. Chang H-Y, Morrow K, Bonacquisti E, Zhang W, Shah DK. Antibody pharmacokinetics
515 in rat brain determined using microdialysis. *MAbs* 2018; 10:843–53.
- 516 20. Guillon A, Sécher T, Dailey LA, Vecellio L, de Monte M, Si-Tahar M, Diot P, Page CP,
517 Heuzé-Vourc'h N. Insights on animal models to investigate inhalation therapy: Relevance
518 for biotherapeutics. *Int J Pharm* 2018; 536:116–26.
- 519 21. Pitance L, Vecellio L, Leal T, Reychler G, Reychler H, Liistro G. Delivery efficacy of a
520 vibrating mesh nebulizer and a jet nebulizer under different configurations. *J Aerosol Med*
521 *Pulm Drug Deliv* 2010; 23:389–96.
- 522 22. Albuquerque-Silva I, Vecellio L, Durand M, Avet J, Le Pennec D, de Monte M,
523 Montharu J, Diot P, Cottier M, Dubois F, et al. Particle deposition in a child respiratory
524 tract model: in vivo regional deposition of fine and ultrafine aerosols in baboons. *PLoS*
525 *ONE* 2014; 9:e95456.
- 526 23. Réminiac F, Vecellio L, Loughlin RM, Le Pennec D, Cabrera M, Vourc'h NH, Fink JB,
527 Ehrmann S. Nasal high flow nebulization in infants and toddlers: An in vitro and in vivo
528 scintigraphic study. *Pediatr Pulmonol* 2017; 52:337–44.
- 529 24. Mager DE. Target-mediated drug disposition and dynamics. *Biochem Pharmacol* 2006;
530 72:1–10.
- 531 25. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev*
532 *Immunol* 2007; 7:715–25.

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