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The Airways: A Promising Route for the Pulmonary Delivery of Anticancer Agents

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1. Introduction

The outcome of lung cancer has not changed dramatically in recent years, despite the availability of new therapeutic agents. Lung cancer is the most common cause of cancer-related death in all industrialised countries (28 % in the USA for 2009). It is usually treated by a combination of surgery, radiotherapy and chemotherapy; and anticancer drugs are generally given intravenously. But this delivery route leads to high drug concentrations in the systemic circulation, with some adverse side effects and low drug concentrations in the respiratory tract. Clearly, a new route for administering anti-cancer drugs is needed: the airways. Drugs for treating chronic respiratory diseases like asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis are commonly delivered *via* the airways. The main advantage of this route is that the drug is delivered directly into the bronchi, bronchioles or deep lungs. Airway delivery should theoretically ensure longer exposure of the intended target to higher concentrations of the drug, while reducing adverse side effects. The patient should therefore benefit from a minimum drug concentration in the bloodstream and other body tissues. The airways therefore appear to be an attractive route for delivering anticancer agents in lung cancer, especially when other treatments have limited success, and in particular pathological situations, such as bronchioloalveolar carcinoma (BAC). This chapter provides an overview of the delivery of anticancer agents by aerosoltherapy for treating lung cancers and metastases, including the current status in the use of aerosoltherapy in oncology and future progress.

2. Challenge of aerosol drug delivery in lung cancer

2.1 Aerosol deposition

Effective drug delivery to the lungs *via* the airways requires a detailed knowledge of aerosols. The main parameters that should be considered are the particle size, the inspiratory flow rate, the volume of the inhalation and the calibre of the patient's airways. The model developed by Weibel divides the lungs into 23 serial branching generations. The first sixteen form the conducting bronchial airways and the last seven, the respiratory zone (or alveolar region) (Weibel et al., 1963). The lungs may also be divided in three parts, upper (apex), middle and lower (base). Thus the major challenge involved in delivering drugs into the lungs is provided by the complex structure of the respiratory tract.

Aerosols generally have a polydisperse particle size distribution, and particle size is one of the most important factors influencing aerosol deposition in the lungs. The mass median aerodynamic diameter (MMAD) is usually used to estimate the particle size distribution. The MMAD describes an aerosol in terms of its mass and size. It is defined as 50 % of the mass of the particles less than MMAD and 50 % greater than the MMAD (Soderlom, 1989). Analysis of lung depositions indicated that almost all particles with a MMAD >5 μm are deposited in the oropharynx. Most of those with a MMAD of 2 to 6 μm are deposited in the bronchi (conducting airways) and fine particles with a MMAD <2 μm are deposited in the alveolar region. Most of the particles smaller than 1 μm are presumably exhaled (Newman et al., 1983).

The depositing of particles in the lungs also depends on several other parameters. One is the inspiratory flow rate (IFR). The particles display high velocity and turbulence when the IFR increases, causing them to be deposited in the conducting airways (Dolovich et al., 2000). Thus drug deposition in the upper airways can be improved by increasing the IFR. In contrast, a low IFR and gas density help aerosols to penetrate into the lung periphery (Anderson et al., 1990). The penetration of particles into the alveolar region also depends on the inhaled volume. Particles are better deposited in the lung peripheries of patients with a high FEV1 (forced expiratory volume in one second) (Pavia et al. 1976). Similarly, rapid exhalation following a short breath hold can influence particle deposition in the large airway. The calibre of the airways also modulates particle deposition. There is excessive mucus secretion and accumulation in some respiratory diseases, such as cystic fibrosis and certain tumours, which leads to obstruction of the airways. This reduction in airway calibre has a negative influence on the deposition and distribution patterns of aerosols. Particle deposition is dramatically decreased in cystic fibrosis patients, even from aerosols with fine particles (<2μm), because of the secretions and reduced airway calibre (Dolovich, 2009).

The diameter of the airways of children is much smaller than those of adults. Hence, particles are deposited mainly in the oropharynx or upper airways because they impact rapidly on the bronchus barrier. The breathing patterns of children also differ from those of adults. Their tidal volume is smaller (volume of air displaced between normal inspiration and expiration) and their respiratory rate higher than those of adults, which prevents drugs from being deposited in the alveolar region. Co-operation and compliance complicate the administration of drug to children *via* the airways. Cries can be assimilated to a rapid exhalation, leading to deposition of drug almost exclusively on the surface of the oropharynx (Schüepp et al., 2004).

Few studies focused on the deposition of inhaled particles in the lungs of the elderly. They have shown that the particle deposition in old and younger patients with lung diseases were similar. However, some pulmonary delivery devices are unsuitable for patients suffering from memory loss and disorders of movement coordination (Allen et al., 2008).

2.2 Technology of delivery devices

Several devices have been developed to generate aerosol particles. They include nebulisers, pressurized-metered dose inhalers (pMDI) and dry powder inhalers (DPI). Nebulisers are widely used for drug inhalation, and there are three types of nebuliser: pneumatic (or jet), ultrasonic and vibrating-mesh. Pneumatic nebulisers use compressed air to aerosolise drug solutions. These solutions are carried into the gas stream and dispersed into droplets due to surface tension (Hess et al., 2008). A fill volume of 4-5 ml is optimal and normal saline is

added when the volume is lower. Ultrasonic nebulisers use a high-frequency piezoelectric system to produce ultrasound, which then nebulises a drug solution. This system is not compatible with all molecules because the ultrasound can destroy certain drugs. In vibrating-mesh nebulisers, the drug solution is compressed by perforated membranes that are vibrating rapidly. All types of nebuliser are only half as efficient with nasal inhalation as with oral inhalation (Everard et al., 1993).

A pMDI is a handheld device that contains a drug in solution or suspension and a gas like hydrofluoroalkane to propel the solution/suspension. It must be shaken before use to mix gas with the drug solution/suspension. The most important component of the pMDI is the metering valve (Hess et al., 2008). This is because the pMDI canister is used inverted, and the valve prevents the drug from leaking due to gravity. The advantages of this system are its small size, ease of use, and its portability. However, good coordination between hand movements and inhalation is needed for efficient drug deposition. Devices with an automatic release have been developed for patients with coordination disorders. A valve holding chamber can also be placed between the MDI and the patient to ensure that aerosol is retained in the chamber and released when the patient breathes.

The drug used in the DPI system is formulated as a dry powder. The devise has a dosing principle and an inhaler (Pedersen, 1996). The powder is broken down into small particles by the force of the patient's inhalation. DPI is available in multi-dose and single-dose models. The single-dose model has a single drug capsule that must be manually inserted into the device by the patient. These drug capsules must not be ingested.

Education is essential for effective drug delivery *via* the airways. Nurses, physicians and pharmacists must all learn how to use inhalers so as to prevent their misuse or inadequate treatment. Mishandling of aerosol devices like the pMDI is associated with decreased asthma control (Giraud and Roche, 2002). Patients must be also educated to inhale properly in order to maximize drug deposition in the intended lung compartment. Most of the anticancer drugs presently being evaluated in clinical trials have been administered to the airways with nebulisers. Nebulisers and endotracheal sprays have generally been used to deliver anticancer drugs in preclinical studies to animals.

2.3 Recommendations for delivering anticancer drugs *via* the airways

Official sources, such as the European Respiratory Society, have not yet established procedures for using anticancer drugs to treat lung cancer. They have only discussed the lack of evidence for using opiates and bronchodilators in palliative care (Boe et al., 2001). Herein, we propose some advices based on official recommendations established for other respiratory diseases (such as COPD and asthma). Certain steps should be scrupulously respected if a drug is administered *via* the airways. The choice of the device is critical because they are not compatible for all drugs. Physicians may prefer not to use pMDI or DPI systems for patients with coordination disorders or memory deficiency. A mouthpiece is the most suitable means of improving lung deposition and is safer for the patient as it avoids injuring the eyes. The manufacturer's instructions (formulation, solution volume and type of driver gas) should be respected to ensure optimal drug deposition, and effective treatment. Nebulisers should be cleaned carefully to prevent bacterial contamination and subsequent lung infections. Maintenance procedures are available for all nebulisers and they should be communicated to the patient. Single-dose nebulisers may be most suitable for hospitalised patients, with replacement every 24 hours.

Great care should be taken when administering anticancer agent *via* the pulmonary route. Anticancer agents are potentially toxic for the lung and may impair the pulmonary function in some patients, but most of them are not very toxic when the inhalation procedure is standardized and the dose well defined. The safety of healthcare workers should be considered. Aerosolised chemotherapy should be delivered in a well-ventilated room with an efficient air filtering system (Gagnadoux et al., 2007). Wittgen et al demonstrated the advantages of combining a mobile HEPA (High Efficiency Particulate Air) filter air cleaning system with a demistifier tent to prevent aerosol propagation during inhalation of nebulised liposomal cisplatin (Wittgen et al., 2006).

3. Aerosolisation of anticancer agents

3.1 Formulation of anticancer drugs for aerosol delivery

Particle size is one of the most critical parameters for ensuring efficient drug deposition in the respiratory tract. Several parameters influence drug targeting to the desired lung area: these are the size, shape, density, electrical charge and hygroscopicity of the aerosol particles (Pilcer and Amighi, 2010). It is also essential that the aerosolised drug remains pharmacologically active. Formulations for nebulised drug traditionally include sodium chloride or other salts to adjust the osmolarity, HCl or NaOH to obtain a stabilised neutral pH and a surfactant such as polysorbates to avoid drug aggregates. But other methods have been developed to produce particles with controlled properties; these include jet milling, spray drying and supercritical fluid techniques. Excipients like lipids and polymers are also used to improve pulmonary deposition (Pilcer and Amighi, 2010).

Some drugs are encapsulated into liposome to increase their resident time into the lungs (Cryan, 2005). Liposomal formulations generally provide sustained drug release, prevent local irritation, and improve drug stability. For example, the resident time in the lungs of liposomal cyclosporine A is nearly 17 times longer than that of the standard compound (Arppe et al., 1998). The lipids most commonly used to produce liposome are lecithins (phosphatidylcholines), phosphatidylethanolamines, phosphatidylserines, and phosphatidylinositols. Formulations with microspheres have also been developed. Although, they are mostly used to deliver drugs whose intended actions are systemic, such as vaccines and insulin, more and more formulations of anticancer agents associated with microspheres are being developed. Microspheres are produced using natural or synthetic polymers. The two most commonly used synthetic polymers are polylactic acid (PLA) and polylacticco-glycolic acid (PLGA) (Cryan, 2005). The rate at which a drug is released from microparticles depends on its dissolution and diffusion.

New formulations are today developed for the controlled release of the drug and to enhance anticancer efficacy. Biotinylated-EGF-modified gelatine was tested as a carrier for cisplatin with better anticancer and less toxic effect than free cisplatin after aerosolisation *in vitro* and *in vivo* (Tseng et al., 2009). Encapsulation of anticancer agent in nanoparticles is also evaluated, in particular through airways delivery with some promising results (El-Gendy and Berckland, 2009, Hureaux et al., 2009, Tomoda et al., 2009).

3.2 Pharmacological properties of aerosolised anticancer agents

Most anticancer drugs resist the physical constraints of aerosolisation, retain their pharmacological properties, and produce particles with aerodynamic properties that are

compatible with lung deposition. A study by Gagnadoux et al. showed that the cytotoxicity of a nebulised formulation of the nucleoside analog gemcitabine (GCB) was similar to that of the native drug when tested against NCI-H460 and A549 Non-Small Cell Lung Cancer (NSCLC) cells (Gagnadoux et al., 2006). Another study of the cytotoxicity of a nebulised farnesol formulation containing polysorbate 80 (Tween 80) in vitro for NSCLC lines (H460 and A549) showed that the anticancer properties of nebulised farnesol were essentially the same as those of the native drug (Wang et al., 2003). The cytotoxic effects of doxorubicin before and after encapsulation were compared *in vitro* using growth inhibition assays. Azarmi et al. (2006) studied doxorubicin (DOX)-loaded nanoparticles formulated as a dry powder by spray-freeze-drying. The cytotoxic effects of free DOX, carrier particles containing blank nanoparticles and DOX-loaded nanoparticles were assessed using H460 and A549 lung cancer cells. The DOX-nanoparticles were more cytotoxic for both cell lines a higher than were the blank nanoparticles and the free DOX. The aerosolisation of therapeutic proteins such as anticancer antibodies has also been tested. The results showed that some inhalers are suitable for limiting the formation of aggregates and preserving the pharmacological activity of the antibody *in vitro* (Maillet et al., 2008). Cetuximab, a chimeric IgG1 that targets the epidermal growth factor receptor (EGFR), was tested with three types of nebulisers: jet, mesh and ultrasonic. The immunological and pharmacological properties of nebulised cetuximab were evaluated using A431 cells. Flow cytometric analyses and assays of EGFR-phosphorylation and the inhibitions of A431 cell growth demonstrated that the mesh and jet nebulisers did not destroy the ability of cetuximab to bind to EGFR or its inhibitory activity.

4. Airways delivery in preclinical studies

4.1 Animal models for aerosol delivery in cancer

4.1.1 Animal model for assessing lung deposition

The structure of the human nasal system is very different from that of all other animals except the non-human primates. The nasal anatomy of primates (human and non-human) is much simpler than that of the majority of animals (Gross et al., 1991.). Rodents cannot breathe through their mouths. Particles must be smaller than 3 μm if they are to reach the airways of rodents (Miller et al., 2000). One way to avoid nasal deposition is to introduce a catheter connected to a high pressure syringe into the trachea to deliver aerosols directly into the lungs. There are two types of tracheo-bronchial anatomy, dichotomous division and monopodial division (McBride et al. 1991). The human respiratory tract is considered to undergo dichotomous branching, while those of rats, mice and dogs are monopodial. This anatomical difference does not seem to influence aerosol deposition in the lungs, but further studies are needed to confirm this. The transition between bronchial airways and the alveolar region is gradual in humans; humans have respiratory bronchioles, while rodents do not (Tyler et al., 1993). Inhaled particles are cleared faster from the alveoli of rodents than from the alveoli of humans because rodents lack bronchioles. However, additional studies are required to determine whether this difference influences the deposition of aerosolised particles in the lungs (Phalen et al., 2008). Total aerosol deposition is better in nasal breathing humans than in oral breathing humans. Upper respiratory tract deposition is similar in nasal breathing humans and in dogs, hamsters, and rabbits. However, pulmonary deposition in nasal breathing humans is comparable to that of dogs and monkeys, but lower than in hamsters and rats. The peak particle size for pulmonary deposition is larger in humans than in dogs, guinea pigs, monkeys, and rats (Phalen et al., 2008)

4.1.2 Animal models for anticancer drug studies

4.1.2.1 Chemically induced lung cancer

Several animal models of lung cancer have been developed and used to understand carcinogenesis and test anticancer agents. One of them is chemically-induced lung cancer. These animal models are usually used only to study carcinogenesis and cancer prevention because of the long time required for tumour development and the poor therapeutic response. Newborn A/J mice injected intraperitoneally with ethyl carbamate (urethane) begin to develop benign lung adenomas within few months, followed by degenerate adenocarcinomas similar to those of humans (Shimkin et al., 1975). Some of the many chemical agents that have been tested for their ability to induce lung tumours and study carcinogenesis are benzopyrene, metals, aflatoxin, and constituents of tobacco smoke such as polyaromatic hydrocarbons and nitrosamines (Liu et al., 2002). But chemical models have rarely been used in aerosol studies except for aerosolised chemopreventive treatment.

4.1.2.2 Transgenic lung cancer

Transgenic mouse models of lung adenocarcinoma expressing a mutant active K-ras transgene are available. Jonhson et al. (2001) created a mouse strain (K-ras LA1) carrying an oncogenic allele of K-ras that had an activating codon 12 mutation (gly to asp) on exon 1, that can be activated only on a spontaneous recombination event in the whole animal (Jonhson et al., 2001). This mutation, probably in combination with other somatically acquired mutations, leads to the development of multifocal lung adenocarcinomas in 100% of the mice, skin papillomas in 23%, and thymic lymphomas in 35%. Lung tumours can first be detected microscopically when the mice are 2 weeks old, and the number and size of tumours increase continuously until they essentially fill the thoracic cavity, causing the mouse to die of respiratory failure (mean survival 300 days). Extrathoracic metastases are rare and occur only at a very late stage of the disease, as in humans with adenocarcinoma (Wislez et al., 2003; Jonhson et al., 2001). The radiological appearance is also very similar to the human disease with multifocal lesions such as nodules with ground glass attenuation (Cody et al., 2005). We are presently using this model to analyze the administration of anticancer drugs *via* the airways.

4.1.2.3 Human lung tumour xenografts

Human lung tumour xenografts are widely used in cancer therapeutic studies. Nude mice or SCID immunosuppressed mice are injected intravenously or into the lungs with human tumour cells. Orthotopic models of lung cancer have also been evaluated for aerosol studies. Lung cancer cells, mainly H460 (large cell lung carcinoma) and A549 (bronchioloalveolar lung carcinoma) or disaggregated lung tumours are implanted endotracheally in immunocompromised mice. Orthotopic models are interesting because the tumours grow directly into the lungs, in an environment mimicking that of human lung neoplastic cells. Models of pulmonary metastases have also been used in aerosol studies. For example, LM7 and LM8 osteosarcoma cells were injected intravenously into immunosuppressed mice, leading to the development of lung metastases (Koshkina and Kleinerman, 2005). Lastly, tumours grow better in the lungs than in the subcutaneous compartment. The cell lines most frequently used in animal studies are H460 (large cell lung carcinoma) and A549 (lung carcinoma).

4.2 Pharmacokinetics of anticancer agents delivered *via* the airways in animals

4.2.1 Lung deposition

The concentrations in the lungs of anticancer drugs delivered by the pulmonary route are higher than the concentrations delivered by any other route. Cisplatin, one of the major drugs used to treat lung cancers, is administered systemically. Delivery of cisplatin *via* a catheter placed in right caudal lung lobe in dogs provided a concentration in the right caudal lobe that was 44 times higher than in other pulmonary lobes (Selting et al., 2008). The pulmonary delivery of other anticancer drugs that are clinically injected intravenously, but are unconventional for treating non small cell lung cancer, was also tested. The deposition of aerosolised liposomal camptothecin, a quinoline alkaloid, in the lungs was assessed in nude mice with colon, breast or lung tumour xenografts. The concentration of the encapsulated camptothecin in the lung was 100 times higher following airways administration than after intramuscular injection (Koshkina et al., 1999). Similarly, the concentration of aeorosolised 5-Fluorouracile (5-FU) in the lung tissue was 1000 times higher than in the serum of hamsters (Hitzman et al., 2006). High concentrations of 5-FU were detected in the trachea and bronchi of dogs after airways delivery, whereas a lower concentration was measured in the peripheral lung (Tatsumura et al., 1993). We have used near infrared imaging to analyze the distribution of cetuximab, an anti-EGFR antibody, in a xenograft model of lung tumour following systemic and pulmonary delivery, (Maillet et al., in press). The antibody accumulated rapidly and durably in the lungs (Figure 1), and the lung concentration was higher following airways delivery (not shown) (Maillet et al., in press).

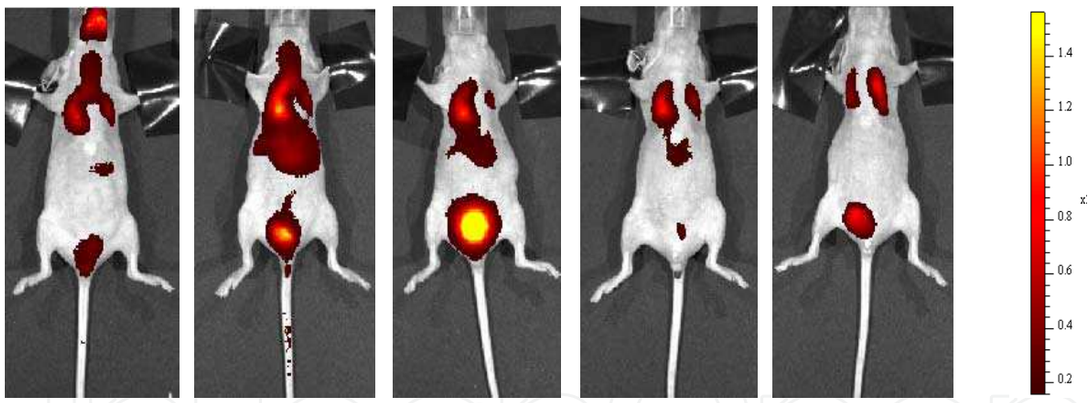

Fig. 1. Lung deposition of inhaled cetuximab at (A) 1h30 (B) 8h (C) 24h (D) 48h and (E) 72h.

4.2.2 Blood passage

Most studies have shown that the concentration of an anticancer drug in the bloodstream is lower after airways delivery than after systemic injection. For example, the concentration of cisplatin in the serum was 15.6 times lower after pulmonary administration than after intravenous (i.v.) injection (Selting et al., 2008). Gemcitabine was given to 3 baboons *via* the airways and its concentration in the blood was 25 times lower than after its systemic delivery (Gagnadoux et al., 2006). Dogs with spontaneous pulmonary metastases were given aerosolized paclitaxel and doxorubicin and the drug concentrations in the bloodstream were measured 1 minute later (Hershey et al., 1999). The serum concentrations were lower than when the drugs were delivered intravenously. However a pharmacokinetic analysis is

required to support this conclusion. We have recently determined the pharmacokinetics of cetuximab delivered systemically and *via* the lungs. Little cetuximab was found in the bloodstream after airways delivery (only 11% of the fraction delivered to the lungs) and its passage was slow with a peak around 48 hours (Maillet et al., in press).

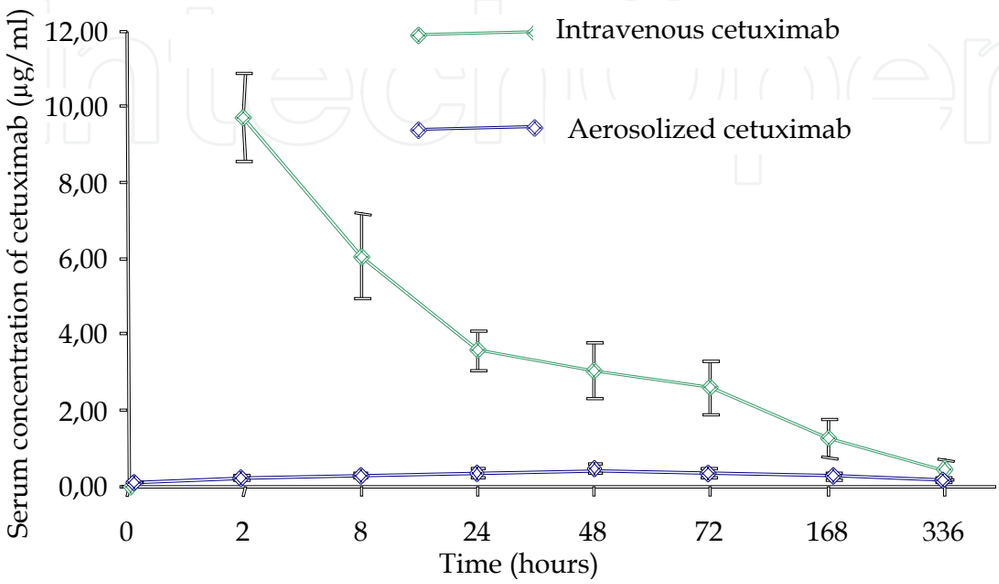

Fig. 2. Serum concentrations of cetuximab delivered systemically or *via* the pulmonary route.

4.3 Efficacy and safety of anticancer agents delivered *via* the airways in animals

4.3.1 Cisplatin

Selting and co-workers aerosolised cisplatin directly into the right caudal lobes of healthy dogs (Selting et al., 2008). They found no haematological toxicity and this delivery of aerosolised cisplatin was generally very well tolerated. Radiological and histological analyses indicated that all the dogs developed mild to moderate pneumonitis in the right caudal lobe where the drug was delivered. Chemotherapy with both cisplatin and gemcitabine was also delivered through the pulmonary route in healthy dogs using the same method (Selting et al., 2011). As previously reported, the only adverse event observed was focal pneumonitis in the right caudal lung lobe. Overall, the local-regional delivery of cisplatin either alone or in combination was well tolerated, suggesting that aerosolised platin-based chemotherapy may be used safely to treat humans. However, this method may be difficult to administer to patients with chronic respiratory failure.

4.3.2 Paclitaxel

Dogs with spontaneous primary or secondary lung tumours were given aerosolised paclitaxel using a jet nebuliser (Hershey et al., 1999). One of the 15 dogs had a partial response and one had a complete response with long term survival. The thirteen remaining had stabilised or progressive disease. In another study, mice with pulmonary metastases were given paclitaxel encapsulated in liposomes using a pneumatic nebuliser (Koshkina et

al., 2001). There were significantly fewer tumours in the treated mice than in the mice given liposome alone. Survival was improved, with 40% of the mice treated with liposomal paclitaxel being alive after 45 days, whereas all the mice treated with liposome alone were dead. A recent study found that adding vitamin E to the aerosolised liposomal-encapsulated paclitaxel enhanced the anticancer response to paclitaxel in murine model of lung metastases from primary breast cancer (Latimer et al. 2009). Lastly, the feasibility of aerosolising paclitaxel encapsulated in lipid nanoparticles was tested *in vitro*. Preclinical studies in animal models of lung cancer are expected to evaluate whether encapsulation leads to the controlled release of the drug (Hureaux et al., 2009).

4.3.3 Gemcitabine

Gagnadoux et al. evaluated the dose-limiting toxicity of gemcitabine delivered endotracheally to Wistar rats with a sprayer (Gagnadoux et al., 2005). The maximum tolerated dose was 4 mg/kg. They detected no toxicity, except for decreases in platelet and red blood cell counts, when gemcitabine had been inhaled once a week for 9 weeks. They also evaluated the antitumor efficacy of aerosolised gemcitabine in nude mice implanted intrabronchially with H460 cells (Gagnadoux et al., 2005). Of the 13 mice given aerosolised gemcitabine, 4 had no visible tumour at the end of the experiment. And those tumours remaining in the treated mice were smaller (2.05 mm) than those in the control group (5 mm). Aerosolised gemcitabine also significantly reduced tumour numbers in a pulmonary metastases murine model of osteosarcoma (Koshkina et al., 2005). Also, dogs with spontaneous lung metastases of osteosarcoma were treated in a preclinical study with aerosolised gemcitabine combined with a standard treatment regimen (Rodriguez et al., 2010). Almost half (46%) of the treated dogs showed over 50% tumour necrosis, but there was no necrosis in the untreated animals.

4.3.4 Doxorubicine

Hershey et al. treated 18 dogs with spontaneous primary or secondary lung tumours with aerosolised doxorubicine using a jet nebuliser (Hershey et al., 1999). There were 4 partial responses and treatment was well tolerated except for some coughing by half of the subjects, cardiotoxicity in two animals, and mild to moderate pneumonitis in almost all the dogs.

4.3.5 Camptothecin

Aerosolised liposomal 9-nitro-20(S)-camptothecin (L9-NC) was first assessed in mice with xenografts of breast, colon and lung tumours injected subcutaneously (Knight et al., 1999). Treatment started 1 to 4 weeks after tumour implantation and was given 5 days a week. The growth of subcutaneous tumours was 7- fold lower in treated animals with breast cancer than in the controls and 7 to 10-fold lower in the mice with colon cancers. The lung tumour xenografts were also smaller in the mice treated with aerosolised L9-NC than in the controls. The antitumor effect of L9-NC was better when it was delivered through the airways than orally in all the systems. L9-NC was also evaluated in nude mice with lung metastases produced by the i.v. injection of melanoma (B16) or osteosarcoma (LM6) cells (Koshkina et al., 2000). The mice were given aerosolized L9-NC 5 days a week for three weeks. The treated mice with lung metastases of melanoma had statistically smaller (mean diameter 32 mm) tumour foci than the control group (85 mm). Similarly, 10 of the 11 control mice with LM6 lung metastase, had visible tumours, whereas none of 11 treated mice had tumours,

suggesting that these tumors were very sensitive to aerosolised L9-NC. Gilbert et al. evaluated the safety of aerosolised L9-NC in healthy dogs. They found no toxicity after treatment with this drug for 5 days a week for 8 weeks (Gilbert et al., 2002).

4.3.6 Temozolomide

Wauthoz and co-workers evaluated aerosolised temozolomide, a recent alkylating agent, in mice with pulmonary metastases obtained by injecting B16 melanoma cells i.v. (Wauthoz et al., 2010). The treatment seemed to be very well tolerated because the maximum tolerated dose was not reached. The antitumor activity of temozolomide was similar whether it was delivered through the airways or intravenously and both routes gave significantly better results than the control group. And three of the mice were long-term survivors. These encouraging results might lead to clinical trials.

4.3.7 Interleukin-2 (IL-2)

IL-2 is a cytokine that induces an antitumor immune response. Anderson et al. evaluated immunotherapy with aerosolised IL-2 using mice with pulmonary metastases of sarcoma (Anderson et al., 1990). The antitumor activity of IL-2 delivered *via* the airways appeared to be better than *via* the other routes tested. Aerosolisation of IL-2 encapsulated in liposomes gave better survival than free IL-2 or liposome alone delivered *via* the same route. Tumour size was reduced by 50% to 85% by aerosolised liposomal IL-2, which was better than the results with free IL-2. This suggested that liposomal IL-2 was better than free IL-2.

The efficacy of airways delivery of free IL-2 and liposomal IL-2 were also compared in healthy dogs (Khanna et al. 1996). There were increases in white cells and effector cells in the broncho-alveolar lavage fluid of dogs treated with liposomal IL-2 but no haematological or clinical toxicity. A preclinical study in which dogs were treated with aerosols of liposomal IL-2 gave spectacular results (Khanna et al., 1997). Aerosolised liposomal IL-2 was used to treat 9 dogs, 7 with pulmonary metastases of a primary osteosarcoma and 2 with primary lung carcinomas. The pulmonary nodules in 2 of the 4 dogs with metastatic pulmonary osteosarcomas were in complete regression. No relapse was observed over 12 and 20 months, respectively. The disease of one of the two dogs with a primary lung carcinoma was stabilized for up to 8 months. Aerosolised IL-2 was widely evaluated in clinical trials to treat pulmonary metastases of renal cell carcinoma but there are few published preclinical studies in animals with IL-2 delivered *via* the pulmonary route. Clinical trials were probably proposed based on results obtained with IL-2 administered i.v. (Rosenberg et al., 1994)

4.3.8 Celecoxib

Celecoxib is a non-steroidal anti-inflammatory drug that inhibits cyclooxygenase 2. Treatment with aerosolised celecoxib was assessed in association with intravenous docetaxel in an orthotopic lung tumour model (A549) (Fulzele et al., 2006). Animals were given aerosolised celecoxib plus i.v docetaxel or oral celecoxib plus i.v. docetaxel, each of the drugs alone or a placebo. The aerosol was produced with a pneumatic nebuliser. Aerosolised celecoxib plus i.v. docetaxel reduced lung tumour volume by 61% compared to the placebo group while oral celecoxib plus i.v. docetaxel reduced it by 54%. Moreover, the combined treatments gave better results than the drugs alone.

4.3.9 Monoclonal antibodies

We have recently evaluated the antitumor activity of aerosolised cetuximab in a xenograft model of lung tumours. Aerosolised cetuximab limited the growth of the lung tumours (Maillet et al., in press). This proof-of-concept study demonstrates that the airways can be suitable for delivering aerosolised monoclonal antibodies to treat lung cancer, but further studies are required to determine whether the pulmonary route really does increase the therapeutic benefit of monoclonal antibodies to patients with lung cancer.

5. Airways delivery of anticancer agents in clinical studies

5.1 Aerosolisation of anticancer drugs in primary lung cancer

5.1.1 Cisplatin

Cisplatin is the standard drug for treating non small cell lung cancers. It is given combined with another chemotherapeutic agent. Aerosolised cisplatin was assessed in a phase I study on 17 patients with lung cancer, including 16 with non-small-cell-lung cancer and one with small cell lung cancer (Wittgen et al., 2007). The cancers were progressing despite all previous treatment. Increasing doses of cisplatin encapsulated in liposomes were given. Each aerosol treatment was for 20 minutes with a jet nebuliser. The particles produced had a MMAD of about 3 μm. Each patient was given cisplatin on 1 to 4 consecutive days over a period of 21 days. The initial dose was 24 mg/m², which was doubled to 48 mg/m² without toxicity. The cycle was then shortened from 3 to 2 weeks, and finally to 1 week. Nebulised cisplatin was administered 3 times a day instead of twice. No dose-limiting toxicity was observed and the maximum tolerated dose was not reached. Dyspnea was reported in 11 patients and a productive and irritating cough in 5. Eosinophilia was observed in 4 of these 11 (36.4%) patients. Cisplatin was detected in the blood of only 4 patients. The disease of 12 of the 17 patients became stable and it progressed in five of them, but the study was not constructed to assess the response to treatment. The dose-limiting toxicity was not reached, precluding a phase II study.

5.1.2 Doxorubicin

5.1.2.1 Safety and pharmacokinetic data

Anthracycline is usually administered intravenously to treat various cancers, including small cell lung cancer. The most severe adverse response is cardiac toxicity. Delivering the drug by inhalation is an interesting alternative route which may limit the cardiac toxicity. A phase I study was conducted by Otterson et al. to assess the safety of inhaled doxorubicin (Otterson et al., 2007). The 53 patients taking part included 16 patients with a primary lung tumour without histology details. The remaining 37 had pulmonary metastases of sarcoma (n=19), osteosarcoma (n=6), colorectal (n=4), thyroid (n=3) and other primary tumours (n=5). They were given doxorubicin with a pneumatic nebuliser fitted with a device that captured any fugitive aerosol to protect health worker. The starting dose was 0.4 mg/m². Inhalation was given every 3 weeks. The majority of adverse events were pulmonary with cough (n=27), dyspnea (n=9), chest pain (n=5), wheezing (n=4), hoarseness (n=3), hemoptysis (n=1), and bronchospasm (n = 1). While 5 patients suffered grade 3-4 pulmonary toxicity such as hypoxemia or decreased lung function tests, it was difficult to differentiate the adverse effects of treatment and the consequences of the pulmonary disease. Two patients suffered severe toxicity in response to a dose of 9.4 mg/m², with respiratory distress in 1 patient and bilateral ground glass infiltrates in the other. The lung function tests

of the whole population were relatively stable and there was little non-pulmonary toxicity. There was no evidence of hematological toxicity. The authors concluded that the recommended dose for a phase II study is 7.5 mg/m². The maximal concentration (C_{max}) of doxorubicin in the blood was low when the dose was less than 3 mg/m². C_{max} increased 1.6 times with doses of 3.8 - 7.5 mg/m². C_{max} was more than doubled with doses of from 7.5 to 9.4 mg/m². The maximal doxorubicin peak in the blood occurred within 5 minutes. This was due to its rapid passage through the alveolar-capillary barrier because doxorubicin is small and lipophilic.

One patient with spindle cell sarcoma gave a partial response, while the diseases of 8 patients were stable after 5 courses: 2 with bronchoalveolar carcinoma, 2 with soft tissue sarcoma, 1 with endometrial carcinoma, and 3 with thyroid cancer. The diseases of 6 patients became stable after three courses. Two patients stopped treatment after first administration. The diseases of the remaining patients progressed.

Very little inhaled doxorubicin enters the blood and probably has little systemic toxicity. It is difficult to come to any conclusion about treatment efficacy because the study was not constructed to do so, and the histological subtypes of cancers varied greatly. However, the stabilized bronchioalveolar carcinoma cases suggest that this histological pattern might be suitable for inhalation treatment with doxorubicin.

5.1.2.2 Efficacy data

Inhaled doxorubicin associated with systemic cisplatin and docetaxel at 75 mg/m² was tested in a phase I/II study on chemo-naive patients with advanced NSCLC (Otterson et al., 2010). Among the 36 patients included in the study, 28 were given dose 1 (6 mg/m²) and 8 were given dose 2 (7.5 mg/m²). The diffusing capacity of the lung for carbon monoxide (DLCO) of two of the patients given 7.5 mg/m² decreased and the recommended dose for the phase II study was 6 mg/m². The 34 patients in the phase II study, included 16 (47.1%) with adenocarcinoma, 5 (14.7%) with squamous cell carcinoma, 1 (2.9%), with large cell carcinoma, 11 (32.4%) with unspecified histology and 1 (2.9%) with a mixed tumour (squamous and adenocarcinoma). The patients were given 1 to 8 treatment cycles. Seven patients were given only one cycle and left the study because their disease progressed (n=3), adverse event (n=2), withdrawal of consent (n=1) or the physician's decision (n=1). There was little pulmonary toxicity except that the lung function of 5 patients decreased, but they did not stop their treatment. Among patients, 24 were evaluable, including 6 (25%) with a partial response and 1 (4%) with a complete response. The remaining 17 patients included 13 (54%) whose disease was stable and 4 (17%) whose disease progressed. Response rate was poorer than expected by the authors (at least 9 responding patients). The median overall survival time was 14.4 months for those given the level 1 dose and 19.5 months for those given the level 2 dose with no statistical difference. The median overall survival time was longer than is usually observed in clinical trials of lung cancer treatment. This discrepancy may be due to bias associated with the selection of the patients or because inhaled doxorubicin plus platinum chemotherapy really had some effect. A phase III study is expected to determine which of these hypotheses is correct.

5.1.3 Campthotecin

Vershraegen et al. **e**xamined the feasibility of using aerosolised liposomal 9-nitro-20(S)-camptothecin (L9-NC) (Verschraegen et al., 2000). They treated 6 patients with primary or secondary lung tumours who had not responded to previous treatment with L9-NC for 5

days a week every 3 weeks. The histology of the lung tumours was not specified, neither were any details about the nebulisation procedure given, except that an air flow of 10 liters per minute was used to generate the aerosol. No toxicity up to grade 2 was observed. The maximum drug concentration was seen 2 hours at the end of the aerosolisation, which rapidly decreased. The disease of two patients stabilized.

The same group then carried out a phase I study with nebulised L9-NC to determine the dose-limiting toxicity (Verschraegen et al., 2004). L9-NC was aerosolised with a pneumatic nebuliser with an air flow of 10 liters per minute and a mouth breathing-only face mask. Patients were treated for 5 days a week as above. The dose was increased by reducing the interval between treatments or increasing the concentration of L9-NC. The patients were premedicated with an inhaled bronchodilatator and steroids after one of them developed a grade 2 bronchial irritation. The starting dose was 6.7 μg/kg. All 25 patients completed the protocol except one who could not undergo nebulisation because of claustrophobia. The 26.6 μg/kg dose was poorly tolerated, with pharyngeal mucositis. The 20.0 μg/kg dose 2 patients to develop grade 3 asthenia but the 13.3 μg/kg treatment was well tolerated. There were mild side effects, such as a cough in 67% of patients, bronchial irritation in 46%, sore throat in 33%, nausea in 62%, vomiting in 33%, fatigue in 50%, anemia and neutropenia in 29%, and skin rash around the face mask in 21%. There was a 20% decrease in FEV1 during treatment which rapidly returned to baseline. Other lung function parameters were stable. The blood plasma L9-NC concentration peaked 2 hours after nebulisation, which was followed by a sustained decrease. The blood concentrations were similar to those obtained after oral administration, without any haematological toxicity. The responses to treatment were reported, but a phase I study is not designed to do so. Two patients had a partial remission, with endometrial carcinoma metastatic to the lungs only, but they suffered a relapse within 8 and 3 years respectively. One of these patients had a partial response of hepatic metastasis. The disease of the patients with lung tumours (3 of 6) was stabilized. Thus, nebulised L9-NC seems to be promising for treating pulmonary metastases rather than primary lung tumours. The recommended phase II study dose was 13.3 μg/kg 5 days a week every week.

5.1.4 5-Fluorouracile

Tastumura et al. were the first to report chemotherapeutic nebulisation for cancer patients (Tatsumura et al., 1983). They treated six patients with lung cancer with 5-FU aerosolised with an ultrasonic nebuliser. Two of them had complete responses and two others partial responses. Only traces of 5-FU were detected in the blood. These encouraging results led to another clinical trial to assess nebulised 5-FU for treating primary lung tumour patients (Tatsumura et al., 1993). A first group of 19 patients with resectable lung cancer was given nebulised 5-FU, 2 hours before surgery to determine the 5-FU concentrations in excised lung tissue. The 5-FU concentration in the tumour tissue was 5-15 times higher than in the normal lung tissue ($p<0.05$). The 5-FU concentrations were higher in proximal tissue and regional lymph nodes than in the remaining tissue. No drug was detected in blood samples. A second group of ten patients with unresectable lung cancer (6 squamous cell carcinomas and 4 adenocarcinomas) were given inhaled 5-FU twice a day for 3 days per week to evaluate their response to treatment. Of these, 6 had objective responses, including two complete responses and four partial responses. The disease of the remaining four patients was unchanged and 3 of them died of their disease. No toxicity was detected. Despite these

promising results, no clinical trial to assess the efficacy of inhaled 5-FU has been published, probably because i.v. injected 5-FU is not indicated for treating lung cancer.

5.1.5 Non-steroidal anti-inflammatory drugs

To the best of our knowledge, no clinical trial has assessed the efficacy of inhaled non-steroidal anti-inflammatory drugs for treating cancer patients, although aerosolised celecoxib plus i.v. chemotherapeutic agents showed good results in preclinical studies.

5.2 Aerosolisation of anticancer drugs in pulmonary metastases

5.2.1 Interleukin-2

The characteristics of clinical trials of inhaled interleukin-2 are summarized in Table 1.

Interleukin-2 (Il-2) was initially administered intravenously to treat patients with melanomas and renal cell carcinoma with some good results (Rosenberg et al., 1994). However, severe toxicity limits its intravenous administration (Mitani et al., 1992). It was logical, therefore, to evaluate IL-2 delivered *via* the airways. It was the first drug delivered *via* this route to be tested in a clinical trial for treating lung metastases (Huland et al., 1994). Patients with pulmonary metastases of renal-cell carcinoma were given IL-2 together with low doses of IL-2 injected subcutaneously (10% of total IL-2 dose) and systemic subcutaneous interferon alpha (IFN alpha). Toxicity was low, grade II toxicity occurred in only one patient who suffered bronchospasms. The pulmonary metastases did not increase during treatment. One of the 15 patients treated showed a complete response, 8 had partial responses, and the lung disease of 6 was stable. Surprisingly, 3 of 7 patients had partial responses of non-pulmonary metastases and one was stabilized. The mean survival time was 19.1 months, whereas the mean survival time of patients with renal carcinoma metastases is usually 9.9 months.

Inhaled natural IL-2 alone was also assessed in a phase I study on 16 patients, including 14 with pulmonary metastases of renal cell carcinomas and 2 non small cell lung cancers (Lorenz et al., 1996). Treatment was initially delivered once a day and progressively increased to reach 5 times a day over 43 days. Aerosol particles had an MMAD of about 2.3 µm. Treatment was relatively safe. Coughs were one of the most common adverse side effects and one patient fractured a rib, but no systemic toxicity was reported. The effect on lung function was mild to moderate. IL-2 was not detected in the blood with the low dose, but increased within 2 to 6 hours with the high dose. The pulmonary metastases of 3 patients went into complete regression, but 2 of them died from non-pulmonary metastases.

The safety and efficacy of inhaled IL-2 was evaluated in a study on 7 patients with pulmonary metastases of renal carcinomas (Nakamoto et al., 1997). The drug was aerosolised with an ultrasonic nebuliser and delivered over 10 minutes for 5 days a week. Treatment was associated with subcutaneous injections of interferon alpha. One patient stopped the treatment before three months because his overall health deteriorated. The disease of two of the remaining 6 became stable, 3 had partial responses and the disease of one patient with cystic renal disease progressed. One patient developed severe toxicity, pulmonary fibrosis appearing within 4 months and treatment was stopped. His metastases grew dramatically and he died 6 months after treatment cessation.

The safety of inhaled IL-2 led Huland et al. to perform a clinical trial on a large cohort (Huland et al., 1999a). The 116 patients with pulmonary metastases of renal carcinoma included this 6-year study were given high doses of aerosolised IL-2, either alone (11%),

with a low dose given subcutaneously IL-2 (33%), or with low-dose systemic IL-2 and interferon-alpha (56%). The overall response rate was 16% for IL-2 alone, 49% for IL-2 plus subcutaneous (s.c.) IL-2, and 35% for IL-2 plus s.c. IL-2 and interferon. The median overall response was 9.6 months. The pulmonary metastases of 15% of patients progressed and those of 55% were stabilized. The authors identified risk factors of poor response in patients treated with inhaled IL-2 (Huland et al., 1999b). Of the 116 patients given inhaled IL-2 (natural or recombinant), 86 had a poor response and at least one of the following risk factors: 1 metastatic location (86%), interval between diagnosis and treatment of <12 months (62%), weight loss prior to therapy (41%), and ECOG (Eastern Cooperative Oncology Group) performance status ≥2 (13%). However, the response rate, including long-term stabilization, was 27 to 57% in patients with these risk factors. Inhaled IL-2 should be proposed for all renal cancer patients with pulmonary metastases. However, patient with multiple nodules and who are tired may have reduced lung deposition of inhaled treatment. Another clinical trial was conducted on 40 patients with progressive pulmonary metastases of a renal cell carcinoma. They were treated with inhaled IL-2 3 times a day for a total dose of 18 million units (MU) plus a low dose of systemic IL-2 (Merimsky et al., 2004). The dose was reduced for one patient because of a cough and dyspnea. The dose was increased to 36 MU for seven patients whose disease progressed. The response rate was poorer than in previous studies supervised by Huland et al. Only one of the 40 patients had a partial response, but the disease of 22 patients was stabilized. The median time to progression was 8.7 months. Toxicity was low including cough, weakness, dyspnea, fever and abdominal pain. The efficacy and safety of inhaled IL-2 were also assessed in a retrospective study on 51 patients with pulmonary metastases of renal cell carcinoma (Esteban-González et al., 2007). The patients were given 3 cycles of 36 MU per day for 5 days per week for 12 weeks. Toxicity was low, always grade 1 or 2. Cough and fatigue were the most common problems. The overall objective response rate was 13.7%. The median progression-free survival time was 8.6 months and the overall survival time was 23 months. Inhaled IL-2 seemed to have an effect but it was not compared to a control group. A retrospective study compared 94 patients with metastases of renal carcinoma treated with inhaled IL-2 to 103 patients treated with systemic IL-2 (Huland et al., 2003). The toxicity in the two groups was radically different. Cough was observed in the inhaled IL-2 group and fever, fatigue, skin lesion in systemic IL-2 group. The 1-, 2- and 3-year survival rates were estimated to be 47%, 28% and 23% for inhaled IL-2 and 26%, 10% and 1% for the systemic IL-2 group. The hazard ratio for inhaled IL-2 was 0.435. The death risk of patients treated with inhaled IL-2 was decreased by 44%.

The largest clinical trial with a drug delivered *via* the pulmonary route to treat lung cancer was the study conducting by Atzpodien et al. (Atzpodien et al., 2006). The 379 patients with metastases of renal cell carcinoma were randomly assigned to group I (143 patients) or group II (236 patients). The group I patients in arm A were given subcutaneous IL-2, subcutaneous interferon-α plus 13-cis-retinoic acid; those in arm B were given the same treatment as arm A plus inhaled IL-2. The patients in group II were assigned to arm C (arm A plus intraveinous 5-FU) or arm D (arm A plus oral capecitabine). The 13-cis-retinoic acid used in this study is a regulator of cell differentiation that has been reported to enhance the antitumor effect of IL-2/IFN-α on renal cell carcinoma metastases (Atzpodien et al, 1995). Patients with pulmonary metastases were preferentially assigned to group I. Arm B patients were given systemic IL-2 and IFN-α plus inhaled IL-2 on days 1 to 5 of weeks 2 and 3 and

	Drugs	Patients	Toxicity	Response rate	Outcome
Huland et al. 1994	Inhaled IL2 + low dose systemic IL-2 + systemic IFN	15 patients with RCC	Grade 2 (7%) Bronchospasm in 1 patient	1 CR 8 PR 6 SD	OS= 19,1 months
Lorenz et al. 1996	Inhaled IL-2 alone	14 patients with RCC 2 patients with NSCLC	Cough Mild to moderate decrement of lung function	1 CR 1 PR 6 SD	NA
Nakamoto et al. 1997	Inhaled IL-2 and s.c. IFN	7 patients with RCC	1 patient with pulmonary fibrosis	3 PR 2 SD	NA
Huland et al. 1999	Inhaled IL-2 either alone or with low dose s.c. IL-2 or with low-dose s.c. IL-2 and IFN	116 patients with RCC	Grade 3 (16%)	CR/PR/SD: -16% IL-2 -49% with inhaled and s.c. IL-2 -35% with tritherapy	PFS= 9.6 months OS= 11.8 months
Huland et al. 2003	Inhaled IL-2 vs systemic IL-2	94 patients with inhaled IL-2 and 103 patients with systemic IL-2	cough	CR/PR/SD: -45% inhaled IL-2 -35% systemic IL-2	Inhaled IL-2 1-year survival : 47% 2- year survival: 28% 3-year survival: 23% Systemic IL-2 1-year survival : 26% 2- year survival: 10% 3-year survival: 1%
Merimsky et al. 2003	Inhaled IL-2 and low dose systemic IL-2	40 patients with RCC	cough, weakness, dyspnea, fever and abdominal pain	1 PR 22 SD	PFS=8.7 months
Atzpodien et al. 2006	Arm A : and systemic IL-2 and 13-cis-retinoic acid Arm B: Arm A and Inhaled IL-2	379 patients with RCC	Equal toxicity for each arm (more than 5% of grade III/IV toxicity). No respiratory toxicity was reported in arm B	Arm A: 12% CR 19% PR 26% SD Arm B: 10% CR 19% PR 36% SD	Arm A : PFS=5 months OS= 22 months Arm B : PFS= 4 months OS= 18 months
Esteban-González et al. 2007	Inhaled IL-2	51 patients with RCC	Grade 1 and 2 exclusively	CR/PR/SD: 13.7%	PFS=8.6 months OS=23 months

Table 1. Main characteristics of clinical trials assessing inhaled interleukin-2. PFS: progression-free survival, OS: overall survival, CR: complete response, PR: partial response, SD: stable disease.

weeks 5 to 8 with a pneumatic nebuliser producing 3 μm particles. A cycle was considered to be 8 weeks and patients were given 3 courses. Sixty patients (15.8%) (arm A: 14%; arm B: 9.2%; arm C: 21.6%; arm D: 15%) did not complete treatment due to disease progression before the first evaluation (2.9%), intolerance (8.7%), death during therapy (1.6%), patients' wish (2.1%), and non-compliance (0.5%). The endpoints were overall survival, progression-free survival and objective response to treatment. In arm A, 8 patients (10%) had a complete response and 15 patients (19%) had a partial response, 28 (36%) had stable disease and the disease of 27 (35%) progressed despite treatment. The overall objective response rate was 29% (95% CI 19, 40). In arm B, 8 patients (12%) had a complete response, 12 (19%) a partial response, 17 (26%) had stable disease, and the disease of 28 (43%) progressed. The overall objective response rate was 31% (95% CI 20, 44). The patients in arms C and D had similar results. Progression-free survival was 5 months in arm A, 4 months in arm B, 0 month in arm C and 4 months in arm D with no statistical difference between them. The overall survival time was 22 months in arm A, 18 months in arm B, 18 months in arm C, and 16 months in arm D with no statistical difference. All four treatments were moderately well tolerated with equal toxicity for each arm (more than 5% of grade III/IV toxicity). No respiratory toxicity was reported in arm B with inhaled IL-2. The treatment based on s.c. IL-2, s.c. IFN and oral 13-cis-retinoic acid was probably too toxic for the advantages of inhaled IL-2 to be detected. Clinical trials associating an oral tyrosine kinase inhibitor like sorafenib with inhaled IL-2 might be interesting. A study on 10 patients reported asthma in response to inhaled IL-2 (Loppow et al., 2007), but only one patient had to stop treatment.

Skubitz et al. demonstrated that interleukin-2 encapsulated in liposomes could be delivered by aerosol to patients with pulmonary metastases (Skubitz et al., 2000). They gave 9 patients liposomes containing IL-2 or placebo with a jet nebuliser and a standard compressor. The IL-2 treatment was inhaled for about 20 minutes 3 times a day. No significant toxicity was observed and inhalation was well tolerated. Further studies are expected. However, it is hard to see the clinical advantage of inhaled liposomal IL-2 considering the impressive results of the free form, unless it could improve IL-2 resident time in the airways, so reducing the number of treatments.

5.2.2 Monoclonal antibodies and tyrosine kinase inhibitor

To the best of our knowledge, no clinical trials have tested inhaled antibodies or tyrosine kinase inhibitors for treating lung cancer patients.

5.2.3 Granulocyte Macrophage Colony Stimulating Factor (GM-CSF)

GM-CSF was first assessed in a dose escalation study on 7 patients with pulmonary metastases (2 patients with osteosarcoma, 2 with melanoma, 1 with leiomyosarcoma, 1 with renal cell carcinoma and 1 with Ewing's sarcoma) (Anderson et al., 1999). They were given GM-CSF aerosolised with a pneumatic nebuliser. One was not treated due to rapid disease progression. The remaining 6 patients were given the drug twice a day for one week, and treatment was stopped for one week. The starting dose was 60 μg. The dose was increased to determine dose-limiting toxicity. Those given the high dose showed a minor increase in their white blood cell count that was not statistically different from their white blood cell count before treatment. Lung function was stable except in one patient with osteosarcoma whose FVC increased. No toxicity was described. Although it was a dose escalation study, treatment response was reported. Of the six patients who completed study, the disease of 4

stabilized for 9 months, 1 had a partial response and 1 had a complete response (patient with Ewing's sarcoma). The authors suggest that the low GM-CSF concentration into the bloodstream after airways delivery may be attributed to GM-CSF sequestration into the lungs by immune effectors. This therapy was given to 45 other patients (40 with pulmonary metastases) to better evaluate the toxicity of inhaled GM-CSF (Rao et al., 2003). They were given nebulised GM-CSF (250 μg) twice a day for 1 week followed by a 1 week rest. Moderate toxicity was observed in 18 patients. The disease of 24 patients was stabilized or partially responded (8 of 13 with a sarcoma, 6 of 14 with melanoma, and 5 of 12 with renal cell carcinoma). The efficacy of aerosolised GM-CSF might be due to stimulation of an antitumor immune response because one patient with pulmonary metastases of melanoma had a 10-fold increase in specific cytotoxic T lymphocytes resulting in a partial response. The authors suggested that there was a dose-dependent response to the treatment and conducted a third dose escalation study with doses higher than in previous trials (Markovic et al., 2008). Only patients with pulmonary metastases of melanomas were included because the authors postulated that there could be an antitumor auto-immunization against the melanoma, as suggested by increase in melanoma-specific lymphocyte T in previous study. Aerosolised GM-CSF was delivered with a pneumatic nebuliser twice daily on days 1 to 7 and 15 to 21 in a 28-day cycle. Doses ranged from 500 to 2000 μg. The author considered aerosolised GM-CSF to be well tolerated.because only 3 of the 40 patients had severe toxicity, including grade 4 dyspnea in 1 patient given 2000 μg and 1 patient given 1750 μg, and grade 3 fatigue in 1 patient given 1000 μg. There were increases in melanoma-specific cytotoxic T lymphocytes in 9 patients (1 given 500 μg, 1 given 750 μg, 2 given 1500 μg, 1 given 1750 μg, and 4 given 2000 μg). Although the data are descriptive, the authors reported that one patient on 2000 μg had a partial response and the disease of 6 became stable. None of the GM-CSF doses used induced antitumor immunity in the majority of patients. Further studies are needed to determine the extent of the antitumor immunity induced by aersosolised GM-CSF in melanoma. A recent clinical trial treated 43 adults and children with lung relapses of osteosarcoma with inhaled GM-CSF. They were given 2 cycles of inhaled GM-CSF, then the tumour nodules were surgically removed. The patients were then given 12 cycles of inhaled GM-CSF (Arndt et al., 2010). Treatment was given 5 days per week every 2 weeks and the dose range was from 250 μg to 1750 μg. The Fas and Fas ligand were assayed in the removed nodules to assess the immunomodulatory effect of GM-CSF. Nine patients had grade 3 respiratory toxicity, including 5 patients on 1750 μg. Thirty seven patients suffered relapses. The median free survival time was 4.3 months and the overall survival rates were 63.1% at 2 years and 35.4% at 3 years. GM-CSF was found to have no immunomodulatory effect, and this potential property of GM-CSF remains controversial.

5.3 Aerosols in palliative cancer care

Breathlessness is a serious problem for cancer patients in palliative care because of the discomfort it causes. Drugs are usually given systemically to relieve this discomfort, but this is invasive and often requires hospitalization.

5.3.1 Morphine and other opiod analgesics

The opiate analgesic morphine is widely used and is given orally, intravenously or subcutaneously to cancer patients to relieve pain. The effect of morphine on breathlessness was suggested more than 20 years ago (Bruera et al. 1990) and reported in a systematic review in 2002 (Jennings et al., 2002). Delivering morphine *via* the pulmonary route might

decrease breathlessness because there are morphine receptors in the lungs. The safety and pharmacology of aerosolised-morphine have been evaluated in cancer patients with dyspnea.

5.3.1.1 Safety

A safety trial conducted on 50 cancer patients with dyspnea revealed no major adverse reactions, such as respiratory distress or vomiting (Tanaka et al., 1999). The 20 mg dose of morphine was delivered with an ultrasonic nebuliser. Dyspnea was measured using a visual analog scale based on the subjective quantification of breathlessness by the patient. Nebulised morphine produced a significant 10% decrease in dyspnea.

5.3.1.2 Pharmacokinetics

Morphine 6 glycuronide (M6G) is an active metabolite of morphine. The pharmacokinetics of inhaled M6G were analysed on 10 healthy volunteers (Penson et al., 2002). M6G was delivered as an intravenous bolus (2 mg), subcutaneously (2 mg) or by inhalation (4 mg). The bioavailability of M6G was 102% after s.c. delivery and 6% after airways delivery compared to intravenous route. The peak of M6G in the blood appeared from 30 minutes to 2 h after s.c. delivery and from 1.2 to 8 hours after airways delivery. A previous study found that the bioavailability of nebulised morphine was 17%, suggesting that little passed into the blood (Chrubasik et al., 1988). A recent study by Krajnik et al. compared two methods of morphine nebulisation (Krajnik et al, 2009). Morphine, at a dose of 5 mg, was given to 10 breathless cancer patients with a nebuliser producing 2-5 μm or 0.5-2 μm particle droplets. The nebuliser producing the larger particles resulted in more metabolite being released into the blood, but a longer procedure was needed to deliver the 5 mg dose and the maximum plasma concentration of the metabolite occurred later. No toxicity was observed. A clinical trial comparing the efficacy of the two devices is required.
The published pharmacokinetic studies on morphine and its metabolite suggest that little passes into the blood.

5.3.1.3 Efficacy

5.3.1.3.1 Uncontrolled trials

The safety profile and poor passage into the blood led to clinical trials to analyse the efficacy of inhaled morphine. Quigley el al. treated 9 cancer patients suffering from breathlessness with M6G in a phase I/II study (Quigley et al., 2002). They were given a single dose of 5 mg, 10 mg, or 20 mg M6G. Treatment was well tolerated. Pharmacokinetic analysis showed a blood peak at 2 hours for all doses with a blood concentration proportional to the dose. The patient status based on visual analog scale of breathlessness and dyspnea (Borg score) was significantly improved, with no difference between doses. The inhalation of a derivative of morphine, hydromorphone, was also evaluated in cancer patients (Coyne et al., 2002). These 32 breathless patients inhaled hydromorphone and were assessed at baseline, 5 minutes and 60 minutes after nebulisation. The patients assessed their change as the same, worse, or improved. Most of them (26; 81%) reported improved breathing and 6 (9%) found no change. Oxygen saturation was improved from 94.6% at baseline to 96.8% at 5 minutes and 96.7% at 60 minutes.

5.3.1.3.2 Controlled trials

The above results should be interpreted with caution because there was no control group (aerosolised placebo or i.v. opioids). A few years ago we performed a trial with inhaled

morphine (20 mg) or normal saline in 10 cancer patients (Grimbert et al., 2004). The patients were given both treatments over 48 hours, separated by a wash-out period. Dyspnea, respiratory rate and oxygen saturation were assessed on a visual analog scale before and after treatment. The scores improved after both treatments, but respiratory rate and oxygen saturation did not change, indicating no specific drug response. We postulated that the improved comfort was due to a nebulisation-dependent humidification of the airways or a placebo response. Our results differ from those of Coyne et al., who described an improved perception of breathing, respiratory rate and oxygen saturation following treatment with nebulised fentanyl citrate (Coyne et al., 2002). This difference may be due to a great variation in the oxygen saturation measured and the subjective dyspnea measurement.

Bruera et al. compared the effects of nebulised morphine and subcutaneous morphine on 11 cancer patients suffering from breathlessness (Bruera et al., 2005). On day 1 they were given nebulised morphine plus a subcutaneous placebo, or nebulised placebo plus subcutaneous morphine. The treatments were reversed on day 2. Both treatments were very well tolerated. The score of the group given morphine subcutaneously decreased significantly from 5 to 3 on a visual analog scale, and that of the nebulised drug group decreased from 4 to 2. However, only 11 patients were studied and the visual analog scale fluctuated during the subcutaneous morphine treatment. A more recent study compared the effects of nebulised hydromorphone, intravenous hydromorphone, and nebulised normal saline on twenty patients (Charles et al., 2008). Patients needing treatment for breathlessness were randomly given 5 mg of nebulised hydromorphone, a systemic breakthrough dose of hydromorphone, or 3 ml of nebulised saline on three different occasions. A placebo given by another route was administrated. The primary goal of a decrease in the visual analog scale within 10 minutes was not reached. There were significant decreases over a longer period but no difference between treatments.

Thus, these studies with a placebo control group have not demonstrated any difference between nebulised opioid and placebo. However both treatments seemed to significantly improve the patients' perception of breathing, which may be attributed to a placebo effect or improved airway humidity.

5.3.2 Furosemide

Inhaled furosemide was used with some success to treat breathlessness asthma patients and healthy volunteers whose breathlessness was induced (Bianco et al., 1989, Nishino et al., 2000).

This finding led to inhaled furosemide being evaluated in cancer patients. A group of three cancer patients reported improved dyspnea when given nebulised furosemide (20 mg) (Shimoyama et al., 2002). Nebulisation of a bronchodilator before giving inhaled furosemide had no effect and the authors excluded a placebo effect. A study on 15 cancer patients suffering from breathlessness assessed the effect of ultrasonically nebulised furosemide (20 mg) (Kohara et al., 2003). Cancer dyspnea was measured with a brief self-rating scale of 12 items. Most patients (n=12; 80%) reported significant improvements in their cancer dyspnea and anxiety. Their heart rates, respiratory rates and oxygen saturation were not improved. The improved dyspnea might have been due to a placebo effect or improved airway humidity, as observed in inhaled morphine studies. A controlled trial on 7 cancer patients treated with inhaled normal saline or furosemide (20 mg) found that the scores of the visual analog scale were better after saline than after furosemide, but without statistical

significance (Stone et al., 2002). Another study that included placebo group confirmed that inhaled furosemide had little effect on the breathlessness of 15 cancer patients (Wilcock et al., 2008). They assessed the patients' perception of breathing before and 60 minutes after treatment; they found no improvement in either group. There were small decreases in FEV1 and vital capacity after nebulised normal saline but not after nebulised furosemide.

5.4 Chemoprevention of lung cancer by aerosolisation

The chemoprevention of lung cancer with aerosolised drugs was first tested in animals. Budesonide, an inhaled steroid, inhibited the formation of lung tumour by over 80% (Wattenberg et al., 1997). Squamous cell carcinoma in hamsters was reduced by 50% with inhaled difluoromethylornitine and 60% with and inhaled 5-FU (Wattenberg et al., 2004).

The results of clinical trials of the chemopreventive effects of inhaled steroid have been equivocal. Lam et al. suggested that budesonide had no effect on bronchial dysplastic lesions and did not prevent new lesions forming in 112 smokers (Lam et al., 2004). In contrast, fluticasone delivered by aerosol gave promising results, with significantly fewer treated patients with increasing numbers of nodules than in the placebo group (Van den Berg et al., 2008). A clinical trial in which 11 subjects with lung metaplasia or dysplasia were given aerosolized vitamin A found complete or partial responses in 56% of cases (Kohlhäufl et al., 2002)

Further studies are needed to determine whether inhaled steroid and vitamin A really do have chemopreventive effects.

Others aerosolised agents such as pioglitazone (an antidiabetic agent), polyphenons E (a mixture of polyphenons) and bexarotene (a retinoid X receptor (RXR) agonist) had promising results in animal studies (Fu et al. 2009, 2011, Yan et al., 2007, Zhang et al. 2011).

6. Perspectives and conclusion

Most of the preclinical studies have shown that aerosolisation preserves anti-cancer properties of a large amount of agents. Administration through the pulmonary route of anticancer drugs is well tolerated in animal models. Lung deposition is better following airways than systemic delivery. Moreover, blood passage is often lower when anti-cancer agents are administered through the airways. To date, the clinical studies of inhaled anticancer agents such as cisplatin, campthotecin, 5-fluoro-uracile, have demonstrated the safety and the pharmacokinetic advantages of airways administration in cancer patients. Moreover, antitumor responses have been observed including complete remission with inhaled interleukin-2 in renal cell carcinoma patients or with inhaled doxorubicin in lung cancer patients. However, studies were constructed with a small number of patients, the histological patterns were mostly heterogeneous with primary and secondary lung tumour mixed in the same trial and different devices or dosage were used to evaluate response to the same agent. Thus, it is difficult to bring conclusions on the real efficacy of anti-cancer agents administered through the airways, but significant effects are obvious. Despite promising results of inhaled anticancer drugs, the aerosol delivery of opioid or furosemide in palliative cancer patients was not convincing. Chemoprevention of lung cancer with inhaled agents is interesting, but further studies are needed to validate this approach.

It would be valuable to systematically integrate aerosol metrology and pharmacokinetic analysis with preclinical studies in order to improve drug deposition at the target site in the respiratory tract, and define the formulation that enables drugs to be retained in the lungs.

Standardisation of administration procedures should be considered to enhance homogeneity of clinical studies. Data on drug deposition is essential to make comparison between patients and drug pharmacokinetics and would be informative in future clinical trials. As reported, combined administration of an inhaled anti-cancer agent with drugs administered intravenously may be relevant to enhance the anticancer effect. It may also be interesting to consider the association of several anticancer agents delivered through the pulmonary route. Selting et al. simultaneously delivered in healthy dogs, aerosolised cisplatin and gemcitabine *via* a catheter in right caudal lung lobe without clinical toxicity (Selting et al., 2011). Along with conventional agent, biotherapeutics such as IL-2 and GM-CSF were assessed with promising preclinical and clinical results. Airways may also be suited for the local delivery of other biologics, such as monoclonal antibodies (Maillet et al. in press). But, researchers now need to evaluate the relative benefits of drug delivery by the airways and the systemic route before aerosolised anticancer agents can become a clinical reality in oncology.

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8. References

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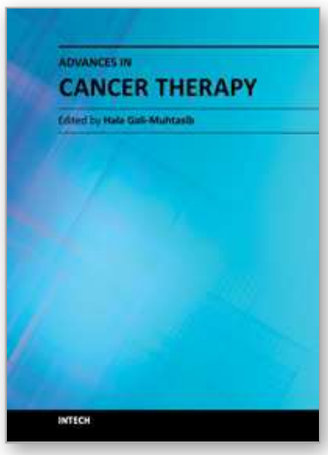

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