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On the issue of transparency and reproducibility in nanomedicine

A full list of authors and affiliations appears at the end of the article.

Following our call to join in the discussion over the suitability of implementing a reporting checklist for bio–nano papers, the community responds.

Below we report short extracts highlighting the main messages of the correspondences we received. The interested readers can find the complete pieces in the accompanying Supplementary Information.

Kimberly S. Butler, Hon S. Leong and C. Jeffrey Brinker

With respect to Minimum Information Reporting in Bio–Nano Experimental Literature (MIRIBEL), we advocate broadening the drug definition to include alternate therapeutic cargos (for example, plasmids, proteins), request certification of reproducibility of nanosystem synthesis and stability, and recommend focus on additional studies required for regulatory agency approval for clinically relevant nanosystems, including stability in physiologically relevant media, degradation and clearance in vivo, and determination of degradation by-product toxicity.

More generally, we recognize that in spite of success in vitro, nanosystems have not realized their potential in vivo[1] where instability mechanisms, including rapid uptake by the mononuclear phagocyte system, non-specific binding and renal clearance, limit tumour-specific delivery. In vivo stability is currently evaluated in costly, time-consuming rodent models. We propose consideration/utilization of the chick chorioallantoic membrane (CAM) model[2] as a rapid, accessible and low-cost alternative approach. The CAM is highly vascularized, mimicking the diverging/converging vasculature of mammalian organs (liver/spleen) known to trap nanoparticles. In a 2013 study, CAM imaging revealed dramatically different circulation behaviours of colloidally stable cationic particles with identical size, shape and zeta potential, differing only by charge distribution/exposure[3]. Nanoparticles with patchy charge were immediately sequestered, whereas uniformly charged nanoparticles remained in circulation, an observation later verified within a rodent model via SPECT imaging[4]. Importantly, the CAM model can also be utilized for nanoparticle-tumour interaction studies[5,6].

While rodent models remain necessary for new investigational drugs, the CAM model confidently serves as an inexpensive, efficient intermediary system in which to qualify

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nanosystems for subsequent mammalian testing. This will reduce the number of mammalian animals utilized and help bridge the in vitro to in vivo void.

May Azzawi, Steve Conlan, Christine Dufés, Andrew Owen, Steve Rannard and Chris Scott

Nano(bio)medicine offers new healthcare paradigm opportunities, and many clinical products already exist. Accurate experimental reporting and analytical/characterization is critical for all science and it is important to not overstate the potential issues for 'nano' research; for example, within its guidance documents[7] the "FDA does not categorically judge … nanomaterials or … the application of nanotechnology as intrinsically benign or harmful." The need for robust assessment is clear, but the case for special attention is not obvious. Maintaining high standards is required for all disciplines, but the need for sub-field-specific checklists is unclear as best practice is already established for the disciplines contributing to nano(bio) medicine; indeed, the scientific community readily identifies poor science through peer review. In line with best practice, we recommend:

- At least two characterization techniques as no single technique can fully characterize a disperse nanoparticle sample.
- Characterization of stored samples as nanomaterials are known to change during storage.
- Inclusion of more than two nanomaterial comparators as publications often rely on limited nanomaterial diversity.
- Standard incubation techniques for tissues and cells to minimize nanoparticle interactions with plastics.
- Capturing observable safety concerns to identify nano-specific toxicities as cytotoxicity studies alone have limited value.
- Reduction in animal use for publishing purposes.

The responsibility for scientific/publication credibility lies squarely and correctly with scientists, the community in the rigour of its peer review and journal editors in their lack of acceptance of hype and claims that are not evidence-based.

Chunying Chen

The use of reporting standards, research guidelines, international standards and checklists is aimed at ensuring the accuracy, reliability, reproducibility and intercomparison of experimental data. The procedures for nanomaterial synthesis, sample preparation, and biointeraction measurements include plenty of details and variables, which determine their therapeutic efficacies. Most of this information is already provided in current high-quality publications. However, data reliability and reproducibility still represent a concern for thorough safety assessment as well as clinical translation of nanomedicine. The discussion around the opportunity of having a checklist gives us a good chance for self-inspection and for reflecting on where we are and what we have done so far.

However, achieving universal standardization practices for nanomaterials is not feasible as strict mandatory requirements may slow down basic research efficiency. For example, lack of sufficient understanding of emerging materials does not allow fast evaluation and standardization. Therefore, the following two points should be emphasized: (a) nanomaterials should be classified according to their intrinsic composition and different kinds of materials should be scrutinized differently depending on the level of understanding of their characteristics; and (b) it may be useful to divide the checklist into a compulsory reporting summary and a list of suggested self-checking points related to the maturity of the material development in the field. The mandatory checklist should be implemented for those materials characterized by widely accepted parameters. For new nanomaterials, a series of self-checking points might be more appropriate, to permit reliable developments. Density in culture and biological fluid characterization of new nanomaterials, for example, could be optional. Instead, purity and dispersion agents of these pristine nanomaterials should be better provided.

Marina A. Dobrovolskaia and Serguei V. Kozlov

Implementing MIRIBEL may standardize the way the manuscripts are written and the formats to present the data, which is an indisputable bonus. However, it will not necessarily improve data reproducibility or have other expected benefits of its implementation. Good laboratory practice (GLP) studies are more extensively documented than the average basic research study, due to the rigorous mandatory requirements to characterize the study details (for example, documentation of each reagent lot number and expiration date; sample's and reagents' stability, storage, number of freeze/thaw cycles; verification of calculations by a second person; instrument calibration; temperature control; controlled electronic records) and to provide adequate supporting infrastructure (that is, quality control and quality assurance personnel independent of the study's principal investigator). GLP requirements also include validation of each experimental procedure. Such validation relies on multiple parameters, including but not limited to controlling for inter- and intra-assay variability, robustness, ruggedness and inter-analyst variability. GLP studies take longer to design, schedule and complete, and they are unavoidably more expensive than their comparable non-GLP counterparts. Following the GLP standards ensures the results reproducibility, as long as there is no change in the source of reagent or qualification/training of staff conducting such studies. If any change needs to occur, GLP requires re-validation or cross-validation. At this point, most academic labs have neither the infrastructure nor adequate budgets to support GLP studies. Switching academic labs to GLP is an unrealistic project that may lead to decreased operational efficiencies and impede the capability of basic science investigators to fulfil their main mission: teaching and training a young generation of scientists. Therefore, it appears that improving scientists' training, reviewers' selection, editorial policies and requirements for data quality is a more reasonable — albeit more demanding and time-consuming — strategy.

Adriele Prina-Mello, Ruth Schmid, Peter Wick, Fanny Caputo, Patrick Boisseau, Rachael M. Crist and Scott E. McNeil

The European Nanomedicine Characterisation Laboratory (EUNCL) and the REFINE consortium effort, funded by EC-H2020, are aimed at developing a regulatory science framework for nanomedicine. EUNCL/REFINE, jointly with the National Cancer Institute's Nanotechnology Characterization Lab (NCI-NCL), are bridging the gap between publication and translation by identifying common pitfalls in nanomedicine development, defining quality attributes for pre-clinical assessment and sharing lessons learned[8–11].

Recognizing the value of the MIRIBEL reporting suggestions, we emphasize that each particle is unique and may have different testing requirements. It is the view of EUNCL/REFINE and NCI-NCL that the developmental path to translation should have a series of pass/fail analyses early in the process[8,9]. Built on our combined experiences, aspects such as sterility and endotoxin contamination, physicochemical characterization in complex biological media[11], and multiparametric investigations of immuno- and cytotoxicity responses can uncover potential show-stopper toxicities[12], calling for refinement of the formulation. Only then should the in vivo efficacy/safety studies commence[13]. Further, we support the adoption of orthogonal methodologies[11], which can be highly informative with regards to the overall properties and performance of the product.

Success is best achieved through a rigorous approach that is well-defined, thorough and makes use of validated assays (http://www.euncl.eu/about-us/assay-cascade) and experimental standard operative procedures (https://ncl.cancer.gov/resources/assay-cascade-protocols) with defined quality acceptance criteria. Failure to adopt these criteria often leads to confounding results, lack of reproducibility and, ultimately, lost time and money. Supporting these minimum reporting and characterization recommendations will greatly advance nanomedicine development, which has been curtailed by the lack of comprehensive characterization data in the literature.

Bengt Fadeel and Lang Tran

We agree that checklists that take into account characterization of the test material as well as the test system may serve as useful tools for authors and reviewers. After all, it is common sense that one should know the test material as well as the model system. The EU-funded project BIORIMA (biomaterial risk management), with more than 40 partner institutes (https://biorima.eu), aims to provide a risk management framework for nanobiomaterials (NBMs) — that is, engineered nanomaterials that are produced for biomedical applications such as advanced therapy medicinal products and/or medical devices. One important aim is to develop and validate test methods reflective of the eventual deployment of NBMs as part of such applications. Indeed, we would add that one should know and describe the application of the nanomaterial that is subjected to biological testing, as this information will undoubtedly inform the choice of test methods/systems. Again, this is common sense: the evaluation of nanomaterials and the study of bio–nano interactions needs to be tailored to their intended use.

Steffen Foss Hansen, Nanna B. Hartmann, Marlene Ågerstrand, Lauge P. W. Clausen, Lars M. Skjolding and Anders Baun

The MIRIBEL reporting standard is very comprehensive, and if all the components are reported, the reliability and possibilities for comparison of studies will certainly increase. There is an inevitable trade-off between having a fully comprehensive and potentially burdensome checklist for all areas of bio–nano research and one that is less ambitious and only covers specific areas. For instance, ecotoxicologists might refrain from using MIRIBEL since several components may not be applicable. Having several reporting checklists might be preferable and could facilitate continuous updates for example, for inclusion of specific demands for using realistic environmental concentrations when testing nanomaterials in ecotoxicological studies.

The number of nano-ecotoxicology publications has increased rapidly but the reliability of the reported findings has been questioned. This emphasizes the importance of a thorough evaluation of study credibility and introduction of reporting standards, such as MIRIBEL. To broaden the applicability of MIRIBEL, the NanoCRED reporting checklist (http://scirap.org) is recommended as a supplement to better encompass essential details for ecotoxicity tests with engineered nanoparticles. This would include, for instance, more specific demands for control experiments helpful to elucidate the ‘nano-effect’ (for example, negative and positive controls, solvent controls; and for metals, ionic and bulk controls). It would also include more emphasis on the analytical chemical information provided to verify exposure — for example, concentrations and transformations of the nanomaterial during tests. Finally, it would be beneficial if MIRIBEL was aligned with efforts within, for example, the Organisation for Economic Co-operation and Development and the European Chemical Agency to develop guidance on reporting parameters for effects studies of nanomaterials.

Zhen Gu

While a one-size-fits-all standard approach might not be the best way forward, a personalized checklist specific for novel discoveries or inventions might be considered. At this stage with the limited clinical outcomes in nanobiotechnology and nanomedicine, promoting high-impact innovations to meet the urgent clinical needs and address the key translation challenges has the highest priority. In the past few years, any dynamic progresses in this field have been made from investigating in-depth interactions of nano-vehicles and physiological environments to developing new drug delivery routes targeting diverse indications. Associated with these advances, many specific measurement methods have been developed, for which editors and reviewers may work together to facilitate the formation of a unique checklist. This should highlight the major merits of a certain work, upon potential discussion with authors. On the other hand, for a general checklist itself, further ‘classification’ could be taken into account. Instead of pointing out the right path with numerous details, it could be more valuable to precisely show readers which steps/reagents affect most the results and which could be substituted by alternative routes. Such a checklist with classified items could efficiently help enhance reproducibility and guide further development and optimization of techniques.

Dimitrios A. Lamprou and Clare Hoskins

Nanoparticle characterization is in need of standardization. Data on nanoparticle size in particular is a real concern in the preparation of nanotechnologies for biological application. With this in mind, we think that all papers should show data on both wet and dry samples. In fact, while microscopy images often fail to define coatings boundaries with underestimated sizes, light scattering overestimates. Reporting both datasets will further the global understanding giving clarity on biological response to shape outcomes. It also needs to be understood that the addition of even one drug/ligand/protein onto the surface of particles can result in a completely different size and zeta potential that may completely alter biological properties.

Common preclinical in vitro assays used for translation of small drug compounds into pharmaceutical products are often not fit for purpose for nanomedicine development — particularly for cytotoxicity assessment. Especially when using larger or highly cationic nanoparticles, these assays are a poor mimic of in vivo cytotoxicity, as gravity/charge may lead to their increased surface contact with the cell membrane in monolayered cultures. Additionally, a wealth of data suggests that coloured particles (particularly inorganic nanoparticles) interfere with the absorbance-, fluorescence- and luminescence-based cell viability assays. This can lead to false positives. Where cell viability is the determinant factor on whether to progress nanotherapies further into in vivo trials, reliable scrutiny and validation is required to ensure consistency and accuracy of results and to minimize the use of animals in research.

Leaf Huang and Wantong Song

Parallel to the Editorial ‘Reopening the dialogue’[14], we generally agree with the MIRIBEL principles proposed by Caruso et al. for publishing accounts of bio–nano research[15]. In recent years, research in nanotechnologies within the context of the interdisciplinary bio–nano field has grown rapidly. Establishing a study and reporting standard will enhance the quality and integrity of the published research, promote reuse and improvement of the results, and enable the comparison across various nanomaterials. But we do not think setting a standard like MIRIBEL will necessarily result in more clinical translation of bio–nano research. We see nano as a technology for solving problems in biomedical research, but we do not endorse ‘doing nano for the sake of nano’. From this aspect, we think problem-driven nanobiomedicine design is a more important prerequisite in bio–nano research for improving the success rate of clinical translation. Only a design aiming to solve a clinical problem, in combination with MIRIBEL, will possibly result in clinical translation. To be practical, extensive interdisciplinary communication is a must in future nanobiomedicine development. Involvement of clinicians in the peer review process may help to improve the quality of a bio–nano paper.

Huiliang Cao, Xuanyong Liu and Klaus D. Jandt

Setting a uniform ‘minimum information standard’ for all types of nanomaterials research is challenging because a bio–nano interface is generally defined by multiple interconnected

parameters with respective action ranges, showing time-dependent transformations and differing with respect to the intrinsic nature and specific applications of each material. Here, we suggest that the nanoscience and nanotechnology communities implement the following two steps to improve the reproducibility, comparability and reusability of the vast bio–nano data pool. First, researchers should specify the ‘indications for use’ of the nanomaterials to refine the delivery approaches, dosages, cell lines and additional microenvironments of the material’s intended applications associated with experimental assays, and lay a fundamental basis to enable comparison and reuse of the results by different groups. Second, they should classify the material properties into three categories — that is, short-range actions (which passively affect the concerned biological systems merely when they come in contact with the nanomaterial), remote actions (which actively reach possible biological systems even when distant from the nanomaterial) and coupling actions (which relate to the additive or non-additive interactions among the sub-systems in the nanomaterial). This would establish an ‘action network’ for visualizing those interacting, interplaying and transforming factors involved in the definition and description of a specific bio–nano interface, and allow customization of the associated metrics and characterization assays for improving reproducibility. Every action network report would generate one primary piece of comparable and reusable ‘information’ for the nanomaterial designed for a certain use, and collecting a large volume of such basic pieces has the potential to advance knowledge and understanding of bio–nano interactions and their diverse applications within nanobiomedicine.

Wen Jiang and Betty Y. S. Kim

Despite the large amount of literature published in the past decade, bionanomedicines have largely failed to justify investigations beyond the preclinical stages due to issues relating to reproducibility or insufficient robustness of the experimental findings. An increased focus on developing a reporting list of experimental conditions in bionanomedicine literature aims to minimize variability and improve reproducibility.

While more stringent reporting requirements may improve transparency, identifying attributable causes that undermine the inability to duplicate findings under similar experimental conditions is necessary to improve reproducibility. More often, irreproducible experimental results arise from the possibility that the original findings were discovered by chance and the precise experimental conditions were not properly defined or cannot be replicated. Many experimental findings reported in bionanomedicine literature tend to hold true only for a narrowly defined set of experimental conditions. Therefore, equally important to reporting a minimal experimental checklist is including a standard that emphasizes the ranges of the variables tested, to allow better determination of the robustness of experimental results. Similarly, one should supply a justification for omitting particular sets of experimental results and, ideally, provide the outliers. The next phase is likely to require the establishment of standardized parameters for reporting experimental outcomes. Collectively, these efforts will raise the bar with respect to the burden of proof to support the claims made in bionanomedicine research and will be integral to our effort to advance the field.

Korin E. Wheeler, Andrew J. Chetwynd and Iseult Lynch

The MIRIBEL standards are the next logical step in the ever-advancing field of nanoscience to ensure the collection of high-quality reproducible data, inform new discoveries and facilitate data-driven modelling. MIRIBEL encompasses material characterization, biological (system) characterization and details of experimental protocols. However, it overlooks one major insight from the field thus far: nanomaterial transformations in biological and environmental matrices. These transformations can include dissolution, agglomeration or alteration of nanomaterials through interaction with biomolecules. There is growing interest in characterizing the newly acquired biomolecular coating, or biocorona, formed upon exposure of nanomaterials to biofluids and natural waters, and evaluation of its impact on controlled delivery, membrane efficiency and other applications. At minimum, the biocorona alters the charge, chemistry and biochemical surface of nanomaterials, attenuating their biological activities. Given the importance and complexity of nanomaterial transformations, we propose the addition of a fourth component to MIRIBEL standards: material characterization post-biofluid exposure that includes, where relevant, the constituents of the acquired biomolecule corona, and the dynamic corona evolution upon entering new environments (for example, following uptake and biodistribution). For protein corona characterizations, which are the most widespread and advanced in the field, these additional standards form a conduit between the nanosciences, biological mass spectrometry and associated bioinformatics. The last, in particular, could benefit from incorporating established reporting guidelines — for example, MIAPE (http://psidev.info/miape) into corona characterization studies. Inclusion of characterization of nanomaterial transformations into MIRIBEL will begin to address the ‘reproducibility crisis’ by correlating biological responses to nanomaterials with the characteristics of the actually exposed entities.

Sayed Moein Moghimi

Inconsistencies in nanomaterials reporting standards have major roots in inadequate training and lack of familiarity with relevant biological and analytical methodologies and their limitations when applied to the bio–nano arena. The experienced bio–nano researcher is well aware of the heterogeneity surrounding nanomaterial production and the challenges regarding nanomaterial characterization, and hence appreciates stochastic biological performance. Since this is science of diverse complexity, standardizing methodology and reporting will be a daunting task. Additionally, inception of data repositories will fuel frustration. Equivocal standardization may slow down innovation, especially where nanoparticles act as functional tools for fundamental studies in biology. Notwithstanding, there are numerous publications from the ‘drug delivery’ community that go well beyond the proposed ‘minimum reporting standard’ and thoroughly report on nanomaterials characteristics and experimental conditions. Some of these studies have further assessed biological performance through systematic approaches and identified attributes that led to better production of viable, reproducible, affordable and clinically acceptable formulations. The pharmaceutical industry has further highlighted challenges in production, characterization and regulatory tasks surrounding the so-called nanosimilars. We must openly acknowledge and embrace the experience and wealth of knowledge present within

this community and implement them into the broader bio–nano arena. Thus, the proposed mandatory checklist and a nanomaterial repository for data organization fall short of a working conceptual framework, will be too restrictive and, at the extreme, may violate an author's right to proprietary information. Focusing on strategies that could better train interdisciplinary scientists in biological and analytical techniques, including validation approaches to methodology optimization, is a more important solution.

André Nel, Tian Xia and Paul S. Weiss

We endorse the importance of providing appropriate material characterization, biological characterization, and experimental protocol details regarding the biological behaviour, safety and therapeutic use of engineered nanomaterials at the nano–bio interface[16–20]. We are not convinced, however, that the call for standardization could simply be implemented as a list of 'minimal information' to be provided. It is important, in our opinion, to consider the wide range of nanomaterial applications in the context of the claims being made, and to reflect on the possibility that mandatory lists could create problems, if applied uncritically or rigidly for the evaluation of manuscripts making diverse claims. For example, while several of the characterization criteria in the MIRIBEL checklist refer to intrinsic or as-synthesized materials properties, the acquisition of 'extrinsic' material properties in different biological media or physiological environments receive minimal coverage (for example, a protein corona, colloidal stability, hydrodynamic diameter, charge, dissolution properties). In addition, recent advances in nanosafety or nano-environmental health and safety research show that even after considering a wide range of intrinsic and extrinsic properties, new structure–activity relationships can emerge that reach beyond traditional property lists[21–24]. For therapeutic nanoparticles, key properties such as colloidal stability, drug retention/leakage, pharmacokinetics, surface modifications (for example, PEGylation, ligands) are omitted that may critically impact drug delivery at the disease site[25]. All considered, the discussion about minimal reporting information is timely and appropriate, but the MIRIBEL checklist should consider numerous reminders and editorials that have been written on the subject, rather than being implemented as a mandatory list for all communications and biological applications of nanomaterials.

Bruno Sarmento, José das Neves, Hélder A. Santos, Luis Santos, Samir Mitragotri Steve Little, Dan Peer, Mansoor M. Amiji and Maria José Alonso

The Nanomedicine and Nanoscale Delivery Focus Group of the Controlled Release Society (NND-FG-CRS; https://www.controlledreleasesociety.org/focus-groups/nanomedicine-and-nanoscale-delivery-nnd) aggregates a community of over 200 members from academic, industrial and regulatory settings interested in fostering an integrative and progressive discussion on the development of nanomedicines. It believes that, in general, mandating the MIRIBEL reporting checklist has the potential to contribute to the establishment of comprehensive measures to allow faster and effective translation of nanomaterials into the clinics. Still, the characterization of the starting materials and their quality, ideally of good manufacturing practice (GMP) grade, and a detailed quality characterization sheet should be included. Moreover, the selection of raw materials and active pharmaceutical ingredients

(APIs) must be clearly regulated and documented, and the production methodologies of nanomedicines during the early stage of drug product development should be chosen with the straightforward industrial framework in mind, instead of the more complex lab-scale setting. Additionally, the document should anticipate future guidance and description of the most relevant critical quality attributes for scaling-up methodologies and for clinical translation.

To allow industrial production, processes for the purification of nanoformulations that do not compromise their quality specifications must be established. These, and potential methods for nanomedicine's sterilization, should also be ready for industrial implementation.

Finally, standardization of the terminology used in MIRIBEL would also be welcomed. Helpful efforts towards this objective have been undertaken in the recent 'Definitions in Biomaterials' conference held under the auspices of the International Union of Societies for Biomaterials Science and Engineering. International experts (re-)examined existing scientific terms and formulated new definitions in the broad field of biomaterials, which will be released soon as a consensus compendium in order to make the scientific concepts on a partially overlapped field of science as bio–nano interfaces[26] uniform. Additional insights on the classification of materials would reinforce the scope of these standards.

Alke Petri-Fink, Sandor Balog, Aaron Lee, Barbara Drasler and Barbara Rothen-Rutishauser

Analytical standardization as proposed by the MIRIBEL guidelines introduced in ref.[15] may be a powerful tool for improving bio–nano research quality and consistency, both of which are critical to understanding and evaluating nanoengineered materials. However, metrological protocols for the characterization of particulate nanomaterials cannot be generalized, since the optimal approach may be material- and environment-specific. This is important to consider in particular when analytical techniques are used outside of their original field of application as comprehensive assessment of their limitations and potential drawbacks are lacking. Reporting of data evaluation methods and their justification in conjunction with sharing of raw data is potentially an effective strategy for improving data quality and continued refinement of analytical approaches. Addressing reproducibility and reliability in bio–nano research requires an understanding of nanometrological uncertainty alongside a complete description of biological test systems. Mitigating the impact of biological variation is essential to ensure robust, reliable and reproducible data that necessitate an appropriate level of reporting. A greater emphasis should be placed on improving current in vitro culture practices within the reporting standard to include detailed characterization of cultures and procedures, as well as consideration of possible interferences with nanomaterials in common reagent-based assays. Many of the issues discussed stem from the interdisciplinary nature of bio–nano research, which highlights the relevance of teams and reviewers with complementary expertise. While MIRIBEL offers an initial approach to provide benchmarks for bio–nano research, overreliance on checklists can stifle creativity and set irrelevant standards without consideration of study design and objectives.

Stefan Wilhelm, Handan Acar, Roger G. Harrison, Chuanbin Mao, Priyabrata Mukherjee, Rajagopal Ramesh and Lacey R. McNally

Successful clinical translation is the key objective in nanomedicine research. To achieve this goal, researchers need to be able to bridge the gap between preclinical and clinical studies. This process may be facilitated by reporting checklists for published studies that provide standardized minimum information. Ideally, this information should be made available via curated online and open-access repositories. Such practice will allow researchers to apply in silico modelling and data mining on large experimental datasets to better understand and predict complex nanotechnology–biology interactions. An improved understanding of these interactions may guide the engineering of next-generation nanomedicines. Another aspect of how the translation of nanomedicines from preclinical to clinical stages may be facilitated is by thorough documentation, verification and justification of selected in vitro and in vivo models. The selection of biological models should be driven by their clinical faithfulness and relevance to increase reliability of preclinical results for downstream clinical translation. Important parameters to consider here include: (i) rigorous cell line authentication; (ii) mycoplasma testing; (iii) the integration of nanomedicine testing strategies that reflect clinical disease more accurately; and (iv) the incorporation of relevant testing in the appropriate organ and tissue microenvironment. Stating such information for published studies in the corresponding reporting document and implementation of standardized reporting guidelines could have lasting impact on nanomedicine research with improved reproducibility and reliability. Ultimately, standardized reporting of experimental details in bio–nano research could facilitate successful clinical translation of nanomedicines.

Sara Busatto, Paolo Bergese, Mauro Ferrari and Joy Wolfram

It is important to highlight specific considerations related to biogenic nanoparticles (BiNPs), such as lipoproteins and extracellular vesicles (EVs), as the present version of MIRIBEL is primarily focused on synthetic nanoparticles (NPs). A more explicit dialogue between communities working with synthetic NPs and BiNPs should be upheld to promote reproducibility, quantitative comparisons and meta-analyses in nanomedicine. BiNPs have promising diagnostic, therapeutic and drug delivery applications, as they can be representative of the pathophysiological status of the secreting cell, remain intact in the blood circulation and display endogenous targeting properties. BiNPs differ in many ways from synthetic NPs, necessitating specific considerations for standardized reporting, which include characterization of the biological source material (percentage of viable cells, mass of tissue or volume of the biological fluid) and description of methods used to separate BiNPs from other components in the biological source material (equipment, separation conditions, performed steps and storage conditions). Furthermore, the obtained BiNP formulation should be characterized in regard to sterility, purity, particle number and biomolecular content (for example, protein/lipid amount). Finally, intended and unintended changes in the biological properties of BiNPs as a result of separation steps, drug loading, targeting and labelling should be evaluated. Specific guidelines for certain types of BiNP already exist — for example, the position paper by the International Society for EVs (ISEV) on the minimal information for studies of EVs (MISEV2018)[27] and the EV-TRACK (Transparent Reporting

and Centralizing Knowledge) repository, an online expandable open-source knowledge base[28].

Ronnie H. Fang and Liangfang Zhang

The MIRIBEL guidelines set forth by Faria et al.[15] are practical and would not significantly raise the barrier to publication. In most cases, they would only require a few additional measurements or simply increased diligence during certain stages of the research process. Their implementation would impact the various groups of researchers that work in the field of bio–nano in different ways. For example, material scientists who develop new nanoplatforms would likely benefit most from the biological portion of MIRIBEL, which would aid in the design, execution and interpretation of their proof-of-concept and validation studies. Clinicians and other scientists, particularly those who prefer to take a 'black box' approach when applying nanotechnologies towards specific biological and medical problems, would be required to report fundamental characterization data that would help others working in nanomedicine to better understand the implications of their work in a broader context. While the MIRIBEL guidelines are largely reasonable, it should be noted there are certain dangers with excessive standardization, especially in the nanosciences where innovation is a major driver of the research. A key function of publication is the dissemination of new, interesting and thought-provoking ideas. Innovation and standardization at times sit in direct opposition to each other and therefore must be carefully balanced. Overall, we believe that the MIRIBEL guidelines will help to bridge the gap between disparate groups of researchers and encourage data reproducibility, which would be a highly welcome development.

Jie Zheng, Chuanqi Peng, Bujie Du and Mengxiao Yu

Engineered nanoparticles often have intrinsic heterogeneities in size, surface chemistry and shape; thus, one could ask whether a small variation among engineered nanoparticles could induce a significant change in their bio–nano interactions and transport in vivo. To answer this question, in the past decade, we have been using ultrasmall metal nanoparticles with well-defined size, surface chemistry and charge to interrogate the differences in their transport and interaction in the kidneys. Our findings suggest that seemingly small variations among these nanomaterials could result in significant differences in bio–nano interactions and transport in vivo. For instance, we recently observed that a seven-atom decrease in the particle size in the sub-nm regime can enhance the interactions of nanoparticles with the glomeruli and slow down their glomerular filtration. Moreover, we also found that a slight difference in the kidney injury stages can result in the distinct nanoparticle transport and interactions in vivo. Not limited to the kidneys, tumour retention and clearance of these nanoparticles are also strongly correlated with subtle differences among them. These observations pass an important message to us that those seemingly small differences/variations in both nanomaterials and biological systems might not be trivial and should not be overlooked in the reports. Therefore, in addition to MIRIBEL, we should always encourage the community to more precisely and quantitatively report nanomaterials, physiological conditions and disease stages. With the joint efforts of the community, we

truly believe that both our fundamental understandings of bio–nano interactions and the clinical translation of nanomedicines will be accelerated.

Danielle M. Charron and Gang Zheng

MIRIBEL is a reasonable, conservative approach to add a minimal level of uniformity to nanobiomedicine research reports but will not substantially improve research quality without synchronous improvements in the review process. MIRIBEL offers both flexibility and a clear framework for integrating specific guidelines and benchmarks from the wide range of disciplines that fall under the nano–bio umbrella. Where previous guidelines have failed due to their overly specific technical recommendations, MIRIBEL recognizes the breadth of the field and we believe a mandatory checklist in this format will not unduly burden researchers. MIRIBEL contains no exceptional guideline and few that are unique to nanobiomedicine. This makes for an appropriate checklist but spotlights shortcomings in the review process. As a community, we should be concerned that the basics are being omitted frequently enough that a mandatory checklist is under consideration. While we anticipate the checklist will improve data reporting, it will have no impact on research quality if the contents are not critically reviewed by referees and editors. We should be realistic also about the impact MIRIBEL will have on facilitating systematic comparisons across the literature. Unlike clinical studies, preclinical studies are fundamentally not suited to meta-analyses and aggregates of datasets are biased due to unreporting of negative data. A mandatory checklist should, therefore, be implemented with the primary purpose of improving data reporting and be evaluated on those terms. The MIRIBEL checklist is a good starting point.

Supplementary Material

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