Analysis of Nanoparticle Delivery to Tumours

S. Wilhelm, A. J. Tavares, Q. Dai, S. Ohta, J. Audet, H. F. Dvorak, W. C. W. Chan.

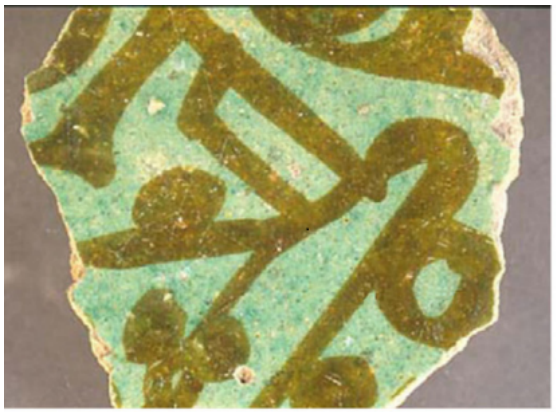
Nature Reviews Materials, 2016, 1, 1.

Presented by Alexander Chatterley

21st of May 2016

What are Nanoparticles?

- Defined as a particle that has a size between 1-100 nM.
- Have been used unknowingly throughout human history. First instance being reinforcement of clay with asbestos more than 4500 years ago.
- More recently they have been used to colour glass by the romans in the 4th century and decorate glaze ceramics in Mesopotamia in the 9th century.



Materials Today. 2013, 16, 7, 262.

What are Nanoparticles?

- One of the first major reports on nanoparticles was by Michael Faraday in "Experimental Relations of Gold (and other Metals) to Light".

- Prepared a two phase solution of $Na[AuCl_4]_{aq}$ and phosphorus in CS_2. Observed a colour change from bright yellow to ruby red – consistent with colloidal gold.

Philos. Trans. R Soc. Lond., 147 (1857), 145.

Synthesis of Nanoparticles

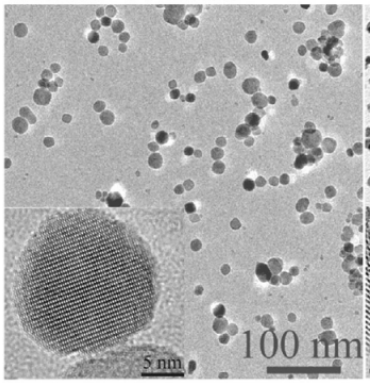
- Nanoparticles come in many forms, popular materials include:
  - Silica
  - Metal oxides
  - Quantum dots.
  - Organic polymers and dendrimers.
- Silica nanoparticles are formed by the hydrolytic condensation of tetraorthosilicate to form particles with a controlled size and pore diameters.
- Organic nanoparticles are formed by emulsion methods.

Synthesis of Nanoparticles

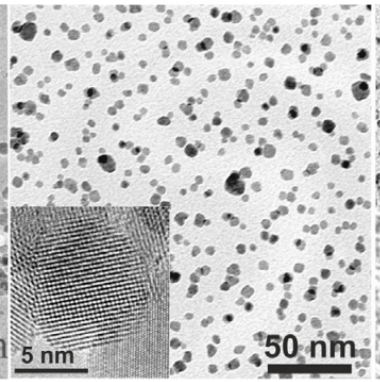
«Molecular» Precursors + Organic Solvents → (50 - 250 °C) → **Metal Oxide Nanoparticles**

Metal Halides	Alcohol (e.g. Benzyl Alcohol)
Metal Acetates	Ketones (e.g. Acetophenone)
Metal Alkoxides	Amines (e.g. Benzylamine)
Metal Acetylacetonates	...

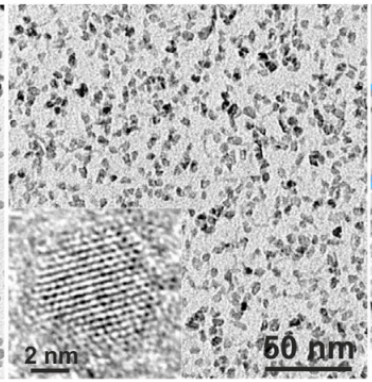
Metal Oxides



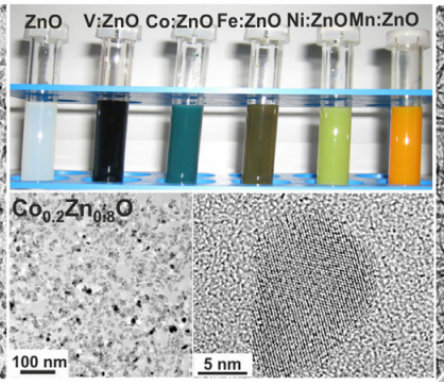
Magnetic Nanoparticles:
Fe_3O_4
MFe_2O_4 (M = Ni, Co, Mn)



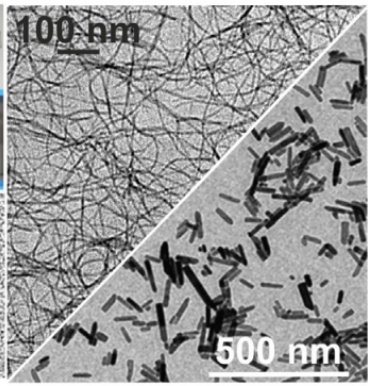
Conducting Nanoparticles:
SnO_2-doped In_2O_3 (ITO)
Sb-doped SnO_2 (ATO)
Al-doped ZnO (AZO)



Ferroelectric Nanoparticles:
$BaTiO_3$, $SrTiO_3$
$LiNbO_3$, $Pb(Zr,Ti)O_3$



Diluted Magnetic Semiconductor & Doped Metal Oxide Nanoparticles:
Doped ZnO



Different Sizes & Shapes
$W_{18}O_{49}$ Nanowires
ZnO Nanorods

Drug Delivery

- Targeted drug delivery first envision by Dr Paul Ehrlich after visiting Maria von Webers opera “Der Freischütz”.
  - Opera’s antagonist was the “Freikugeln” who always hit their target.
  - Envisioned a “Zauberkugeln” or magic bullet that would always hit its target within the body.

- Another early pioneer was Professor Peter Paul Speiser at ETH Zurich.
  - Was able to encapsulate proteins and viruses in nanospheres generated from organic molecules using polymerisation.

International Journal of Pharmaceutics, **2007**, 331, 1.

Drug delivery and Cancer

- **Problem: Majority of cytotoxic chemotherapeutics effect both healthy and malignant tissues within the body.**

- Solution: Deliver chemotherapeutics directly to malignant tissues using a smart delivery system (magic bullet).

- How? Attach chemotherapeutics to nanoparticles that can release payload under certain conditions.
  - pH
  - Enzymatic catalysis
  - Irradiation

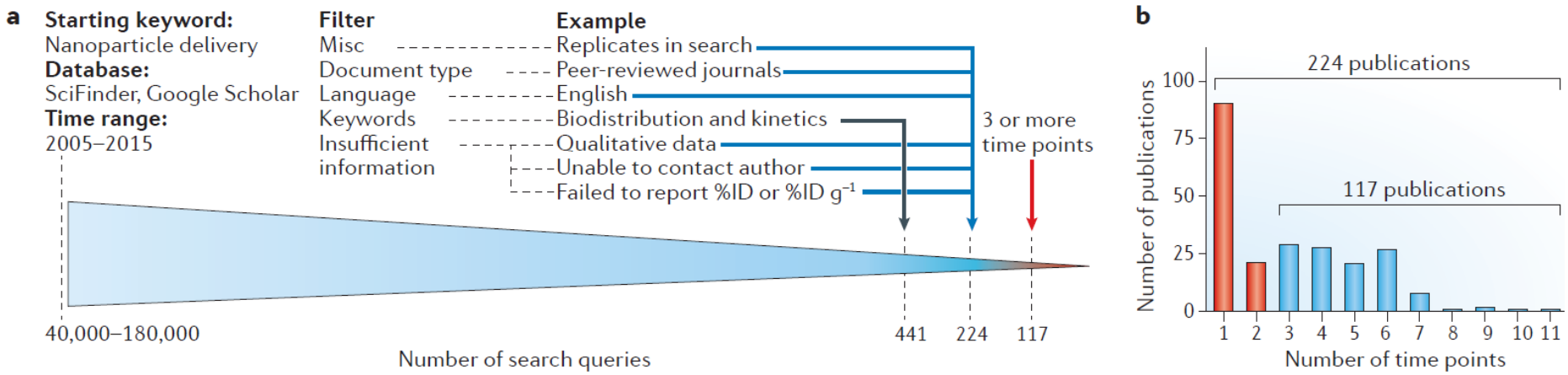
Pharmacol Rep. **2012** 64(5) 1020.

This publication.

- This paper examines the literature concerning nanoparticle delivery from the past 10 years.
  - It discusses the advances in targeted delivery (or lack thereof).
  - Discusses ways of enhancing target selectivity.
  - Proposes a thirty year plan to enhance research.

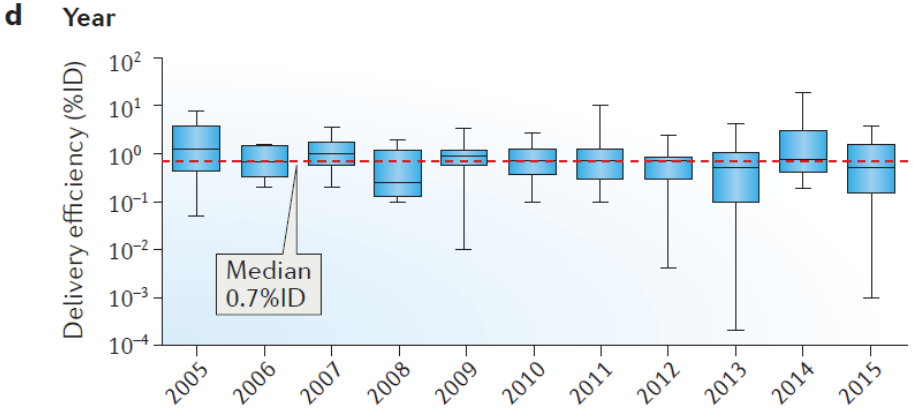
Methodology

- Authors examined the literature using the following criteria.

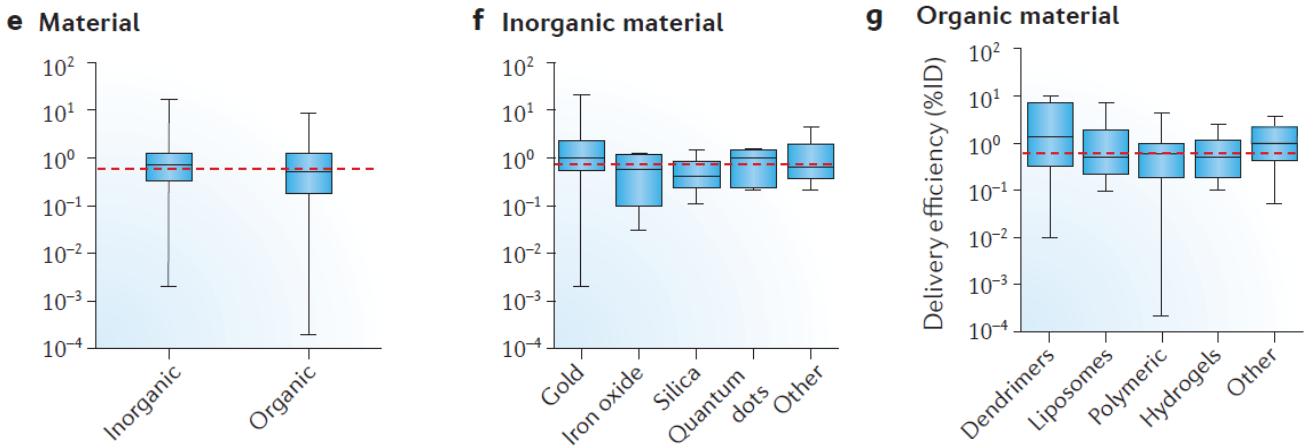


- Arrived a 117 publications suitable for examination in this article.
  - Found that, on average, 0.7% of injected dose (ID) reached the tumour.

Data trends

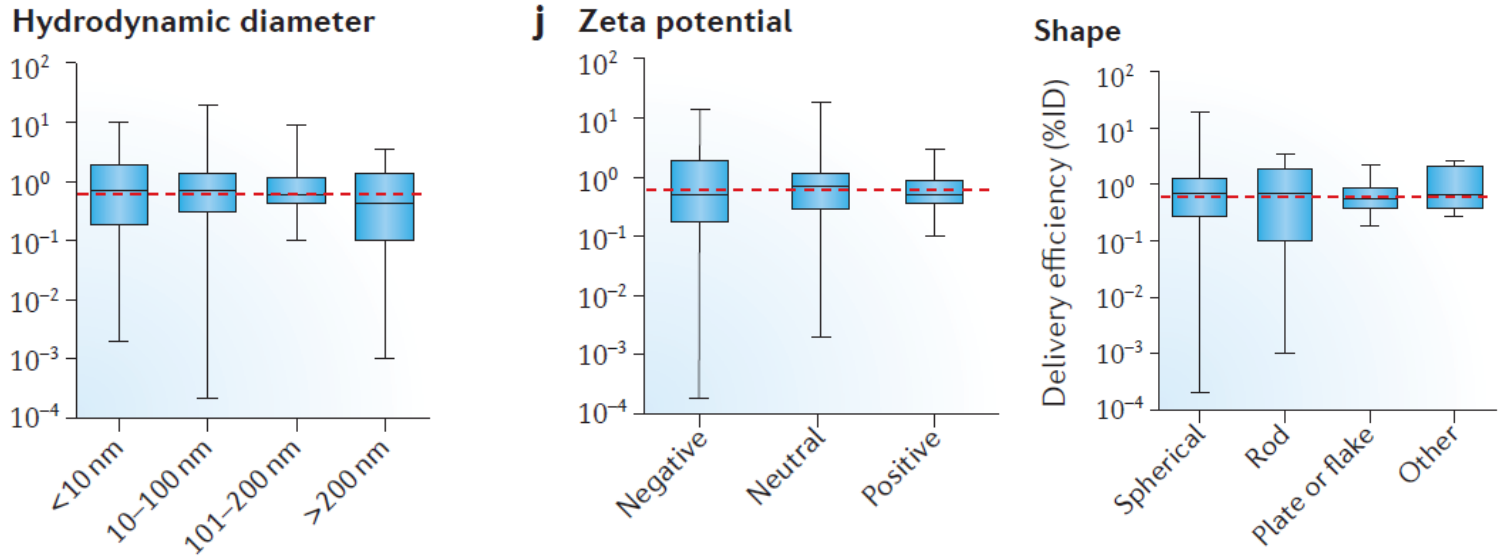


- This average has not significantly changed in the past ten years.



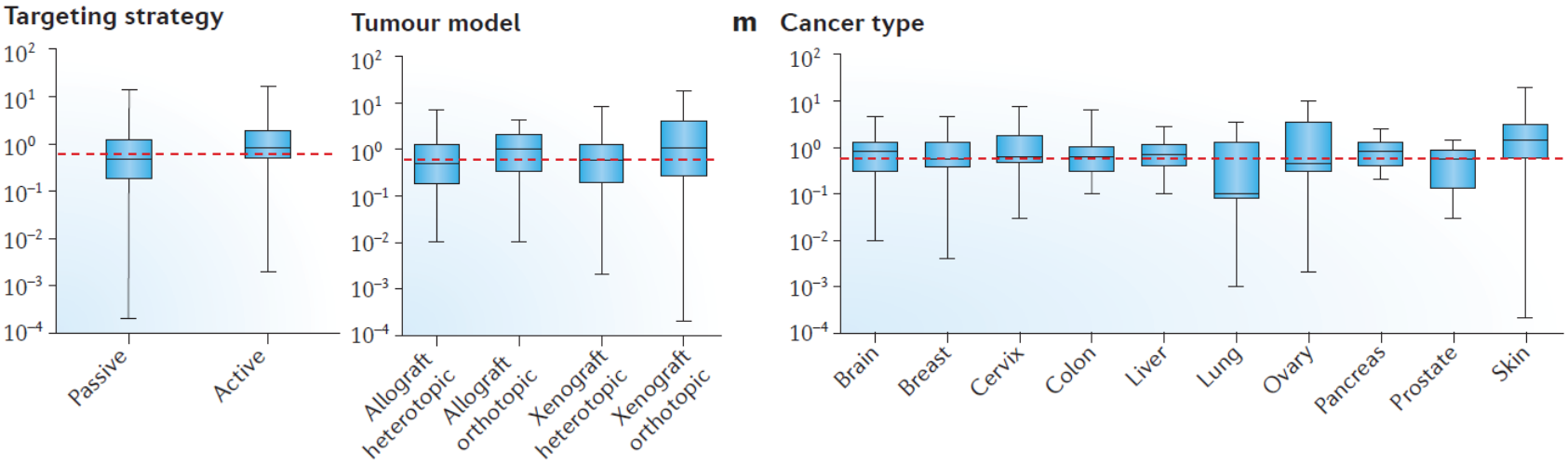
- Inorganic materials provide a higher delivery efficiency than organic (0.8% vs 0.6% ID).

Data trends



- Particles between 10-100 nM performed better than larger particles (0.7% vs 0.6% ID).
- Neutral particles tended to have a better efficiency than negative or positive ones (0.7%, 0.6% and 0.5% ID respectively).
- Rod shaped particles performed the best when compared to spheres, flakes and other shapes. (1.1%, 0.7%, 0.6% and 0.9% ID respectively).

Data trends



- Active targeting methods performed outperformed passive targeting methods (0.9% vs 0.6% ID).
- Orthotopic allo- and xenografts performed better than other methods.
- Higher levels of efficiency shown against cervical, ovarian, pancreatic and skin cancers.

Consequences of low delivery efficiency

- Evaluation for human dose for both an drug encapsulated in a nanoparticle and loaded onto the surface.

- Assumptions:
  - 60 nM diameter.
  - Drug has a MW of 500 g/mol^{-1}
  - IC_{50} 1µM
  - 1% delivery efficiency
  - Tumor volume: 0.5 cm^3 of a 20g mouse.

- Encapsulation: 20% wt of drug encapsulated
  - 1.2×10^{12} nanoparticles or 6.5 mg kg^{-1}

- Surface loading: 1 drug/nm^2
  - 2.8×10^{12} or 15.7 mg kg^{-1}

Consequences of low delivery efficiency cont.

Applying this to an average human using a body surface-area based dosing strategy:

- Encapsulation: 20% wt of drug encapsulated
  - Injection volume of 90 mL
- Surface loading: 1 drug/nm^2
  - Injection volume of 213 mL
  - Assuming nanoparticle concentration of 5 nM.
- This causes serious problems
  - Problems synthesising that amount of nanoparticles.
  - Prohibitive cost.
  - Technical difficulties due to injection volume – higher concentrations can impact particle stability.
  - Large quantity of nanoparticles may result in toxicity.
  - Possible that higher volumes than calculated will be required as nanoparticles may interact with other components in tumour matrix.

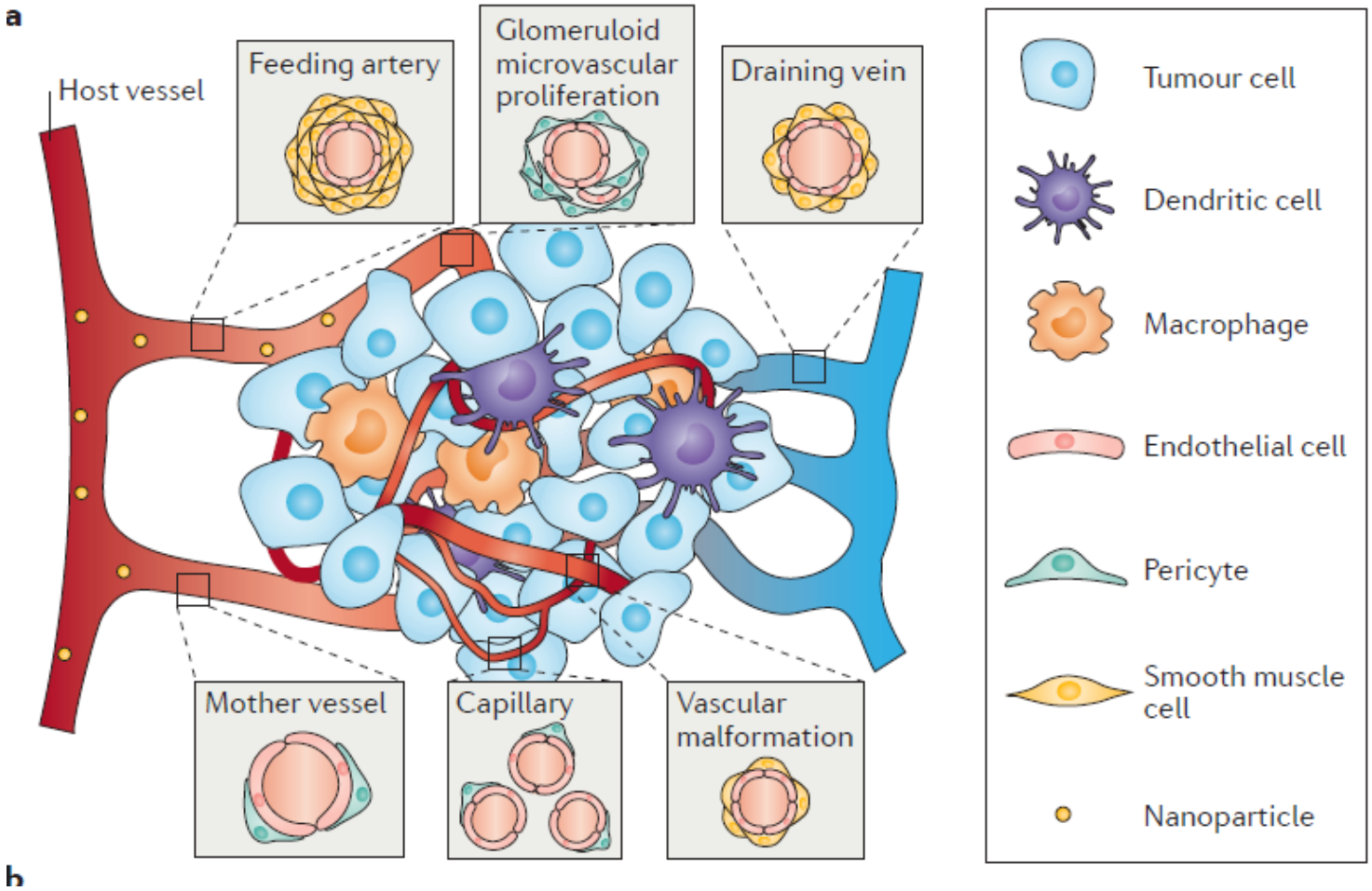
The Solution!

The authors propose a solution to all of these concerns – raising the average ID efficiency from 1% to 10%.

How to do this?

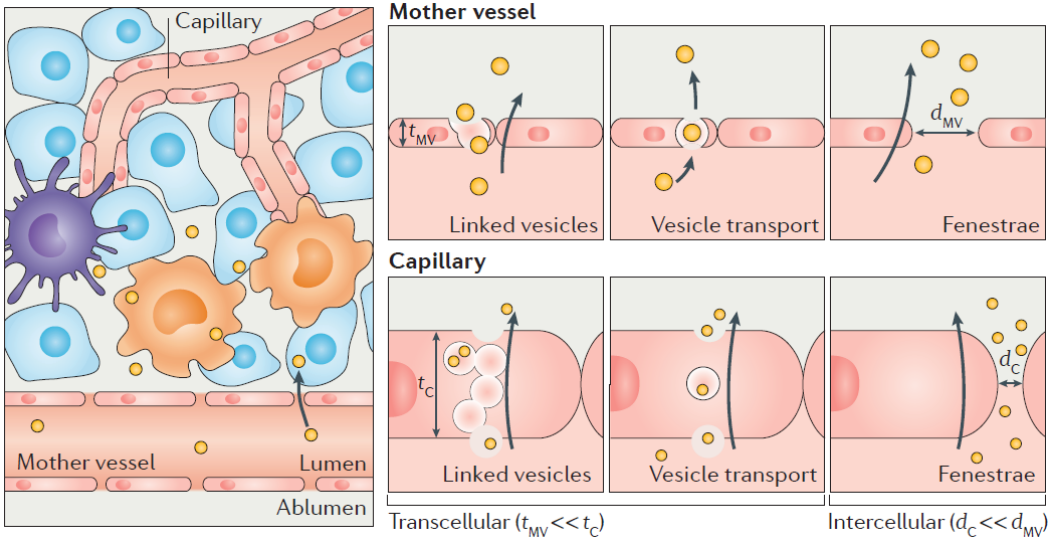
- A greater focus on targeted delivery by elucidating tumour targeting mechanisms.
- Increase mechanisms to evade nanoparticle clearance.
- Implement a 30 year development plan.

Nanoparticle Extravasation



Nanoparticle Extravasation

- Nanoparticles are most likely to enter a tumour through the mother vessels via either intercellular extravasation or transcellular extravasation.
  - Mother vessels leak both plasma and proteins into the tumour through intercellular gaps.
  - This results in a very low blood flow, allowing nanoparticles to cross over due to prolong residence time by seeping through the gaps.



- Other transport mechanisms exist such as active transport through cells. If one could target the transport transcellular mechanisms, it would allow targeted delivery to the tumour. This represents an attractive target for increasing the delivery efficiency.

Intratumoral Targeting

Once particles have crossed into the tumour matrix they then need to cross into tumour cells.

- **Problem: Tumour matrix is highly dependent on the type of tumour.**
  - Solid tumours have a rigid matrix supported by collagen, fibronectin, fibirin, etc.
  - Tumours can have a internal pressure 10-40 times greater than normal cells due to poor lymphatic drainage. This can greatly effect the transport of chemotherapeutics within the matrix.
  - General consensus is that smaller particles penetrate deeper than larger ones.
- **Solution: A complete and through study of different tumour types and matrices.**
  - Currently only 2D images are used, full 3D imaging and elucidation of nanoparticle fate is required for further development.

Nanoparticle Clearance

Nanoparticles are primarily cleared by the mononuclear phagocytic system (MPS) and kidneys.

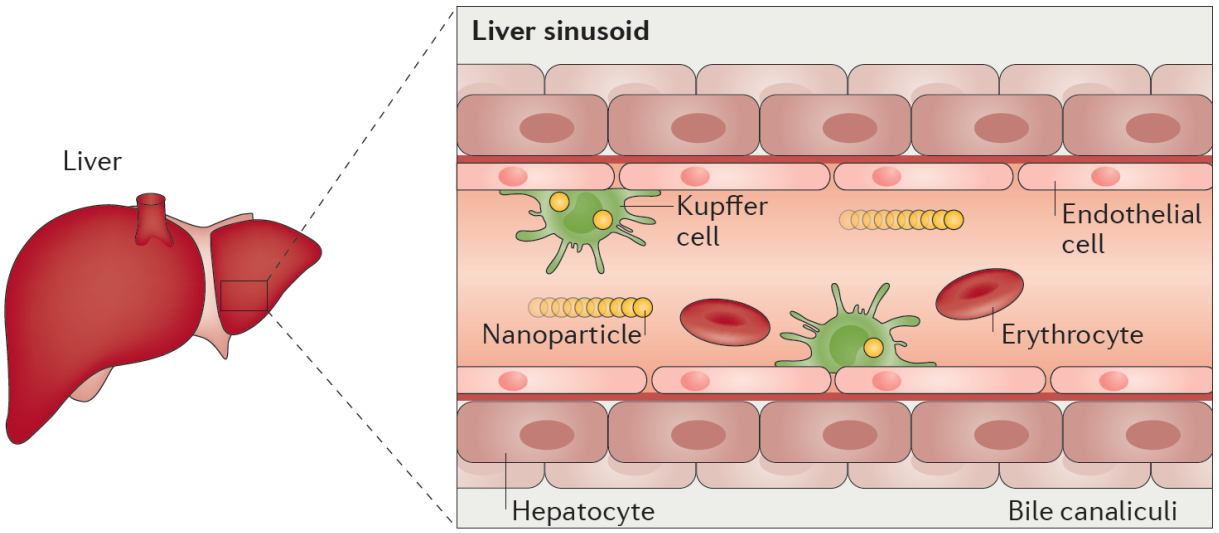
- The MPS system comprises of the following organs:
  - **Liver**
  - **Spleen**
  - Lymphatic system
  - Skin
  - Bone marrow

Further improvements to delivery efficiency can be made by reducing the clearance ability of these two systems.

MPS System

Macrophagic cells in the liver and spleen engulf nanoparticles (primarily by phagocytosis) removing them from system circulation (similar to first pass metabolism of drugs).

- Large inorganic nanoparticles can reside in macrophages for extended periods of time (possible tox issue?)
- Smaller and organic nanoparticles are rapidly broken down.

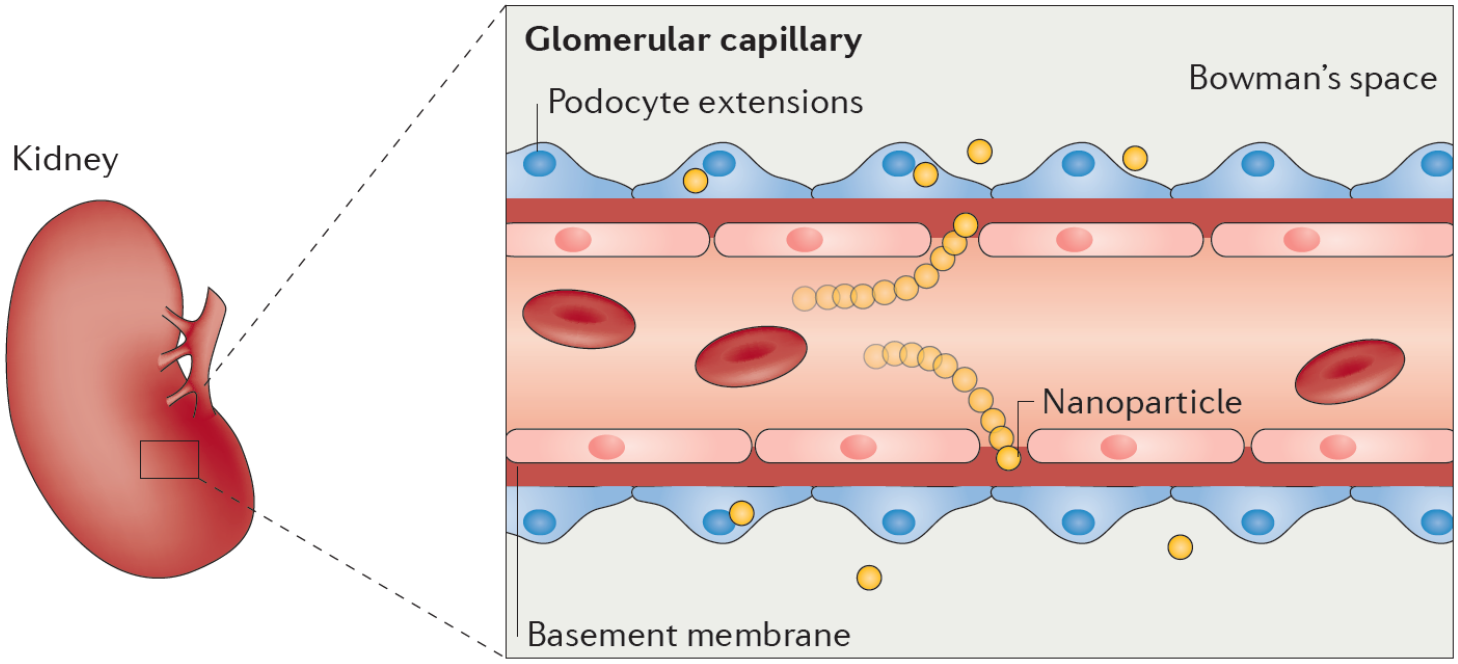


- Larger nanoparticles are sequestered more rapidly.
- Cationic particles are sequestered the fastest follow by anionic particles.
- Smaller nanoparticles circulate through the body more than larger particles.
- **Possible to overcome these problems using PEG coatings to hide the particles.**

Renal clearance

Nanoparticles are also filtered by the renal system.

- Particles smaller than 4-6 nM are filtered out of the blood and are eventually passed in the urine.



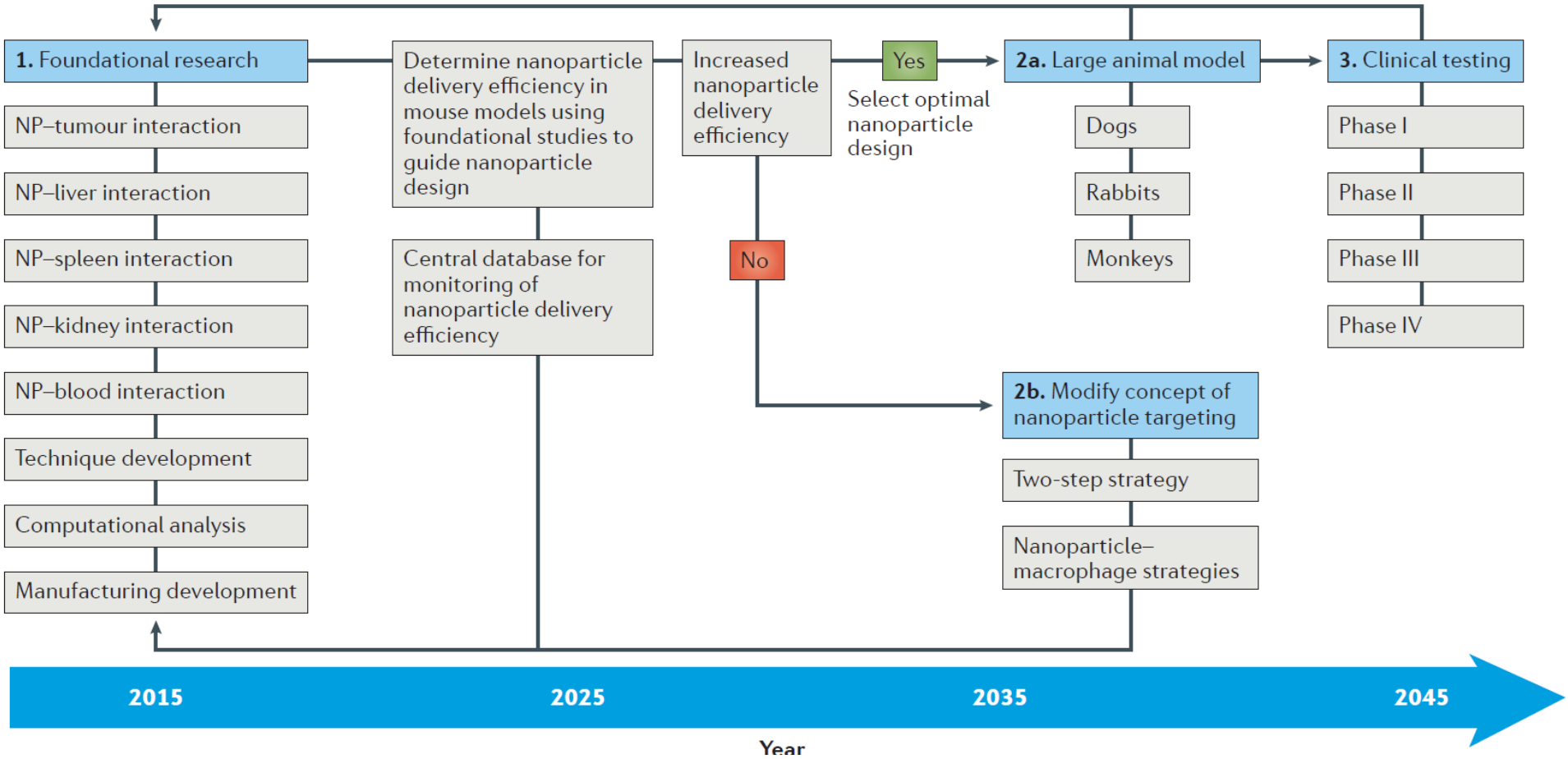
Thirty year plan

Despite more than a decade of research and $1 billion, there has been very little progress in this field. Many regard a 1% delivery efficiency to be a nonspecific interaction rather than specific targeting.

- Only a few nanoparticle formulations have been approved – Abraxane and Doxil for example.

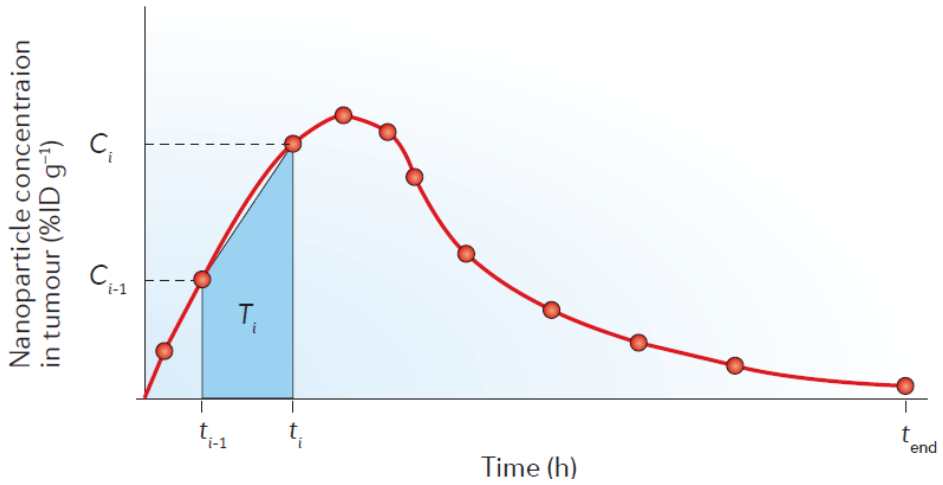
Authors propose a thirty year plan to further develop nanotechnology into a useful force for the treatment of cancer and other disease states.

Conclusion


1. Foundational research
NP–tumour interaction
NP–liver interaction
NP–spleen interaction
NP–kidney interaction
NP–blood interaction
Technique development
Computational analysis
Manufacturing development
Determine nanoparticle delivery efficiency in mouse models using foundational studies to guide nanoparticle design
Central database for monitoring of nanoparticle delivery efficiency
Increased nanoparticle delivery efficiency
Yes
Select optimal nanoparticle design
No
2a. Large animal model
Dogs
Rabbits
Monkeys
3. Clinical testing
Phase I
Phase II
Phase III
Phase IV
2b. Modify concept of nanoparticle targeting
Two-step strategy
Nanoparticle–macrophage strategies
2015
2025
2035
2045
Year

Questions?

c



$$\text{Trapezoid } (T_i) = 0.5\,(C_i + C_{i-1})\,(t_i - t_{i-1}) \quad (1)$$

$$\text{AUC}_{\text{Tumour}} = \sum_{i=1}^{n} T_i \quad (2)$$

$$\text{Delivery efficiency} = \frac{\text{AUC}_{\text{Tumour}}}{t_{\text{end}}}\,(m_{\text{Tumour}}) \quad (3)$$

Datasets

Table 1 | **Delivery efficiency and the number of data sets used from Figure 1d–m**

Category	Delivery efficiency [%ID]*	Number of data sets
All data sets	0.7	232
Year		
2005	1.4	8
2006	0.7	8
2007	1.0	24
2008	0.3	8
2009	0.9	11
2010	0.8	14
2011	0.7	27
2012	0.7	14
2013	0.5	35
2014	0.8	38
2015	0.5	45
Material		
Inorganic	0.8	86
Organic	0.6	137
Inorganic material		
Gold	1.0	45
Iron oxide	0.6	8
Silica	0.4	13
Quantum dots	0.9	5
Other	0.6	14
Organic material		
Dendrimers	1.4	7
Liposomes	0.5	27
Polymeric	0.6	62
Hydrogels	0.5	18
Other	0.9	23
Targeting strategy		
Passive	0.6	175
Active	0.9	57

Table 1 (cont.) | **Delivery efficiency and the number of data sets used from Figure 1d–m**

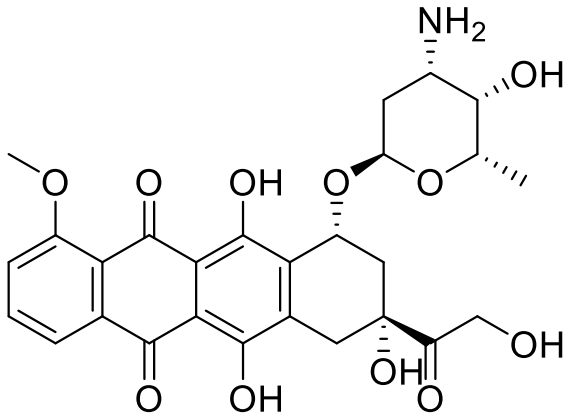
Category	Delivery efficiency [%ID]*	Number of data sets
Hydrodynamic diameter		
<10 nm	0.7	14
10–100 nm	0.7	115
100–200 nm	0.6	54
>200 nm	0.4	34
Zeta potential		
Negative	0.5	65
Neutral	0.7	118
Positive	0.6	14
Shape		
Spherical	0.7	188
Rod	0.8	23
Plate or flake	0.6	12
Other	0.7	9
Tumour model		
Allograft heterotopic	0.7	90
Allograft orthotopic	1.0	13
Xenograft heterotopic	0.6	90
Xenograft orthotopic	1.1	38
Cancer type		
Brain	0.8	28
Breast	0.6	63
Cervix	0.6	20
Colon	0.6	24
Liver	0.7	15
Lung	0.1	10
Ovary	0.5	8
Pancreas	0.8	10
Prostate	0.6	8
Skin	1.3	35

Zeta potentials were reported at pH 7.4. Negative, neutral and positive zeta potentials are defined as <−10 mV, −10 to 10 mV and >10 mV, respectively. *Median.

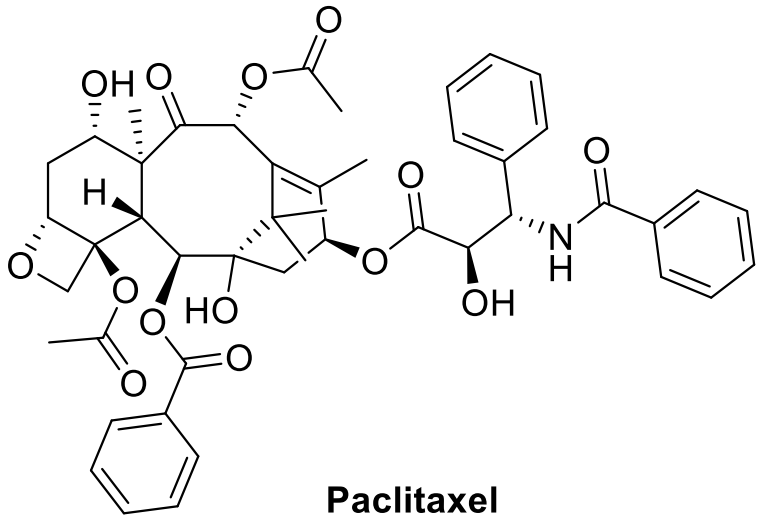
Table 2 | *P* values for effects on delivery efficiency*

Effect parameter	*P* value
All materials	
Cancer type	<0.0001
Targeting strategy	0.0082
Material	0.0210
Hydrodynamic diameter	0.0633
Shape	0.0992
Tumour model	0.2748
Zeta potential	0.3782
Material and tumour model	<0.0001
Cancer type and hydrodynamic diameter	<0.0001
Material and targeting strategy	0.0178
Hydrodynamic diameter and hydrodynamic diameter	0.0478
Organic Material	
Cancer type	<0.0001
Tumour model	0.0001
Organic material	0.0088
Hydrodynamic diameter	0.0185
Shape	0.0479
Zeta potential	0.1493
Targeting strategy	0.7350
Cancer type and hydrodynamic diameter	<0.0001
Tumour model and hydrodynamic diameter	<0.0001
Zeta potential and zeta potential	0.0068
Hydrodynamic diameter and hydrodynamic diameter	0.0078
Inorganic material	
Inorganic material	<0.0001
Targeting strategy	0.0040
Hydrodynamic diameter	0.0086
Cancer type	0.0180
Zeta potential	0.1491
Shape	0.9013

*P values for main effects, quadratic effects and two-factor interaction effects on delivery efficiency were obtained using analysis of variance (ANOVA) in combination with a multiple regression model for 'all materials', 'organic materials' and 'inorganic materials'. A Box-Cox transformation was performed on the delivery efficiency and the parameter 'hydrodynamic diameter' was log-transformed. Multiple factor interactions could not be solved for 'inorganic materials' owing to limitations of the data sets. A detailed description and interpretation of the multivariate analysis is described in the Supplementary information S3–S5 (multivariate analysis).



Doxorubicin



Paclitaxel