International Journal of Universal Pharmacy and Bio Sciences 9(5): September-October 2020

INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES

IMPACT FACTOR 4.018*

ICV 6.16*

Pharmaceutical Sciences **Review Article……!!!**

NANOMEDICINES: INNOVATION IN HEALTHCARE

Shirole Prasad U.*[1] Patil Prashant B.[2], Bachhav Rishikesh S.[3]

[1,2]Department of Pharmaceutics, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.

[3]Department of Pharmacology, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik- 422213, Maharashtra, India.

KEYWORDS:

Nanotech, Nanomedicine, Liposomes, Anti-Cancer Therapy.

FOR CORRESPONDENCE:

Shirole Prasad U. *

ADDRESS:

Department of Pharmaceutics,
R. G. Sapkal College of Pharmacy, Anjaneri,
Nashik- 422213,
Maharashtra, India.

ABSTRACT

A growing number of applications and products containing or utilising nano materials have become accessible in recent years. This has raised concerns that new threats to humans or the environment could emerge from some of these materials. It is a requirement for a consistent description to discriminate against nano materials from other materials to include protections for nano materials in the legislation. The 'Recommendation on the concept of a nano material' was published by the European Commission in October 2010, primarily to provide unambiguous requirements for the identification of materials for which unique regulatory provisions may apply, but also to encourage clarity in the understanding of the term 'nano material.' This paper discusses the current status of various European Union regulatory systems with regard to nano materials and the major issues related to the regulation of nano materials are discussed. This will help to better understand the consequences of the decisions that policymakers have to make for more nano material regulation. Potential concerns that need to be solved and areas of study that science should pay tribute to are highlighted. These concerns include knowledge of circumstances where there might be nano-related risks for materials that fall beyond the concept, guidance and further development of measurement techniques, and coping with life-cycle changes.

INTRODUCTION:

The term ‘nanotechnology’ first came to wide public prominence in a 1986 book by K. Eric Drexler entitled ‘Engines of creation: the coming era of nanotechnology’ . The concept was based on an idea first propounded by Nobel laureate Richard Feynman in a presentation he gave in 1959 entitled ‘There’s Plenty of Room at the Bottom’ [15].

Nanomedicines have become a promising innovation priority for drug development opening new indications and treatment options for medicinal products. At the same time such nanomedicines authorized many years ago might have competition by follow-on versions if appropriate regulatory evaluation pathways existed.[2]. As these nanomedicines are highly complex products, and difficult to characterize, it is a challenge to have robust and consistent manufacturing processes in place, which define the product's profile and finally its quality, safety and efficacy. For nanocrystals, the main aspects to consider have been recently addressed in review from FDA authors summarizing the experiences from submissions over the last 35 years.

Since its inception several decades ago, nanotechnology has drawn increasing attention from both the academic and industrial sectors for applications not only in materials science and engineering, such as light-emitting devices and solar cells, but also in the biotechnology and medical fields including disease diagnostics, prevention, and treatment. [3] Accordingly, the level of interest in nanotechnology shown by both academic and industrial investigators has led to the increased development of novel nanotechnology platforms for medical applications, sharp increases in government funding, and venture capital investment . Nanomedicine as a newly created sub-term, refers to the application of engineered nanomaterials to the medical eld.

Nano-medicine, which involves the use of nanotechnology in drug development, overs ever more exciting promises of new diagnoses and cures. It has been defined as the monitoring, repair, construction and control of human bio- logical systems at the molecular level, using engineered nanodevices and nanostructures (Miller, 2003). Fig. 1

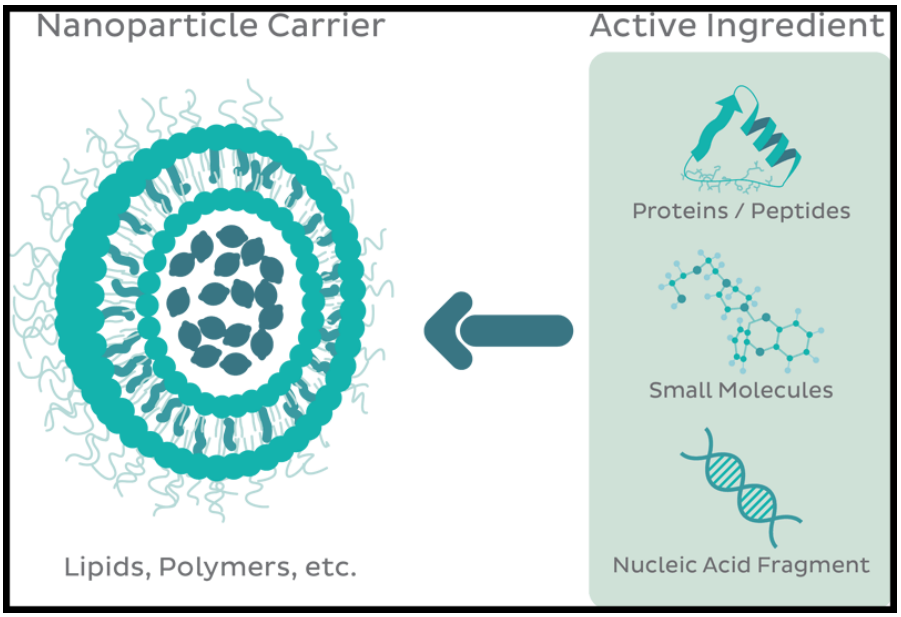

Fig.1. Ingredient of the nanoparticles

Potential Advantages :-

Nanomedicines might someday provide answers to long- standing problems in medical research, ranging from poor drug solubility to a lack of target specificity for therapeutic compounds. Nanomedicine also has tremendous promise as a noninvasive tool for diagnostic imaging, tumor detection, and dr ug deliver y because of the unique optical, magnetic, and structural properties of NPs that other tools do not possess. [3]

One of the most exciting applications of nanomedicine is the use of multifunctional NP complexes for simultaneous non-inva- sive targeting, imaging, and treatment.[5] Multifunctional NPs for cancer treatment can potentially include a variety of tumor targeting ligands as well as imaging and therapeutic agents that allow noninvasive monitoring and treatment.[5] Multifunctional NPs that include fluorescent dyes can also provide *in vivo* imaging of biologic events during drug administration as well as potential diagnostic labels for the early detection and local- ization of tumors.

Physical Features of Nanoparticles [20]

The physical characteristics of NPs can differ in many ways that influence function. A discussion of several of these physical features follows.

Size

NPs are inherently small , ranging from 1 to 100 nm with at least one dimension, although they can also be particles of a micrometre (μm) scale. NPs have new structural, optical , and electronic characteristics which are missing in several larger molecules or bulk sol- ids. They have enhanced solubility as well, so they can be used to reinvestigate equivalents of bulk drugs considered to have low solubility. The

ability to transform insoluble or poorly soluble drugs into soluble aqueous suspensions may be supported by this property, reducing the need for toxic organic solvents. Increased bioavailability and circulation time is another primary advantage due to the small size of NPs. Studies have shown that, as opposed to larger particles, particles under 200 nm have longer circulation periods, independent of any surface modifications present.

Shape

NPs, including spheres, discs, hemispheres, cylinders, cones, tubes, and wires, come in a number of types. NPs may be hollow, porous, or solid as well. On the basis of interactivity, loading capability and transport capabilities, these characteristics of NPs can be picked. For example, for drug therapies or imaging contrast agents, a hollow NP can be an attractive carrier.

Surface Area

A large surface area relative to size is one aspect of NPs that gives them unique physical properties. Total surface area grows exponentially as particle size decreases. An increase in surface area means that a higher proportion of atoms are placed compared to the core on the particle surface. In contrast with typical larger molecules, or bulk solid equivalents, this phenomenon makes NPs more reactive. Increased surface area is also responsible for the enhanced water solubility and bioavailability that often occur with NPs.

The large surface area of NPs also allows them to be designed to include a broad range of surface characteristics, including conjugation with electrostatic charges or biomolecules. Such surface features can be strategically selected for targeting and other purposes and are therefore determined on that basis.

Permeability

If NPs are properly built, their small size can allow them to cross physiological barriers in order to deliver drugs to locations that are not typically accessible by conventional means. For example, by passing through neo vessel pores that are less than 1 μm in diameter, the increased permeability of an NP can enable it to transport cancer drugs into tumours. Through the use of various uptake mechanisms, the increased permeability of NPs can also enable them to cross the blood-brain barrier.

Development of Nanopharmaceuticals

At present, after its initial discovery / development, the time scale for a medication to reach the market can take up to two decades[15]. Among other factors, ample trained science and medical workers should be able to spend a decade or two of their lives on a single project; the basic scientific idea should be novel, with adequate security of intellectual property, and an economic business strategy should reassure investors of potential profits. Business conditions should be correctly measured, and profit / risk ratios should be sufficiently high. It is also important to explain the distribution and shelf life of the

therapeutic agents [16]. Many key steps along the way, including intellectual property, technical problems, total costs and even the ethics and regulatory affairs of the matter.

From laboratory to market

In order to protect the intellectual property of inventors and businesses, it is necessary to file patents during all phases of the drug discovery and commercialization process, and also save money and time from being spent in litigation or even losing court cases. Nevertheless, taking into account the long time it takes to receive a drug's regulatory approval, pass the requisite clinical trials, and eventually bring a new product to the market, Allowing a 20-year period of patent protection (depending on the country) decreases the period of commercial exclusivity to 12 years or less. The time available to make money for the company is therefore often too limited to risk the capital required [15]. In particular, taking into account the additional risks of nanopharmaceuticals, this problem needs to be addressed by the authorities. The duration of market exclusivity should be increased in the light of ethical concerns, as well as the economic effect of clinical trials on the overall cost of the production of nanopharmaceuticals, preclinical in vivo animal testing and human clinical trials.

Fundamentals of nanotechnology based techniques in designing of drug

Nanoscale drug design has been extensively studied and is by far the most advanced technology in the field of nanoparticle applications because of its potential benefits, such as the ability to alter properties such as solubility, profiles of drug release, diffusivity, bioavailability and immunogenicity. This can lead to the enhancement and development of convenient routes of administration, reduced toxicity, reduced side effects, improved biodistribution, and extended drug lifespan.[12]. The drug delivery systems that are engineered are either aimed at a specific location or intended for the controlled release of therapeutic agents at a specific location. Their creation includes self-assembly, where they spontaneously develop from building blocks in well-defined structures or patterns. They will need to resolve obstacles such as mononuclear phagocyte system opsonization / sequestration [11].

Delivery and pharmacokinetics of nanomedicines

Changes in the pharmacokinetic characteristics of nanomedicines are due to changes in the pharmacokinetic properties of their active pharmaceutical ingredients (APIs), which can improve their effectiveness and reduce adverse reactions, including a longer stay in the body and greater dissemination to target tissues (Onoue et al . 2014).

It is possible to increase anti-cancer efficacy by increasing tumour permeability and retention time by using the enhanced permeability and retention (EPR) effect.The EPR effect also enables nanomedicines to be selectively delivered to the target tissue through conjugation to an antibody, protein, peptide, or polysaccharide that can be used to alter the delivery of nanomedicines to target

tissues using receptor / ligand interactions or other physio-logically specific target cell interactions, drug efficacy modulation, or adverse reactions.Improved stability is achieved by nanomedicines coated with hydrophilic material and their opsonization or aggregation in mucus is avoided. Nanomedicines may be maintained in vivo by inhibiting macrophage-induced or mucosal instability, e.g. in lung tis-sue for extended periods of time by particle size, regulating and preventing mucus ciliate elimination, which can lead to degradation or macroscopic effects in lung mucosa.[10].

Method

In order to evaluate nanomedicine innovation in India, a scientometric study was conducted by implementing a two-step approach involving quantitative and qualitative analysis over a 5-year period from 2010 to 2015. Figure 1 demonstrates the technique adopted. In order to classify various stakeholders, industry dynamics and patent filings, the quantitative research was performed via keyword-based primary and secondary searches. We used 'nano' and 'India' as keywords to catch all alternate phrases that are used for nanomedicine. Details on the government's funding schemes was mined from the website of the respective agency and its annual reports. Google search has been used to collect information about Venture Capital (VC) firms and angel investors that fund nanomedicine-based businesses.

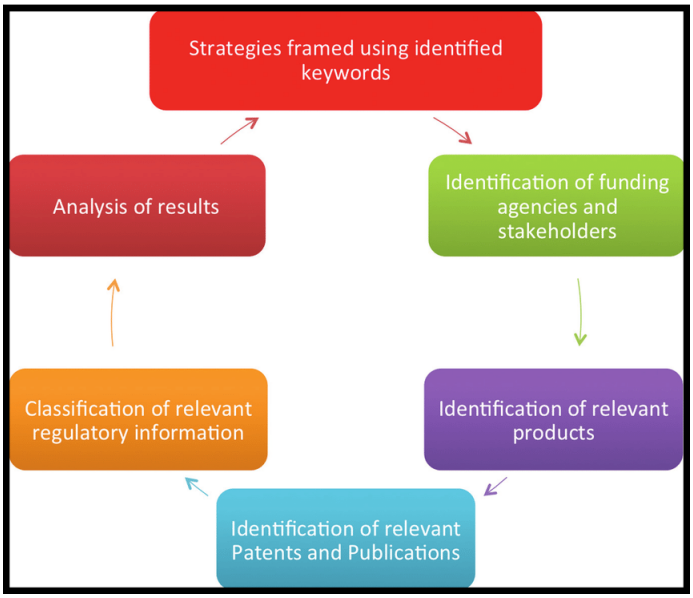

Fig. 2 Methodology adopted for mapping nanomedicine innovation landscape in India.

Factors for assessment of nano-materials [2]

Adequacy of characterization of the material structure and its function. Complexity of the material structure. Understanding of the mechanism by which the physicochemical properties of the material impact its biological effects (e.g., effect of particle size on pharmacokinetic parameters) . Understanding the in vivo release mechanism based on the material physicochemical properties..

Predictability of in vivo release based upon established in vitro release methods. Physical and chemical stability.. Maturity of the nanotechnology (including manufacturing and analytical methods).. Potential impact of manufacturing changes,including in-process controls and the robustness of the control strategy on critical quality attributes of the drug product . Physical state of the material upon administration.

Nanoparticle Behaviour in Live Animals

When injected into the blood, most nanomaterials are taken up by the phagocytic cells of the MPS within minutes or hours. The addition of poly(ethylene) glycol (PEG) to the surface of nanomaterials will prevent this rapid clearance. The addition of PEG significantly increases the blood half-life of all nanomaterials independent of surface charge by preventing opsonization. In general, by increasing the length of PEG, the blood half-life of gold nanoparticles is also increased, which allows the protective layer to thicken (3). The synthesis of these "stealth" nanoparticles, which are long-circulating, increases aggregation in the target tissue. In addition to nanoparticle surface PEGylation, the blood half-life often depends on the form, scale, and surface chemistry of a nanoparticle. Rod-shaped micelles, for instance, have a lifespan of circulation ten times longer than that of spherical micelles micelles. For intravenously administered nanoparticles, due to the variable size of interendothelial pores lining the blood vessels, diameter is an important determinant of pharmacokinetics and biodistribution. Nanoparticles smaller than 6 nm in diameter are easily removed from the body because the kidneys can excrete them (4).If a nanomaterial consists of degradable materials such as polymers, lipids or hydrogels, if the diameter is greater than 6 nm, the kidneys can not remove it. In the spleen and liver, nanoparticles with diameters greater than 200 nm grow where the MPS cells philtre them.

It is also possible to accumulate nanoparticles in tumours and use them to provide therapeutic compounds or contrast agents for imaging purposes (Figure 2). Tumors have large angiogenesis-generated fenestrations between the en-dothelial cells of blood vessels and can retain particles found in the blood. This reaction, called enhanced permeation and retention (EPR), allows the accumulation of nanoparticles within the tumour if the liver or spleen does not clear or excrete them through the kidney. A diameter of between 30 nm and 200 nm is required to generate long-circulating nanoparticles that can accumulate within tumour tissues (4). Researchers can induce the passive aggregation of nanomaterials within a tumour using this method. In fact, by adjusting the diameter of a nanoparticle, it is possible to monitor the total aggregation and depth of penetration into the tumour. Researchers determined that with decreasing size, the capacity of the nanoparticle to manoeuvre between the tumour interstitium after extravasation increased. Larger nanoparticles (100 nm), by comparison, do not extravasate well outside the blood vessel because they remain trapped between cells in the extracellular matrix. The

smallest nanoparticles (20 nm) thus penetrate deep into the tissue of the tumour but are not retained for more than 24 hours. Surprisingly, it does not appear that the addition of a targeting moiety to the surface of the nanoparticles enhances the aggregation inside the tumour or alters the bio-distribution. The successful targeting of nanoparticles alters the localisation of intratissue nanoparticles and their increased internalisation through cancer cells. Some studies, however, have shown active targeting increases tumour accumulation of 20-nm nanoparticles. Because of these contradictory findings, it is currently uncertain how nanoparticle accumulation in the target tissue is affected by active targeting.

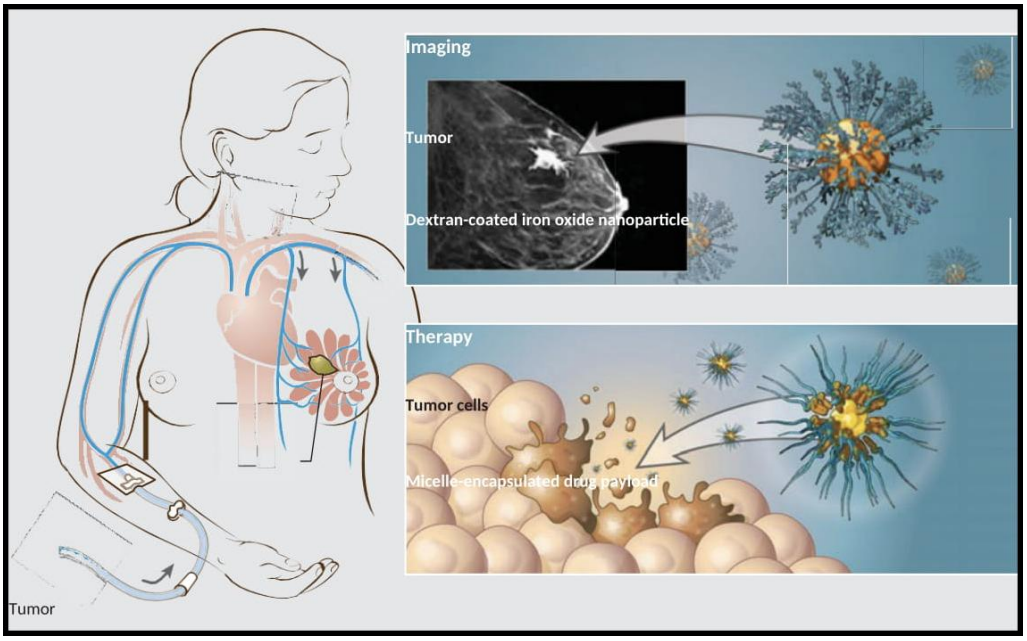

Fig. 3. Nanoparticles in tumor-specific delivery. Nanoparticles can be injected into a patient's blood and accumulate at the site of the tumor owing to enhanced permeation and retention.

Cancer Nanomedicine on the Market

1. Liposomal Anthracyclines. Several nanoparticle technologies based on liposomal anthracycline encapsulation are considered to be active cytotoxic agents for different types of cancers. Doxil (also sold in Canada and Europe as Caelyx, Janssen Biotech, Inc.), Myocet (sold by Sopherion therapeutics, LLC in North America and Cephalon, Inc. in Europe), and DaunoXome (Galen Ltd.) are three of the most well known and marketed technologies.

2. DermoDox. Although not currently licenced in any industry, as discussed above, Celsion's DermoDox is very similar to Doxil and Myocet, in that it consists of doxoru-bicin encapsulated inside the aqueous inner core of bilayer liposomes. The property that makes DermoDox special and has gained so much attention is that they release their pay-loads of doxorubicin within seconds when the liposomes are heated to temperatures of 39.5 C. It is property enables the payload of doxorubicin to be delivered

as a burst to a tumor site without the associated systemic toxicity observed with the administration of the free drug itself .

3. Abraxane. Abraxane (marketed by Celgene) is a nanoparticle-based cancer treatment that utilises albumin nanoparticles as the carrier of the active agent, unlike the liposomal technologies previously mentioned. The active agent in Abraxane is paclitaxel, a diterpene that, based on its capacity to stabilise microtubules that cause mitotic arrest, has dramatic chemotherapeutic effects. Although paclitaxel has been widely used in different types of cancer as a chemotherapeutic agent, its original formulation (also known as Taxol and containing ethanol) contained solvents[4].

4. Rexin-G. The nano-medicine platform targeting the cancer collagen matrix is a liposome platform with a high-affinity collagen-binding motif derived from von Willebrand factor (vWF) coagulation genetically engineered into liposome surface proteins[3]. A replication-incompetent, pathotropic (disease-seeking) tumour matrix (collagen-) targeted retro vector encoding an N-terminal deletion mutant of the cyclin G1 gene with potential antineoplastic activity is the model product of this platform, Rexin-G, an Epeius proprietary product.

5. Oncaspar. Asparaginase (ASNase), Catalyzes asparagine hydrolysis into aspartic acid, a naturally occurring enzyme expressed and formed by microorganisms. ASNase was established as a possible chemotherapeutic agent for acute lymphoblastic leukaemia (ALL) in 1961 when a rapid and sometimes total regression was observed in guinea pig serum-treated lymphoma-bearing mice [4]. With the successive series of clinical trials, the promise of ASNase as a childhood ALL therapeutic was combined with

6. Cancer Imaging Nanomedicine: Resovist and Orem Feridex / End-. Superparamagnetic iron oxide (SPIO) nanoparticle agents for MR imaging are among the very few fully licenced inorganic-based cancer nanomedicines. Over the past decades, SPIO nanoparticle MR imaging contrast agents have been of high interest in research and clinical applications[54]. Though there are still some in clinical trials or experimental research phases, various regulatory bodies, including the US-FDA, have licenced a few SPIO products.[4]

Table 1. FDA approved nanomedicines for anti cancer therapy.

Trade name	Compound	Nanocarrier
Abraxane	Paclitaxel	Albumin bound paclitaxel
DaunoXome	Daunorubicin	Pegylated Liposome
Doxil	Doxorubicin	Pegylated Liposome
Bexxar	anti-CD20 conjugated to idodine131	Radio immuno conjugate
Zevalin	anti CD 20 conjugated to yittrium-90	Radio immuno conjugate
Zeladex	goserelin acetate	Polymer rods
Myoset	Doxorubicin	Non-pegylated liposome
Oncasper	PEG-L-asparaginase	Polymer-protein conjugate
Ontak	IL-2 fused to diphtheria toxin	Immuno toxin fusion protein
SMANCS	Zinostatin	polymer protein conjugate

Nanomedicine in the face of market failure -

Over the years, the mainstream pharmaceutical industry has undergone several significant disasters. Probably the first to touch mainstream consciousness was Thalidomide. Thalidomide was first sold as a sedative under the brand-name Contergan in 1957 (West Germany). The tragedy happened as it was used in pregnant women to relieve morning sickness. In West Germany, shortly after the medication started to be sold, between 5000 and 7000 babies were born with limb malformations. Around 10,000 cases of children with phocomelia due to thalidomide have been recorded worldwide; about 50 percent of the 10,000 survived. The Vioxx controversy involving non-steroidal anti-inflammatory drugs (NSAIDs) was another major tragedy. NSAIDs are broken down into non-specific NSAIDs that inhibit both COX1 and COX2 (diclofenac, ibuprofen, naproxen and aspirin), while COX2-specific NSAIDs (celecoxib or Celebrex, valdecoxib or Bextra, and etoricoxib or Arcoxia) are also present. Another COX2 inhibitor named rofecoxib (Vioxx) was on the market, but Merck took it out of the market in 2004 in the midst of litigation and blamed it for causing between 88,000 and 139,000 heart attacks, 30-40% of which were fatal. The estimated cost of the settlement was in excess of $5 billion.

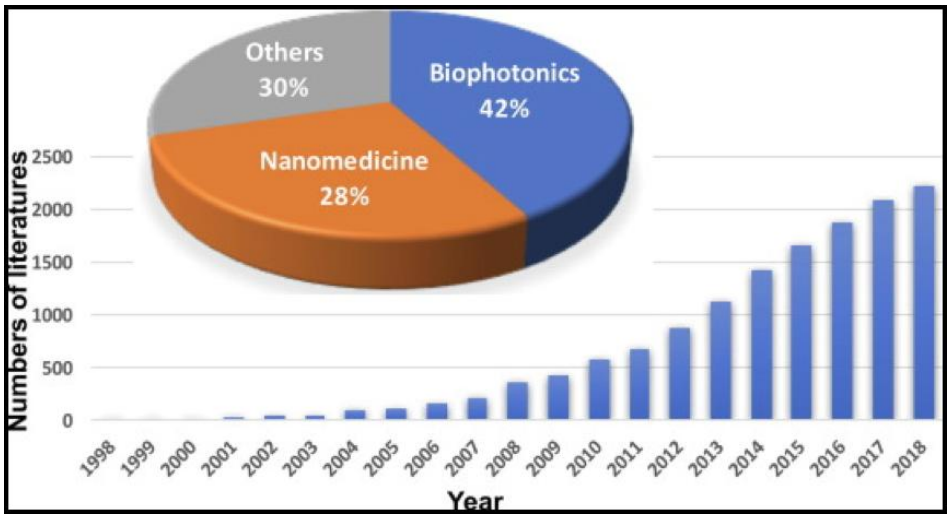

Fig.4. Upconversion and downconversion nanoparticles

Challenges on way to the market

In various areas and facets of the operation, the reasons for such an undesirable state of affairs possibly lie. NPs' in vivo behaviour, which is likely to be somewhat different from their in vitro behaviour, is one of the key challenges. The key problems that need to be extensively explored using various animal (in vivo) models are cellular interactions, tissue transportation, diffusion and biocompatibility.

The complexity and heterogeneous nature of tumours is another challenge , especially for tumor-targeted nanoformulations. In terms of gene expression profiles, molecular patterns and degree of drug resistance, variations between different tumours could impede penetration and decrease the effectiveness of tumor-targeted NPs. This difficulty could result in an unsuccessful clinical trial (despite promising animal preclinical data) and the rejection of the nanoformulations tested.

After it has been determined that clinical trials for a new nanoformulation need to be organised and conducted, numerous drug regulators will arrive on the scene with their stringent regulations. While they may provide a source of useful information and advice, particularly in the design and creation of clinical trials, their regulations generally include gaps between national and ethnic groups, and individuals from different ethnic groups are often asked for different clinical trials. Another roadblock may also be the provision of precise and detailed information on the nanocharacterization and protection of nanomedicines to the drug authorities.

Future perspectives on nanomedicines considering their pharmacokinetic properties

In view of the considerations for the production and use of nano-medicinal drugs, evaluation of the essence of formulations, pharmacokinetic properties and the approval procedure for nanomedicinal drugs are indispensable measures to achieve clinical significance. Therefore, we propose a simple algorithm to direct the recommended ADME evaluations of nanomedicines, based on recent

developments in nanomedicine growth and FDA and EMA guidelines. [10] Stability in the production process and virtual human conditions in the proposed algorithm decide whether or not the ADME properties of the drugs of interest are evaluated. Depending on administration routes and delivery, assessment varies. Evaluation, for example, varies depending on whether orally administered nanomedicines are present in nano forms or in gastrointestinal tract non-nano forms. Thus, the proposed algorithm provides critical and practical checkpoints in nanomedicine development and assessment. [21].

Conclusion

In order to ensure legal , social and regulatory acceptance and public trust, adequate risk assessment in relation to the health and safety of NPs used in medicine is necessary. It is important to fill certain awareness gaps concerning the health impact evaluation of therapeutic NPs. There is currently no consensus on the most effective toxicity assessments, exposure evaluation models and structured monitoring methods to determine potential hazards to human health from therapeutic NPs. The long-term consequences of prolonged exposure to these NPs in humans must be examined. For several of these therapeutic NPs, methods must be built for detecting NPs in situ. The biotransformation of therapeutic NPs in the human body, their interaction with biological processes, and the adsorption, distribution, metabolism, transformation, degradation and excretion (ADME) of NPs in living systems should be investigated in conjunction with the characterization of NPs in terms of distribution of scale, surface properties, biopersistance, and stability of original and modified NPs in different biological media.

References:

1. Pooja Bhatia*, Suhas Vasaikar and Anil Wali A landscape of nanomedicine innovations in India Nanotechnology Rev 2018; 7(2): 131–148.
2. Stefan Mühlebach , Regulatory challenges of nanomedicines and their follow-on versions: A generic or similar approach ? Regulatory Science Lead Non Biological Complex Drugs at Vifor Pharma Ltd, Flughofstrasse 61-63.
3. Ruibing Wang, Paul S. Billone, and Wayne M. Mullett , Nordion Inc., 447 March Road, Ottawa, ON, Canada K2K 1X8 Hindawi Publishing Corporation ,Journal of Nanomaterials, 7-8.
4. Alexandre Albanese, Peter S. Tang, and Warren C.W. Chan Institute of Biomaterials and Biomedical Engineering; and Chemistry, University of Toronto, Toronto, Canada Annu. Rev. Biomed. Eng. 2012. 14:1–16.
5. Meliha Ekinci* and Derya Ilem Ozdemir* Department of Radiopharmacy, Ege University, Turkey Current Approaches in Nanomedicine , ISSN: 2637-6660 , 183.

6. Thierry Bastogne, PhD Université de Lorraine, CRAN, UMR 7039, Vandœ uvre-lès-Nancy, France CNRS, CRAN, UMR 7039, Vandœ uvre-lès-Nancy, France, Quality-by-design of nanopharmaceuticals – a state of the art Nanomedicine: Nanotechnology, Biology, and Medicine 13 (2017) 2151–2157.
7. Mahdi Adabi, Majid Naghibzadeh, Mohsen Adabi, Mohammad Ali Zarrinfard, Seyedeh Sara Esnaashari, Alexander M. Seifalian, Reza Faridi-Majidi, Hammed Tanimowo Aiyelabegan & Hossein Ghanbari ,applications in nanomedicine, Artificial Cells, Nanomedicine, and Biotechnology, 45:4, 833-842.
8. Eric A.J. Bleeker, Wim H. de Jong, Robert E. Geertsma, Monique Groenewold, Evelyn H.W. Heugens, Marjorie Koers-Jacquemijns, Dik van de Meent, Considerations on the EU definition of a nanomaterial: Science to support policy making , Regulatory Toxicology and Pharmacology 65 (2013) 119–125.
9. Vivian S.W. Chan , Nanomedicine: An unresolved regulatory issue , Regulatory Toxicology and Pharmacology 46 (2006) 218–224**.**
10. Young Hee Choi[1] · Hyo- Kyung Han[1] , Nanomedicines: current status and future perspectives in aspect of drug delivery and pharmacokinetics , ISSN 2093-6214 , Journal of Pharmaceutical Investigation (2018) 48:43–60.
11. Falk Ehmann*[1], Kumiko Sakai-Kato[2], Ruth Duncan[1], Dolores Hernán Pérez de la Ossa[1], Ruben Pita[1], Jean-Marc Vidal[1], Ashish Kohli[3], Laszlo Tothfalusi[1], Alan Sanh[4], Next-generation nanomedicines and nanosimilars: EU regulators' initiatives relating to the development and evaluation of nanomedicines Nanomedicine (2013) 8(5), 849–856.
12. SaraSoares[1],JoãoSousa[1,2],AlbertoPais[3] andCarlaVitorino, Nanomedicine: Principles, Properties, and Regulatory Issues , August 2018, Volume 6, Article 360.
13. A. E. Nel , Implementation of alternative test strategies for the safety assessment of engineered nanomaterials, doi: 10.1111/joim.12109**.**
14. Lucienne Juillerat, Lise Marie Fjellesbo, Maria Dusinska, Andrew Richard Collins, Richard Handy, Micheal Riediker , Biological impact assessment of nanomaterial used in nanomedicine. Introduction to the NanoTEST project , doi:10.3109/17435390.2013.826743.
15. Fatemeh Farjadian , Amir Ghasemi , Omid Gohari , Amir Roointan , Mahdi Karimi* & Michael R Hamblin**,[7,8,9,] Nanopharmaceuticals and nanomedicines currently on the market: challenges and opportunities , ISSN 1743-5889.

16. Andresen TL, Berg RH (2013) Current Challenges and Future Directions in Nanomedicine. JSM Nanotechnol Nanomed 1(2): 1013.

17. Vivek Agrahari ,Challenges associated and approaches for successful translation of nanomedicines ***(Lond.)*** (2017) 12(8), 819–823.

18. Astier A, Barton Pai A, Bissig M, Crommelin DJA, Flühmann B, Hecq JD, Knoeff J, Lipp HP, Morell-Baladrón A, Mühlebach S. How to select a nanosimilar. Ann N Y Acad Sci. 2017 Nov;1407(1):50-62.

19. Ruben Pita,[1,2] Falk Ehmann,[1] and Marisa Papaluca[1] , Nanomedicines in the EU—Regulatory Overview The AAPS Journal (# 2016) 100-105.

20. Ventola CL. The nanomedicine revolution: part 1: emerging concepts. P T. 2012 Sep;37(9):512-25. PMID: 23066345; PMCID: PMC3462600.

21. Patra, J.K., Das, G., Fraceto, L.F. *et al.* Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnol* **16,** 71 (2018).