Avoiding Collateral Damage from Magic Bullets

Enhancing Delivery to Target Requires Stealth, not Affinity

Ted Parton
21 May 2018
RSC/DMDG/DMG
Paul Ehrlich first developed the concept of the “Zauberkugeln” in 1900, in his work on the immune system – in effect the antibody.

Early interest in staining microbes with dyes.
In 1905 he progressed to work on chemicals more toxic to microbes than animals - trypanosomes (sleeping sickness).

Also in 1905, the spirochaete cause of syphilis was identified; studies were expanded.
“So it was that another arsenical – arsphenamine – was discovered, synthesized in 1907 by Alfred Bertheim, and tested on spirochaetes by Ehrlich's assistants. The two assistants who first tested it concluded that it was useless, and it was therefore put aside until Ehrlich asked his Japanese assistant, Sahashiro Hata, to repeat the experiments. Hata found that arsphenamine was superior to all the other drugs that had been tested, prompting Ehrlich's fury that the inadequate methods used by his former assistants had resulted in the delay in this discovery.”

Ehrlich first used the English term “magic bullets” at a lecture in London in 1908. In a lecture given in 1913 it was translated as “bewitched bullets” (BMJ) and “... the antisubstances discovered by Behring, which after the manner of bewitched balls, fly in search of the enemy.” (The Lancet)
Success of nanomedicine

30-100nm Pegylated liposomal doxorubicin – Caelyx® (1996) or Doxil® (1995) (also Myocet® non-pegylated)

“Caelyx is doxorubicin hydrochloride encapsulated in liposomes with surface-bound methoxypolyethylene glycol (MPEG). This process is known as pegylation and protects liposomes from detection by the mononuclear phagocyte system (MPS), which increases blood circulation time.”
(EMEA, EPAR - Caelyx Product Information)

One ml of Caelyx contains 2 mg doxorubicin hydrochloride in a pegylated liposomal formulation.
Dosed at 20 - 50mg/m². Maximum lifetime dose 500mg/m²
Composition of Doxil (Caelyx)

2mg/mL doxorubicin

9.58mg/mL HSPC - fully hydrogenated soy phosphatidyl choline

3.19mg/mL mPEG-DSPE (PEG2000)
α-(2-[1,2-distearoyl-sn-glycero(3)phosphooxy]ethylcarbamoyl)-ω-methoxypoly(oxyethylen)40, sodium salt

3.19m/mL cholesterol
Ammonium sulfate, sucrose, histidine
HCl/NaOH for adjustment to pH6.5
Dilute into 5% glucose for infusion

Theory is that PEG prevents plasma proteins binding to surface

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Pegylated liposomal doxorubicin PK

# Liposomal doxorubicin PK

From McPherson & Evans. Breast Cancer: Targets and Therapy 2009:1 1–18

<table>
<thead>
<tr>
<th>PK of doxorubicin:</th>
<th>V (L/m²)</th>
<th>CL (L/h/m²)</th>
<th>T_{1/2,z} (h)</th>
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<tbody>
<tr>
<td>CremophorEL/ethanol</td>
<td>700-1100</td>
<td>24-73</td>
<td>30</td>
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<tr>
<td>liposomes</td>
<td>34</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>pegylated liposomes</td>
<td>1.93</td>
<td>0.03</td>
<td>74</td>
</tr>
</tbody>
</table>
130nm nanoparticles of paclitaxel in human albumin, though suspension in water leads to smaller particles (30nm (range 10-60))

Previously paclitaxel was formulated in CremophorEL/ethanol which caused side effects

nab-Paclitaxel appears to be nanocrystals of paclitaxel coated in human albumin, which may have some effect on cellular uptake, but broadly PK is similar to PK of solvated paclitaxel
Magic bullets

Magic bullets –
  harm bad guys
  do not harm good guys

Ideally ...
  antibiotics
  antivirals
  many kinase inhibitors
  monoclonal antibodies

The target can be anywhere or everywhere; there is no need to know where.
Magic mortars

Magic mortar – hits bad guys, misses good guys. eg proton-beam therapy.

But – you **must** know the range and direction.

Many NP concepts also depend on knowing where the tumour is - magneto(thermo)therapy - photodynamic therapy.

The need to follow where the NPs go has led many to combine imaging with therapy – so called theranostics.
Oncologists have tried to take better aim.

Regional limb perfusion with melphalan (nitrogen mustard):
– ligate major blood vessels, cannulate major artery upstream of tumour and major vein downstream, and perfuse.

Negative outcomes:
1 - whole limb may be necrotized;
2 - drug in tissue is released on reconnection of normal circulation with toxic results.
Hepatocellular carcinoma (HCC, primary liver cancer) is an exception, using microcatheters in techniques called

TransArterial Embolization (TAE) and

TransArterial ChemoEmbolization (TACE)
Aggregated human serum albumin – macro-aggregates (20-30µm).

They are differentially captured by hepatomas.

Aggregates are infused directly into the hepatic artery.

Sinusoidal blood is 75% venous and 25% arterial.

Tumour capillary blood is 75-80% arterial.

Higher density of aggregates in tumour capillaries results in micro-embolism, and very long retention, probably on account of the greatly reduced circulation.

Dose is matched to the tumour volume to avoid other embolisms. Gradually the aggregates are dismantled by proteases.
Blocking capillaries generally
DC Beads (BioCompatibles Ltd)

70-150, 100-300, 300-500 & 500-700 µm DC Beads
polyvinyl alcohol hydrogel
crosslinked with acrylamide
modified with sulfonate groups
bind chemotherapeutic drugs,
doxorubicin, irinotecan.

The drug is soaked into the beads before use.
After TACE, it is released over 1 month.

The beads themselves are not cleared.

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Lipiodol is iodinated poppy-seed oil – di-iodinated ethyl esters of C16 and C8 fatty acids.

It is used as a contrast agent in X-ray imaging.

Retained in HCC tumours for weeks, in the form of microembolisms of the tumour capillaries.

This effect is enough to form a repeatable treatment for HCC
Blocking capillaries in HCC – 4

Smancs

Neocarzinostatin
highly unstable DNA-disrupting diene-diynne-epoxide,
MW660, stabilized by a 100pM affinity 113-AA protein,
secreted by *Streptomyces macromyceticus*.

*Smancs* is neocarzinostatin covalently bound to a
styrene-maleic acid co-polymer.

It is approved in Japan for hepatic cancer,
formulated with Lipiodol,
infused using TACE
“New prostate therapy recommended on NHS”

Actually not new, but the recommendation by NICE was new.

They write:

“Under X-ray guidance, the prostate is approached through the left or right femoral artery. Super-selective catheterisation of the small prostatic arteries is done using fine microcatheters through the pelvic arteries. Embolisation involves the introduction of microparticles to completely block the prostatic vessels. Embolisation agents include polyvinyl alcohol (PVA) and other newer synthetic biocompatible materials.”

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Blocking capillaries generally
DC Beads (BioCompatibles Ltd)

While I was writing these slides…

http://www.bbc.co.uk/news/health-43877002
“New prostate therapy recommended on NHS
Actually not new, but the recommendation by NICE was new.
They write:
“Under X-ray guidance, the prostate is approached through the left or right femoral artery. Super-selective catheterisation of the small prostatic arteries is done using fine microcatheters through the pelvic arteries. Embolisation involves the introduction of microparticles to completely block the prostatic vessels. Embolisation agents include polyvinyl alcohol (PVA) and other newer synthetic biocompatible materials.”

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Ehrlich also coined the word chemotherapy

Their narrow therapeutic index limits their utility.

Damage to non-target tissue can be:
- Cosmetic (hair loss, rash)
- Debilitating (diarrhoea, emesis)
- Life-threatening (cardiotoxicity, hepatotoxicity, renal toxicity, neutropenia)

These are often cumulative, limiting the number of cycles.

Targeted delivery of drugs to tumours holds out the promise of improved therapeutic windows.
Examples of magic bullet claims

“Magnetic nanoparticle-induced hyperthermia with appropriate payloads: Paul Ehrlich’s “magic (nano)bullet” for cancer theranostics?”

EVERYTHING (Cancer centres, Switzerland and Texas)

“'Magic bullet’ nanomedicine developed for acute lung injury” (Queen’s Uni)

INHALED POWDER

“Nanoparticles: A golden bullet for cancer. Nanoparticles provide a targeted version of photothermal therapy for cancer” (Washington Uni, St Louis)

“Magic Beacons and Magic Bullets: The Medical Applications of Functional Nanoparticles” IMAGING, THERANOSTICS (Uni Leicester)

“Scientists to investigate 'magic bullet' cancer therapy” (Uni Sheffield)

RECEPTOR BINDING (SR-B1)
Magnetic nanoparticle-induced hyperthermia with appropriate payloads: Paul Ehrlich’s “magic (nano)bullet” for cancer theranostics?

- Hyperthermia at 39–45 °C is a potent radiosensitiser and has been shown to improve therapeutic outcomes in various tumours through its synergy with chemotherapy.
- Nanoparticles could extravasate passively into the tumour tissues in preference to the adjacent normal tissues by capitalizing on the enhanced permeability and retention effect.
- Tumour targeting might be further augmented by conjugating tumour-specific peptides and antibodies onto the surface of these nanoparticles or by activation through electromagnetic radiations, laser or ultrasound.
- Magnetic nanoparticles can induce hyperthermia in the presence of an alternating magnetic field, thereby multifunctionally with tumour-specific payloads empowering tumour specific radiotheranostics (for both imaging and radiotherapy), chemotherapy drug delivery, immunotherapy and gene silencing therapy.
- Such a (nano)bullet could realise the “magic bullet” conceived by Paul Ehrlich more than a century ago.
- This article discusses the various aspects of this "magic (nano)bullet" and the challenges that need to be addressed to usher in this new paradigm in modern cancer diagnostics and therapeutics.

Claims for receptor-targeting: SR-B1

Targeting the SR-B1 Receptor as a Gateway for Cancer Therapy and Imaging Frontiers in Pharmacology 2016

Texas:
“In addition, the SR-B1 receptor has been shown to serve as a potential gateway for the delivery of therapeutic agents when reconstituted high density lipoprotein nanoparticles are used for their transport to cancer cells and tumors.”

Sheffield:
“... smart nanoparticles that are taken up by two of the main receptors, known as SR-B1 and CD36. These nanoparticles could then be used to carry therapies directly to the cancer cells, without affecting healthy cells.”

“These nanoparticles could be the necessary 'magic-bullets' to save many lives affected by the worst types of cancers. Our proposal takes a step closer to patient-tailored strategies, allowing the delivery of therapeutic bio-molecules and restricting side effects of existing drugs.”

These receptors are widely distributed, wherever cells need cholesterol, and the hypothesis rests on tumour cells having a voracious appetite for cholesterol.

Any stratagem that attaches the particle to the tumour is called “targeting” and an ACTIVE process, compared to the EPR effect which is regarded is PASSIVE.
Summary of claims for Targeted NPs

Promises made that nanoparticles will “deliver” drug to the tumour

No mention of keeping the drug AWAY from other tissues

Justified by the “Enhanced Penetration and Retention (EPR) effect”. (Nanoparticles which find their way into interstitial fluid in a solid tumour tend to reside there longer than in normal tissues.)

Some surface agents are designed to encourage selective cellular uptake or drug-release by cancer cells.

Discussion points include:
- Tumour vasculature is “leakier” than normal capillaries
- Tumour vasculature is “incomplete” – way in, but no way out
- Tumours have no lymphatic system
- Interstitial fluid in tumour is high pressure
- Necrotic regions are more acidic

When your carrier does not work all the time, call it personalised medicine

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Key Performance Indicators of a Magic Bullet

1 Fraction of dose that reaches target (% ID)
   Ideal 100%; requires calculation of dose to match size of tumour
   Improvement would show progress, assisting design of carriers
   What would be an acceptable fraction?

2 Area under the concentration-time curve at the target
   Retention of drug at target while it is cleared elsewhere is beneficial
   Reduced frequency of treatment is beneficial

3 AUC target / AUC most sensitive healthy tissue
   Keeping drug out of sensitive tissues is beneficial

4 Drug and carrier are cleared harmlessly after tumour is destroyed

5 Active drug is released efficiently but only at the target

NOTE: Confusion arises over detection and quantification of particles rather than active drug. Surrogate particles are often detected and counted.
Key Indicators of a Magic Bullet 1 Fraction of dose that reaches target

This measure is confounded by the size of tumour and the size relative to body weight
A 1g tumour is 2.8% of the mass of a 35g mouse; 5% of a 20g mouse

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**Table:**

<table>
<thead>
<tr>
<th></th>
<th>No anesthetic</th>
<th>With anesthetic</th>
<th>Ratio of change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rb/g in tumors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H mammary carcinoma</td>
<td>2.15 ± 0.35*b</td>
<td>3.9 ± 0.55</td>
<td>1.8</td>
</tr>
<tr>
<td>WHT Sarcoma F</td>
<td>1.5 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Rb/g in kidneys</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H mice</td>
<td>12.9 ± 1.6</td>
<td>37.1 ± 2.8</td>
<td>2.9</td>
</tr>
<tr>
<td>WHT mice</td>
<td>15.0 ± 1.5</td>
<td>28.4 ± 2.4</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>HSA-(^{125})I % of volume occupied in tumors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H mammary carcinoma</td>
<td>5.0 ± 0.25</td>
<td>4.15 ± 0.5</td>
<td>0.83</td>
</tr>
<tr>
<td>WHT Sarcoma F</td>
<td>3.8 ± 1.1</td>
<td>3.14 ± 0.8</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>HSA-(^{125})I % of volume occupied in kidneys</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H mice</td>
<td>11.0 ± 0.9</td>
<td>6.0 ± 0.6</td>
<td>0.56</td>
</tr>
<tr>
<td>WHT mice</td>
<td>9.4 ± 0.4</td>
<td>7.2 ± 0.6</td>
<td>0.77</td>
</tr>
</tbody>
</table>

\* Percentage of injected **Rb (minus radioactivity in tail) per g of tumor.
\* Mean ± S.E. (12 to 16 mice).

Lessons from Imaging

Injected materials for imaging require differential concentration

Signal to noise ratio is defined by the difference between signal in tumour and signal in surrounding healthy tissues and the noise level

Unlike therapeutic uses, a temporary difference in concentration is sufficient for imaging, and observations can be made at an optimum time after administration - in one case 40 minutes, in another 3 hours after administration

On the other hand, still need to minimise exposure in healthy tissues and ensure that reagents are cleared safely

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PET scans not high definition - positron travels a few millimetres before meeting an electron, annihilating it and emitting a pair of $\gamma$ rays.

Increased glucose consumption characteristic of most tumours, due to over-expression of GLUT-1 & increased hexokinase activity.

Scans 60 minutes after 18FDG dose but 4-6 hours for some tumours.

Some cancers do not respond at any time eg prostate, others variable.

A detailed protocol must be followed:
- no food/sugar for 6 hours before injection;
- blood glucose measurements (raised glucose leads to muscle uptake)
- 1 litre of water during 2 hours before injection; empty bladder;
- no exercise, keep still, no talking, stay calm
- precise timing of scans after injections
- calculated dose for bodyweight
18FDG PET/CT

Maximum Intensity Projection (MIP) of man with oesophageal cancer.

Observe high responses from brain, kidneys and bladder, and moderate signal for liver.


A 56-year-old man who came for initial staging of esophageal cancer. The MIP (left panel) and axial fused (right upper panel) and axial CT (right lower panel) images of 18F-FDG PET/CT showed the primary mid-esophageal tumor with no evidence of FDG-avid distant metastases.
18FDG PET/CT

Woman with history of breast cancer.

Left – normal intensity image, with no apparent tumours

Right – reduced intensity image shows metastases in brain

Almuhaideb et al, (2011)
Standardized Uptake Value

SUV is the radioactivity in the volume of interest (in kBq/mL) divided by the radioactivity in the dose (in kBq/g bodywt) so it is in units of g /mL but treated as dimensionless (1g ≈ 1mL).

If distribution was uniform in soft tissues, SUV = 1.5 – 2

In liver, mean SUV = 2 - 3 and maximum SUV = 3 – 4

SUV(tumour) > SUV(neighbouring tissue) to be detectable.

Tumours can have SUV > 20, but many are in the questionable region.

This is a time-dependent property of the tumour depending on the changes of distribution that happen after injection. Liver, kidneys peak 5 – 10 minutes
SUV over time in 20 breast cancer patients
from Beauieu et al, J Nucl Med 2003; 44:1044–1050. Liver, kidney peaks 5-10 minutes
A 0.5g tumour is 1.4% of the mass of a 35g mouse
The equivalent in a 70kg patient would be a 1kg tumour or 2000 0.5g tumours
The vascular density of tumours decreases as tumours grow

Vaupel, Cancer Research 47, 3496-3503 (1987)

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A 66-year-old woman who came for restaging of breast cancer. MIP image (left panel) and coronal fused images (right panel) of 18F-FDG PET/CT showed extensive hepatic and bony metastases.
Estimating PK Properties of a Perfect Magic Bullet

Assume only the tumour extracts drug and to save time extracts it perfectly

\[ E_T = 100\%; \quad E_H = E_R = E_? = 0 \]

The only clearance mechanism is now through the tumour, determined by the tumour size and rate of perfusion – the tumour blood flow.

For a 1g tumour, a high perfusion is possible eg 1mL/min/g - \( Q_T = 1mL/min \)
For a 100g tumour, a low perfusion is likely eg 0.01mL/min/g - \( Q_T = 1mL/min \)

Assume the drug does not leave the blood (5L) except by tumour extraction
Then the half-life of extraction by the tumour is 58 hours ...
Estimating PK Properties of a Perfect Magic Bullet

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Assume the drug does not leave the blood (5L) except by tumour extraction

Then the half-life of extraction by the tumour is 58 hours ...

in which time (1 half-life) the entire blood volume has passed through the

- liver 1000 times
- kidneys > 800 times
- spleen > 150 times
- heart at rest > 150 times
- heart exercise > 900 times
- adrenals ~ 30 times

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Human blood flows - Vaupel, Kallinowski & Okunieff
Cancer Res 1989; 49:6446-6465

![Diagram showing blood flow rates for various organs and conditions.](image_url)
Estimating PK Properties of a Perfect Magic Bullet

In terms of relative blood flows, the scales for tissue:tumour are:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver</td>
<td>1450 x</td>
</tr>
<tr>
<td>kidneys</td>
<td>1240 x</td>
</tr>
<tr>
<td>spleen</td>
<td>240 x</td>
</tr>
<tr>
<td>heart at rest</td>
<td>240 x</td>
</tr>
<tr>
<td>heart exercise</td>
<td>1320 x</td>
</tr>
<tr>
<td>adrenals</td>
<td>77 x</td>
</tr>
</tbody>
</table>

Clearly extraction ratios for these tissues must be extremely low, and very much lower than for the tumour, for even a moderate fraction of a dose of nanoparticles to reach a tumour.

No amount of enhancement of binding of nanoparticles to tumour cells is going to deliver a substantial fraction of the dose unless the extraction by major organs is reduced.
“A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs.”
Matsumura & Maeda, Cancer Res. 1986; 46(12 Pt 1):6387-92. Kumamoto University, Japan

Intracutaneous tumour $10^6$ cells 8-10mm, 8-10 days in 35g mice.

“Tumoritropic accumulation” observed using $^{51}$Cr-labelled proteins and Evans Blue (up to 16 per molecule of albumin).

This aspect of the EPR effect seems to be consistent in mice

In the following year, looking at labelled proteins 12 – 160kDa, he coined the term Enhanced Permeability & Retention
The EPR Effect – a big story

A PubMed search on “EPR effect (NOT dosimetry)” revealed around 850 papers including 6 in the first 10 days of May this year.

Papers including H Maeda as an author: 68

and in many of those, he has taken to calling it “the so-called enhanced permeability and retention effect”
Challenging papers

Most papers challenge the results of decades of work but only in order to justify the concept behind their own nanoparticle design (eg Kudgus 2014 showing benefit of PEGylation of Au NPs) thereby recognising the problem but simultaneously holding out the hope for success in the short term. These differ:

2010: Florence “Nanosystem drug targeting: Facing up to complex realities”

2011: Bae & Park “Targeted drug delivery to tumors: Myths, reality & possibility”

2016: Petersen “Meta-analysis of clinical and preclinical studies comparing the anticancer efficacy of liposomal versus conventional non-liposomal doxorubicin”

2016: Warren Chan published a Perspective - “Analysis of nanoparticle delivery to tumours”.

This elicited Scott McNeil’s Correspondence “Evaluation of nanomedicines: stick to basics” and Chan’s reply to that criticism.
The most used models for solid tumours are xenografts of human immortal cell lines, in immunocompromised mice, and most often grown to 10mm diameter before use.

For experimental convenience, cell lines are selected which grow quickly through neovascularisation.

Are these fundamentally different from treatable tumours found in patients?
Are they different in ways which suit nanoparticle delivery of cytotoxic cpds?

In 2016, Petersen, Alzghari, Chee, Sankari and La-Beck (Department of Immunotherapeutics and Biotechnology, Texas Tech University Health Sciences Center School of Pharmacy, Abilene, TX, USA) published:

Of Mice and Men

Does this represent genuine advantage in mouse tumour models compared to clinical trials or is it a case of publication bias?

Clinical trials are more likely to report negative results than academic animal studies are.

Pegylated liposomal doxorubicin compared to conventional doxorubicin in MICE
Overall P Value < 0.0001

Liposomal vs conventional formulations of anthracyclins, cisplatin, paclitaxel or irinotecan in PATIENTS
Overall P value 0.34

Key Indicators of a Magic Bullet: **1 Fraction of dose that reaches target**

This measure depends on the size of tumour relative to body weight.
A 0.5g tumour is 1.4% of the mass of a 35g mouse.

<table>
<thead>
<tr>
<th>Year</th>
<th>% ID</th>
<th># data</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>1.4</td>
<td>8</td>
</tr>
<tr>
<td>2006</td>
<td>0.7</td>
<td>8</td>
</tr>
<tr>
<td>2007</td>
<td>1.0</td>
<td>24</td>
</tr>
<tr>
<td>2008</td>
<td>0.3</td>
<td>8</td>
</tr>
<tr>
<td>2009</td>
<td>0.9</td>
<td>11</td>
</tr>
<tr>
<td>2010</td>
<td>0.8</td>
<td>14</td>
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<td>2011</td>
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<td>2012</td>
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<td>2013</td>
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<td>35</td>
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<tr>
<td>2014</td>
<td>0.8</td>
<td>38</td>
</tr>
<tr>
<td>2015</td>
<td>0.5</td>
<td>45</td>
</tr>
<tr>
<td>All</td>
<td>0.7</td>
<td>232</td>
</tr>
</tbody>
</table>

Strongest predictor of % injected dose was cancer type.
From Wilhelm & Chan, Nature Reviews, Materials, 1, 16014 (2016)
This paper also reviews what is known about the various mechanisms by which nanoparticles are extracted by macrophages in the liver and spleen and by the kidneys. They also attempt to provide some data concerning the ways in which the nature of nanoparticles influence these extractions.

- the relationship between particle size and renal clearance;
- the relationship between particle size and liver uptake;
- the influence of surface charge on liver uptake
- the influence of surface PEG coating

One of Chan’s conclusions was a recommendation to pool data and knowledge as part of a 30-year plan to overcome the problems
My Observations

- Clearance is considered a mechanism, not a parameter
- Generally PK is examined through distribution studies
- Clearance is confused with excretion (where measured)
- Rather than use PK experience, *ad hoc* calculations and histograms are made and new words are coined (biokinetics?) which usually add nothing to understanding
- Temporary relative concentrations (e.g., tumour to blood ratio) are confused with delivery (%ID) and exposure (AUC ratio)
- For a useful review of passive targeting, see Bazak et al, *Molecular & Clinical Oncology* (2014) 2: 904-908
Opportunities

• Many investigators now realise the need to address extraction by RES/MPS and kidney, and have the resources to investigate

• Collectively a lot is known and published, and it could be put together into the best options

• As one problem is solved, new challenges will appear; there are no physiological cut-offs, so when liver/spleen extraction is reduced, renal filtration may become noticeable

• Good progress has been made with cellular uptake – important to improve the extraction ratio at the tumour
Impact of particle size on biological systems.

<table>
<thead>
<tr>
<th>Size</th>
<th>Biological systems and remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 nm</td>
<td>Abundant small pores present in normal tissue endothelium [44]</td>
</tr>
<tr>
<td>25 nm</td>
<td>Relatively few large pores present in normal tissue endothelium [44]</td>
</tr>
<tr>
<td>20–50 nm</td>
<td>Average size of polymeric micelles without loaded drugs</td>
</tr>
<tr>
<td>100 nm</td>
<td>Frequently tested size of drug-loaded polymeric micelles [45].</td>
</tr>
<tr>
<td>150 nm</td>
<td>Proposed cutoff size for particle extravasation in liver [46]. Liver has blood vessels with</td>
</tr>
<tr>
<td></td>
<td>fenestrations of 100–175 nm [47].</td>
</tr>
<tr>
<td>200 nm</td>
<td>Nanoparticles less than 200 nm have significantly longer circulation time due to low uptake by</td>
</tr>
<tr>
<td></td>
<td>the reticuloendothelial system (RES) [48,49].</td>
</tr>
<tr>
<td>380 nm</td>
<td>A tumor-dependent functional pore cutoff size ranges from 200 nm to 1.2 μm, but the pore</td>
</tr>
<tr>
<td></td>
<td>cutoff size of porous blood vessels in majority tumors is known to be 380–780 nm [50]. Thus,</td>
</tr>
<tr>
<td></td>
<td>the range for the EPR effect should be similar.</td>
</tr>
<tr>
<td>400 nm</td>
<td>Sterically stabilized liposomes of 400 nm in diameter were able to penetrate into tumor</td>
</tr>
<tr>
<td></td>
<td>interstitium [51]. Accumulation of hyaluronic acid-coated nanoparticles (100 nm) in the tumor</td>
</tr>
<tr>
<td></td>
<td>tissue [52].</td>
</tr>
<tr>
<td>500 nm</td>
<td>The maximum size of nanoparticles allowing penetration through cell membranes is known to be</td>
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<td></td>
<td>500 nm [53].</td>
</tr>
<tr>
<td>1 μm</td>
<td>Particles below 1 μm were taken up by Peyer’s patches and then migrated to mesenteric lymph</td>
</tr>
<tr>
<td></td>
<td>nodes [54].</td>
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<tr>
<td>5 μm</td>
<td>The upper limit for rigid particles circulating within the smallest capillaries [38].</td>
</tr>
<tr>
<td>40 μm</td>
<td>Particles larger than 40 μm have been used for embolization therapy [55].</td>
</tr>
</tbody>
</table>
PK of Au NPs in mice: influence of PEGylation on clearance & distribution

30nm gold NPs conjugated with gemcytabine (all) (dose=450µg);
and with IgG – inactive (AIG44)  
$CL_p = 1.5 \text{mL/min}$;

or modified Cetuximab (ACG44)  
$CL_p = 2.1 \text{mL/min}$;

(Note: $Q_{H,p} = 1.0 \text{mL/min}$)

and with 2kDa PEG (ACG44p2K)  
$CL_p = 0.0018 \text{mL/min}$
$T_{1/2} = 11 \text{hrs (I make it 6hrs)}$

CL 1000x lower with PEG  
(beware of errors in this paper)
Remarkable distribution of Au NPs without PEG. It appears that Au NPs go into some tissue and return to plasma.

In the tissue distribution part of the study, PEGylation slowed down the uptake of Au into liver but by 4 hours it was at the same level.

Lowered spleen concentration at 0.5 and 4 hours.

But made kidney concentrations higher.

It is difficult to reconcile tissue distribution with plasma PK.
Pharmacokinetics of nano-sized agents. Nano-sized agents, which are favorable for operating EPR effects, should stay in the blood pool for long time.
Effect of removing Kupffer cells on (Au) nanoparticle tumor delivery.


Clodronate iv in liposomes knocks out up to 80% KCs over 48hrs

T\(_{1/2}\) 0.6h & 8h for Au NPs

Kupffer cells recover after 48hrs

Ted Parton 2018
Effect of removing Kupffer cells on 50, 100 & 200nm Au nanoparticle tumor delivery.

The ratio of tumour uptake with and without Clodronate treatment increases with particle Diameter, a usual trend for extraction by Kupffer cells.
Similar trend in spleen.

Increase in %ID in tumour = 20x

= 140x

Now using zebra fish.

Stab2 is expressed in endothelial cells of liver, spleen and lymph nodes.
Question for nanomedicine designer:

How many of your nanoparticles are left after:
- 10,000 passes through liver and
- 10,000 passes through kidneys and
- 1,000 passes through spleen?

Then – will they be taken up into the tumour, and will they release the active drug?
Mozartkugeln