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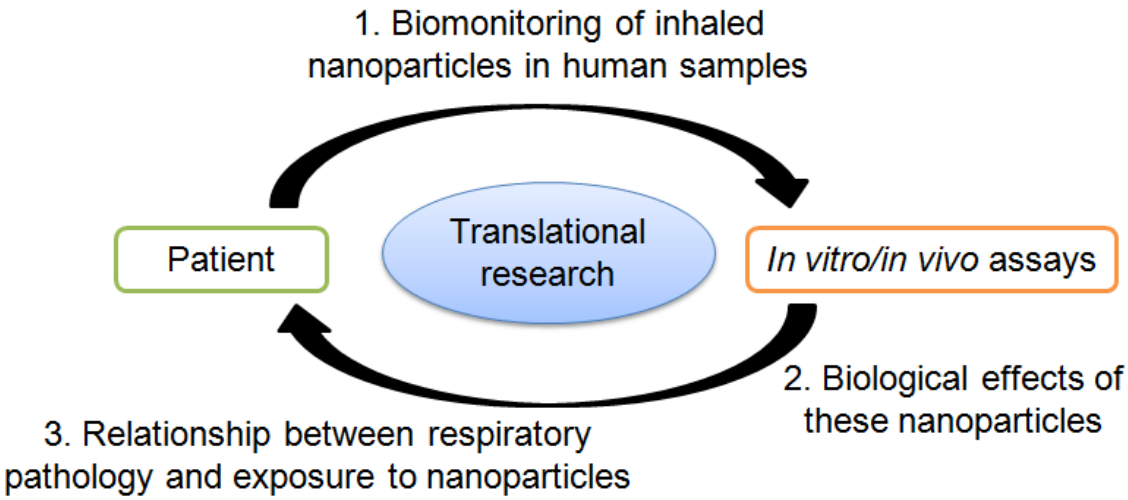
Key-words

Nanotoxicology; *in vitro/in vivo* assays; biological monitoring/biometrology; translational research; nanoparticles.

Table of Content graphic



Abstract

Although necessary, *in vitro* and *in vivo* studies are not fully successful at predicting nanomaterials toxicity. We propose to associate such assays to the biological monitoring of nanoparticles in clinical samples to get more relevant data on the chemical and physical nature and dose of nanoparticles found in humans. The concept is to establish the load of nanoparticles in biological samples of patients. Then, by comparing samples from different patient groups, nanoparticles of interest could be identified and a potential link between a given nanoparticle type and toxicity could be suggested. It has to be confirmed by investigating the biological effects induced by these nanoparticles using *in vitro* or *in vivo* models (mechanistic or dose-response studies). This translational approach from the bedside to the bench and vice-versa could allow a better understanding of the nanoparticle effects and mechanisms of toxicity that can contribute, at least in part, to a disease.

Background

Because of the tremendous development of nanotechnologies during the last decades and consequently the potential exposure of workers and general population to nanomaterials, nanotoxicology is a rapidly evolving research field. However, biological markers are still missing. If it is recognized that biomarkers and biological monitoring need to be used widely in the toolbox of nanosafety to support laboratory testing and risk assessment, it is also admitted that there are considerable hurdles in defining nanoparticle-specific biomarkers[1].

Nanomaterial toxicity is usually investigated through *in vivo* (small animal models) or *in vitro* studies. They are complementary approaches. Animal testing, although tending to be limited due to ethical issues, is necessary especially to define dose-effects and exposure limit values. Conversely, because of their lower cost and because they allow a rapid screening of effects as well as mechanistic studies, *in vitro* models are widely used. One approach commonly adopted is to expose different cell lines to different nanoparticles (some being asked to receive priority attention by international organizations such as Organization for Economic Co-operation and Development [OECD]), generally using a wide range of concentrations defined more or less arbitrarily. These latter are sometimes totally irrelevant, unrealistic in the sense that such doses could not reasonably reach target organs in the body.

Anyway, if many data are now available on the toxicity of nano-objects, many conflicting results are also reported, making it difficult to draw firm conclusions. This is partly due to a lack of consensus in the materials and methods used in the different studies rendering impossible any comparison (many parameters vary and can potentially have an impact on the biological effects triggered by the nanomaterials, such as their nature, dose, size, administration pathway, etc. but also parameters intrinsic to the system: type of cell line, type of culture medium, the varying biological identity of the nanoparticle due to the formation of a protein corona, the agglomeration/aggregation of nanoparticles, etc.)[1,2]. Finally the

complexity of extrapolating conclusions from *in vitro* or *in vivo* assays to humans should be mentioned. Indeed, if there is accumulating evidence from animal studies supporting the concern that exposure to some nanomaterials could be harmful, engineered nanomaterials have not yet been reported to cause health effects in humans[1].

Consequently, complementary strategies are needed to gain new insight in nanotoxicology. In this context, we propose an approach combining biometrology of nanoparticles in patient samples and *in vitro*/*in vivo* assays (here by *in vivo* assays we mean assays involving animal models and not including humans).

Lung biometrology of inhaled nanoparticles, why?

A way to tackle with nanotoxicology could be by monitoring nanoparticles in human biological samples. This approach that can bring useful information is not new and is inspired from an historical example as it has already been widely used in clinical practice to identify the causal link between asbestosis exposure and pulmonary diseases. Indeed, the assessment of asbestosis bodies (AB) in patient lung tissues or in broncho-alveolar lavage fluids (BALF) has allowed defining values specific of pathologies (1000 AB/g in bronchial cancer, 6000 AB/g in mesothelioma and more than 100000 AB/g in asbestosis fibrosis against 67 AB/g in healthy control)[3]. One can easily imagine what such information could bring to the nanotoxicology field where it has been established that if all existing nanomaterials were to be tested for toxicity, it would cost U.S. industries between $249 million and $1.18 billion, and could take as long as 53 years at current levels of investment[4]. In this context such data in humans could be interesting to identify the chemical nature, size and dose of nanoparticles in target tissue, allowing nanotoxicologists to focus their efforts on nanoparticles of interest. This research could be particularly relevant in the case of respiratory diseases potentially induced by biopersistent nanoparticles in the lung. Indeed, the respiratory tract represents the

main entrance pathway of nanoparticles in the body and despite the lack of clear evidence it has been suggested that inhaled nanoparticles accumulated in the lungs could be responsible, or at least could contribute, to idiopathic respiratory pathologies. As a matter of fact, after deposition nanoparticles are cleared by chemical dissolution or physical translocation. But these mechanisms are not 100% efficient and it was estimated that about 10 to 20% of insoluble nanoparticles accumulate in the lung, this biopersistence being responsible for adverse effects[5]. If the impact of engineered nanoparticles is not fully demonstrated the impact of ultrafine particles has been well described[5,6]. Through the analysis of BALF from asthmatic children, a recent study has evidenced that inhaled fine particular matters mostly consisted of carbon nanotubes. These carbon nanotubes were very similar to those found in dust and vehicle exhaust collected in Paris. They were shown to accumulate in lungs potentially contributing to the pathologic condition of the subjects[7]. In this context, the biometrology of biopersistent inhaled nanoparticles in BALF, like what was done with asbestosis bodies, could bring new insights in the understanding of some respiratory pathologies, although the size scale between asbestosis bodies and nanoparticles is different and implies the use of adapted physicochemical techniques of detection. Of course BAL is an invasive procedure that is not without associated risks therefore the idea is neither to collect BALF especially for such studies nor to carry out BAL in healthy people or in patients who do not need it. We simply suggest to use BALF that were performed for a diagnosis purpose and which remaining were stored in biobanks.

In order to establish potential relationships between an exposure to inhaled manufactured nanoparticles and biological effects (development of a pathology for instance), it is crucial to quantify the internal dose of nanoparticles in a tissue and not only an external dose measured by atmospheric metrology or surface sampling. Indeed, following an exposure to nanoparticles we can distinguish the external dose from the internal dose that is also different

from the biologically active dose able to induce biological effects. The assessment of the internal dose is a first step towards the characterization of persistent nanoparticles in tissues and the understanding of this potential source of adverse effects.

Different types of samples can be used, BALF seems to be a material of choice as it represents the gold standard for clinical diagnosis (*e.g.* for pathology caused by asbestos exposure) and it is well admitted that it is representative of the lung content. However, induced sputum rather used in a context of occupational medicine could also be interesting. Finally, although their analysis is currently at a development stage and needs further investigations, the use of exhaled breath condensates could also be considered. Tissue biopsies can also be used but due to their small size and a sampling issue they could be less representative of the lung content. In addition, solid tissues are technically more difficult to handle and adapted protocols should be developed.

Consequently, it seems crucial to develop methodologies adapted to the analysis of nanoparticles to be able to quantify precisely their load in human tissues, to investigate their nature and their potential relationship with idiopathic respiratory pathologies. Therefore, the biomonitoring of inhaled nanoparticles in human biological samples could contribute to a better understanding of pathology potentially induced by biopersistent manufactured nanoparticles in lung tissues.

Lung biometrology of inhaled nanoparticles, examples and challenges

But if this approach is appealing in theory, it appears much more complicated in practice. The challenges of detecting and adequately characterizing the nanoparticulate load of clinical samples and define their pathological significance have already been thoroughly reviewed by Bitounis et al.[8]. One major issue is that precise and true qualitative evaluation of nanoparticles in human biological samples is still hindered by various technical reasons.

Regarding the investigation of nanoparticles in biological samples two types of studies can be found in the literature. First, studies dealing with nanoparticles which nature is known (like in the case of nano-sized wear debris originating from biomedical prostheses[9–11]) and second, exploratory studies aiming at unraveling possible causative links between idiopathic pathological conditions and nanoparticles present in the affected part of the organism[12–14]. Of course it is much easier to deal with the first case and these *ex vivo* studies were the first ever to have detected manufactured nanoparticles in human samples and to have assessed their clinical importance[8]. On the other hand, the analysis of bioptic material retrieved from patients who suffer from diseases of unknown origin could shed light on the potential pathological role of nanoparticles.

Anyway, the quantitative and qualitative analysis of nanoparticles in human biological samples represents a considerable task. Methods and protocols must be adapted to cope with the complexity of biological matrices and the challenging physicochemical nature of nanoparticles. Some techniques such as single particle inductively coupled plasma mass spectrometry (spICP-MS) have been proposed. But although promising, they require further optimizations as their use is intended for particles of homogeneous composition and geometry, and therefore cannot be directly applied to biological samples containing nanoparticles of varied morphology and complex chemistry. Furthermore, cutting-edge technologies may work very well but they are very expensive, time-consuming, they need a lot of developments and are poorly available. One challenge for the biological monitoring of nanoparticles involves a methodological breakthrough. Simple and easily available techniques can be performed at low cost and high screening rate. They may be less sensitive or effective but they can bring information of clinical interest as they can analyze numerous patient samples (through multi-center trials for instance).

Consequently epidemiologic data are still lacking today. To our knowledge, only two studies have established a relationship between exposure to nanoparticles and long-term negative effects in humans. The first one[15] reported in 2009 the case of 7 women who had been suffering from shortness of breath and had amber-colored pleural and pericardial effusions prior to their admission to the hospital, indicating a physical or chemical pulmonary irritation. They all presented lung damage, nonspecific interstitial inflammation, inflammatory infiltration, pulmonary fibrosis and foreign body granulomas of the pleura; at the same time none of them had tumor markers nor virological findings relevant to their clinical condition. They had all been working for several months in the same poorly ventilated environment and had been exposed to an airborne dust of polyacrylic ester which contained known carcinogens (ethylene dioxide) as well as other dangerous substances (toluene), among others. It was also found that the airborne dust contained silica nanoparticles of a diameter ranging from 2 to 30 nm. These nanoparticles were spotted in the chest fluid, in mesothelial cells of the pleural fluid as well as in various cells and structures of the macrophages and in bioptic lung tissue (in blood and lymphatic vessels, endothelial cells). The clinical image of the patients continued to deteriorate months after their removal from their workplace suggesting that the detected nanoparticles were one of the causes behind their condition. However, the conclusion that silica nanoparticles were responsible for the pathological findings is only hypothetic as the patients had been exposed to other toxic substances and that engineered nano-objects were effectively cleared through the lymphatic system during their treatment.

The second study that has reported in 2014 the long-term toxicity of nanoparticles in humans[16] was carried out in a context of occupational exposure as it was focused on welders. Indeed, this specific population of workers particularly exposed to metallic nanoparticles contained in the welding fumes is often affected by pulmonary inflammations and lung tissue remodeling. It was therefore legitimate to wonder if a potential causal link could exist

between exposure to nanoparticles and lung pathologies. To test this hypothesis, Andujar et al. compared the lung tissue content of a group of 21 welders to that of a control group composed of 21 unexposed people. It was observed that nanoparticles and macrophages co-localized in welder lung in a much more important extent than in the control group. Moreover, pulmonary injuries were more severe in welders than in unexposed people. Finally, in welders, an iron, manganese, chromium and to a lesser extent titanium overload was observed compared to the control group. This argued for the fact that nanoparticles present in welding fumes could be responsible, at least in part, for the pulmonary inflammation observed in welders.

Thus, available data are sparse and limited to the description of few clinical cases and do not have a significant statistical power. The biometrology of nanoparticles in clinical samples is not systematically investigated and when it is, it is generally not extensive and limited to electron microscopy observations that do not allow a rapid physicochemical characterization of the nanoparticles nor the determination of the nanoparticle size distribution. Moreover, it is time-consuming and not adapted to high throughput analyses.

Lung biometrology of inhaled nanoparticles, perspectives

As mentioned before, due to the inherent limitations of simplified models *in vitro* and *in vivo* studies are not satisfactory at predicting toxicology of inhaled nanoparticles or their clinical outcomes. On the other hand, biological monitoring of nanoparticles has its own challenges: technical and conceptual. Regarding the techniques and as previously mentioned without trustworthy information to describe the nanoparticulate load of clinical samples, it is impossible to accurately assess its pathological impact on isolated cases or allow relevant epidemiological surveys on large populations[8]. About the conceptual limitations, it should be noted that nanoparticles are often concurrent with micro-sized particles or patients have been

simultaneously exposed to other potentially harmful substances, making it difficult to conclude on the real influence of the nano-objects. In a context where evidence of an unequivocal relationship between human diseases and exposure to nanoparticles are still missing, large epidemiological studies cannot be afforded as they are too personnel, financial resources and time-consuming. Instead, small, high-quality pilot studies based on well-designed and reliable techniques for nanoparticle extraction and characterization from clinical samples could be very useful to the applied nanotoxicology field. If such small-scale studies report plausible causal links between some idiopathic pathological condition and bioaccumulated nanoparticles, larger epidemiological studies should then be undertaken[8].

We believe that great benefits could be awaited by applying a strategy taking advantage of both biological monitoring and *in vitro/in vivo* studies. Figure 1 schematically illustrates the idea. First, the qualitative and quantitative load of nanoparticles in a patient sample is determined. The initial step consists of the extraction of the nanoparticles, *i.e.* the separation from the micro-sized particles and from soluble elements. Second, the samples are analyzed using for instance the dynamic light scattering (DLS) method and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) to establish the size and the chemical nature and concentration of the nanoparticles respectively. These simple but robust and reliable techniques are largely available and allow a rapid high-flow analysis of numerous samples at low cost[17]. The nanoparticle load can then be compared between samples from different patient groups. For example the nanoparticle content of BALF from patients suffering from idiopathic respiratory diseases potentially induced by nanoparticles can be compared to that of patients suffering from pathologies of defined etiology. An overexpression of nanoparticles could suggest a potential contribution to the development of a disease. But it is far from enough to conclude on the potential relationship between exposure to these nanoparticles and pathological effects. Further investigations are required and it is

where the *in vitro/in vivo* model comes in, to potentially confirm the suspected link between a nanoparticle type and toxicity. The nanoparticles of interest (*i.e.* the nanoparticles found in the patient and therefore corresponding to a realistic context) are studied using an *in vitro/in vivo* model to determine their impact on a biological system. Cells or animals can be exposed to the selected nanoparticles (either extracted from the clinical sample or synthesized chemically and exhibiting the same chemical nature and size than those identified by biological monitoring) and many parameters of interest (markers of cell death, inflammatory response, oxidative stress, etc.) can be assessed. The idea is to go from the bedside to the bench and return to the patient. This approach allows a better understanding of the nanoparticle effects and mechanisms of toxicity that can contribute, at least in part, to a disease. Indeed, if researchers from the nanotoxicology field are doing a great job raising data on various nanomaterials toxicity potential, because of the limitations of *in vitro* and *in vivo* models as discussed earlier they are carrying out lab assays that are far from real-life conditions. They would greatly benefit from communicating and working in close collaboration with clinicians who are daily confronted to patients.

Before proposing this approach, we ensured its technical feasibility. To that purpose, we established specific protocols to characterize both qualitatively and quantitatively the nanoparticle content of BALF from 100 patients, including appropriate controls to ensure reliable results (article in preparation). This was a proof-of-concept study which has to be further extended. Indeed, most diseases are multifactorial and the identification of inducers, especially a potential pathogenetic role of nanoparticles, will need a considerably high number of cases. The number of BALF that can be collected and analyzed depends on the research teams involved. Through multi-centric studies involving different French University Hospitals we could recruit a relatively large number of patients (*i.e.* several thousands).

Furthermore, it is possible to imagine the development of international collaborations to recruit even more patients.

Using this strategy, further investigations can be performed. For example, the impact of the nanoparticle dose could be studied by assessing a wide range of concentrations starting from the concentration found in the patient sample to increasing (or decreasing) doses to potentially determine a threshold of toxicity. The type of cell line used as well as the way of exposure could also vary to better analyze the biological effects induced. The advantage of such strategy is that the *in vitro/in vivo* assays are performed using nanoparticles which nature and dose are more representative of real-life and therefore more relevant whereas classical *in vitro/in vivo* assays often use unrealistic doses[1].

Thus, we believe that a combined approach including biological monitoring in humans could be useful to improve our knowledge in the nanotoxicology field. Moreover, this strategy was already adopted by Andujar et al.[16] to confirm the potential biological impact of the nanoparticles they found in welders and suspected to be responsible for lung diseases. Nanoparticles of similar chemical nature to those found in the welder lungs were chemically synthesized and exposed to a macrophage cell line. It resulted that the nanoparticles were able to trigger the production of an inflammatory secretome.

Finally, we should keep in mind that nanoparticle toxicity can be due to their accumulation at a specific site, especially in the lung in the case of inhaled nanoparticles, but it can also be due to the translocation of a small amount of them and their subsequent accumulation in distant organs. This was recently demonstrated in healthy volunteers where inhaled gold nanoparticles translocated from lungs to systemic circulation and accumulated at sites of vascular inflammation[18].

Conclusion

Standard *in vitro/in vivo* assays do not allow concluding if manufactured nanoparticles could induce or contribute to respiratory diseases in humans. To bring partial answer to this issue in the case of biopersistent nanoparticles in lung tissue, we propose a new approach combining biological monitoring of nanoparticles in patients to usual *in vitro/in vivo* assays. This concept represents a major methodological breakthrough as it implies clinicians and nanotoxicology researchers working in synergy to design more relevant nanotoxicity assays. Another technological breakthrough relies in the use of simple methods of detection and physicochemical characterization of nanoparticles such as DLS and ICP-MS which are cheap, easily available and allowing high-flow analyses, in contrast to techniques that although more efficient are expensive, time-consuming, not easily available, etc. To our opinion this strategy is quite promising as it can potentially allow getting new insights in the understanding of the genesis or evolution of pathologies due to nanoparticle exposure. Therefore this strategy, typically fitting with translational studies, mixes applied and fundamental research and benefits could be awaited in the therapeutic and/or prevention fields. Indeed, the knowledge of the toxicity potential of nanoparticles can lead to preventive measures to limit the exposure to the harmful substances.

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Abbreviation list:

AB: Asbestosis Bodies

BAL: Broncho-Alveolar Lavage

BALF: Broncho-Alveolar Lavage Fluid

DLS: Dynamic Light Scattering

ICP: Inductively Coupled Plasma spectrometry

References:

(1) Bergamaschi, E., Poland, C., Guseva Canu, I., and Prina-Mello, A. (2015) The role of biological monitoring in nano-safety. *Nano Today 10*, 274–277.

(2) Forest, V., Cottier, M., and Pourchez, J. (2015) Electrostatic interactions favor the binding of positive nanoparticles on cells: A reductive theory. *Nano Today 10*, 677–680.

(3) De Vuyst, P., Karjalainen, A., Dumortier, P., Pairon, J. C., Monsó, E., Brochard, P., Teschler, H., Tossavainen, A., and Gibbs, A. (1998) Guidelines for mineral fibre analyses in biological samples: report of the ERS Working Group. European Respiratory Society. *Eur. Respir. J. Off. J. Eur. Soc. Clin. Respir. Physiol. 11*, 1416–1426.

(4) Chatterjee, R. (2009) Calculating the costs of nanohazard testing. *Environ. Sci. Technol. 43*, 3405–3405.

(5) Rinaldo, M., Andujar, P., Lacourt, A., Martinon, L., Canal Raffin, M., Dumortier, P., Pairon, J.-C., and Brochard, P. (2015) Perspectives in Biological Monitoring of Inhaled Nanosized Particles. *Ann. Occup. Hyg. 59*, 669–680.

(6) Brunekreef, B., and Holgate, S. T. (2002) Air pollution and health. *The Lancet*. Article.

(7) Kolosnjaj-Tabi, J., Just, J., Hartman, K. B., Laoudi, Y., Boudjemaa, S., Alloyeau, D., Szwarc, H., Wilson, L. J., and Moussa, F. (2015) Anthropogenic Carbon Nanotubes Found in the Airways of Parisian Children. *EBioMedicine 2*, 1697–1704.

(8) Bitounis, D., Pourchez, J., Forest, V., Boudard, D., Cottier, M., and Klein, J.-P. (2016) Detection and analysis of nanoparticles in patients: A critical review of the status quo of clinical nanotoxicology. *Biomaterials 76*, 302–312.

(9) Revell, P. A., al-Saffar, N., and Kobayashi, A. (1997) Biological reaction to debris in relation to joint prostheses. *Proc. Inst. Mech. Eng. [H] 211*, 187–197.

(10) Tsaousi, A., Jones, E., and Case, C. P. (2010) The in vitro genotoxicity of orthopaedic ceramic (Al2O3) and metal (CoCr alloy) particles. *Mutat. Res. 697*, 1–9.

(11) Williams, S., Tipper, J. L., Ingham, E., Stone, M. H., and Fisher, J. (2003) In vitro analysis of the wear, wear debris and biological activity of surface-engineered coatings for use in metal-on-metal total hip replacements. *Proc. Inst. Mech. Eng. [H] 217*, 155–163.

(12) Gatti, A. M., and Rivasi, F. (2002) Biocompatibility of micro- and nanoparticles. Part I: in liver and kidney. *Biomaterials 23*, 2381–2387.

(13) Gatti, A. M. (2004) Biocompatibility of micro- and nano-particles in the colon. Part II. *Biomaterials 25*, 385–392.

(14) Wu, M., Gordon, R. E., Herbert, R., Padilla, M., Moline, J., Mendelson, D., Litle, V., Travis, W. D., and Gil, J. (2010) Case Report: Lung Disease in World Trade Center Responders Exposed to Dust and Smoke: Carbon Nanotubes Found in the Lungs of World Trade Center Patients and Dust Samples. *Environ. Health Perspect. 118*, 499–504.

(15) Song, Y., Li, X., and Du, X. (2009) Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. *Eur. Respir. J. Off. J. Eur. Soc. Clin. Respir. Physiol. 34*, 559–567.

(16) Andujar, P., Simon-Deckers, A., Galateau-Sallé, F., Fayard, B., Beaune, G., Clin, B., Billon-Galland, M.-A., Durupthy, O., Pairon, J.-C., Doucet, J., Boczkowski, J., and Lanone, S. (2014) Role of metal oxide nanoparticles in histopathological changes observed in the lung of welders. *Part. Fibre Toxicol. 11*, 23.

(17) ISO/TR 13014:2012. (2012) Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment. *https://www.iso.org/standard/52334.html*.

(18) Miller, M. R., Raftis, J. B., Langrish, J. P., McLean, S. G., Samutrtai, P., Connell, S. P., Wilson, S., Vesey, A. T., Fokkens, P. H. B., Boere, A. J. F., Krystek, P., Campbell, C. J., Hadoke, P. W. F., Donaldson, K., Cassee, F. R., Newby, D. E., Duffin, R., and Mills, N. L. (2017) Inhaled Nanoparticles Accumulate at Sites of Vascular Disease. *ACS Nano 11*, 4542–4552.

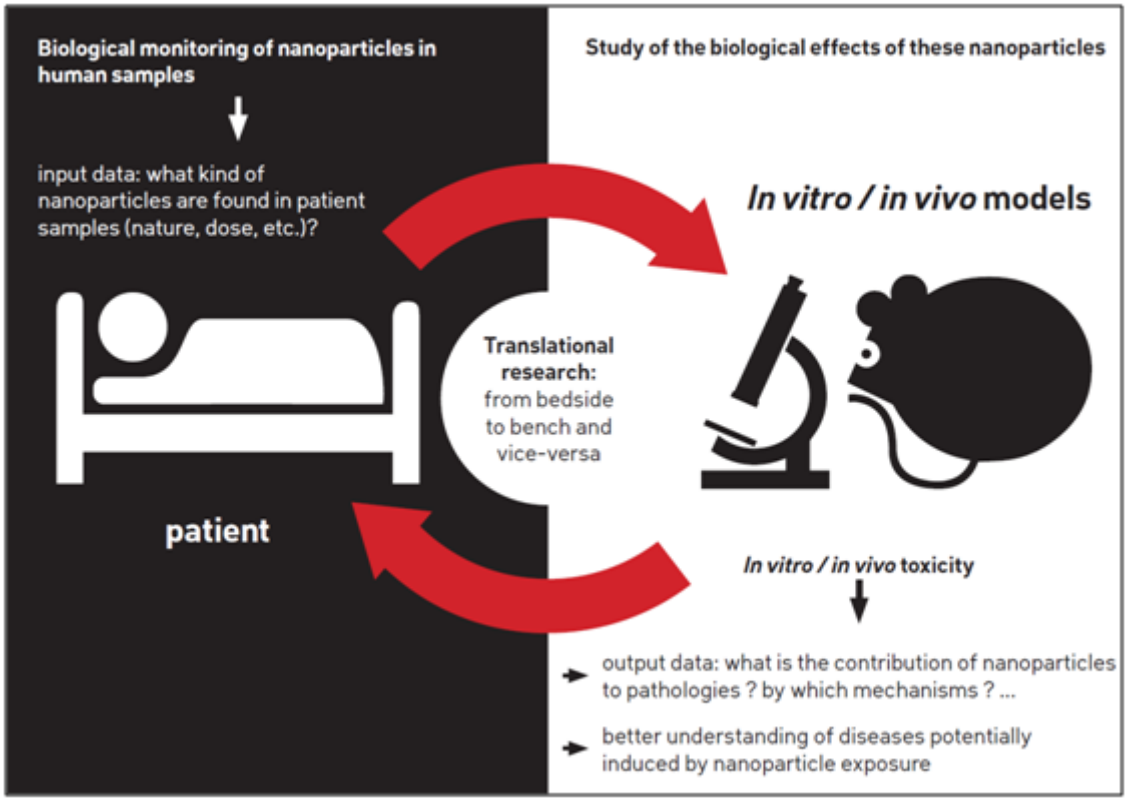
Conflict of interest:

The authors declare no competing financial interest.

Figure legends:

Figure 1 – Aims and means of the proposed approach

Figure 1


Biological monitoring of nanoparticles in human samples
input data: what kind of nanoparticles are found in patient samples (nature, dose, etc.)?
patient
Translational research: from bedside to bench and vice-versa
Study of the biological effects of these nanoparticles
In vitro / in vivo models
In vitro / in vivo toxicity
output data: what is the contribution of nanoparticles to pathologies ? by which mechanisms ? ...
better understanding of diseases potentially induced by nanoparticle exposure