The Primal Essence of Targeted Drug Delivery:
Can It Deliver Enough Drug to Cure?

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PURDUE
UNIVERSITY

Weldon School of Biomedical Engineering **College of Pharmacy**

Knowing the History and Facts

From Brown to Green

1886 1906~1920

https://www.nyhistory.org/community/when-did-the-statue-of-liberty-turn-green
https://www.dailymail.co.uk/sciencetech/article-4652254/The-Statue-Liberty-RED-turned-green.html

Evolution of Controlled Drug Delivery Systems

1950 1960 1970 1980 1990 2000 2010 2020 2030

1952 Spansule®
Dissolution-control

1974 Ocusert®
Diffusion-control

1975 OROS®
Osmosis

1982 Delsym®
Ion exchange

1989 Lupron Depot®
PLGA Microparticle

Nanomedicine

Small Molecules

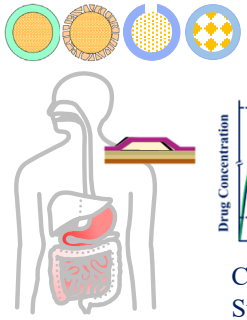
Peptide & Protein Drugs

Targeting

Biological Barriers

Long-Term Treatment

Basic Drug Delivery Mechanisms



Controlled release ≈ Sustained Release

Modulated drug delivery

Drug release kinetics controls pharmacokinetic (PK) profile → **Body controls PK profile**

1974 InFed®
Iron-Dextran Complex
(IRON DEXTRAN Injection USP)

1979 Transderm Scop®
(scopolamine) TRANSDERMAL SYSTEM 1.5 mg

1990 Norplant®
Implant

2000 Mylotarg™
(gemtuzumab ozogamicin) for Injection
Ab-Drug Conjugate

2000 Rapamune®
Nanocrystal
(sirolimus) Tablets

2019 Rebelsus®
Oral Peptide Tablet
semaglutide tablets

1992 Taxol®
Paclitaxel in PEGylated Castor Oil

2005 Abraxane®
Paclitaxel-Albumin Complex

1990 Adagen®
(pegademase bovine) Injection
PEGylated Protein

2014 Movantik
(naloxegol) Tablets
PEGylated naloxol

2018 Onpattro®
(patisiran) lipid complex injection
RNAi in PEGylated Lipid Nanoparticle

1964 Liposome (Bangosome)

1995 Doxil®
PEGylated Liposome

2017 Kymriah® CAR-T Gene Therapy
(tisagenlecleucel)

2021 Comirnaty® PEGylated Lipid Nanoparticle
(COVID-19 Vaccine, mRNA)

Evolution of Controlled Drug Delivery Systems

1950 1960 1970 1980 1990 2000 2010 2020 2030

1952 Spansule®
Dissolution-control

TID (Twice a day)

BID (Twice a day)

Increase in Convenience & Compliance

United States Patent Office

2,738,303
Patented Mar. 13, 1956

1

2,738,303

SYMPATHOMIMETIC PREPARATION

Rudolph H. Blythe, Llanerch, Pa., assignor to Smith, Kline & French Laboratories, Philadelphia, Pa., a corporation of Pennsylvania

Application July 18, 1952, Serial No. 299,566

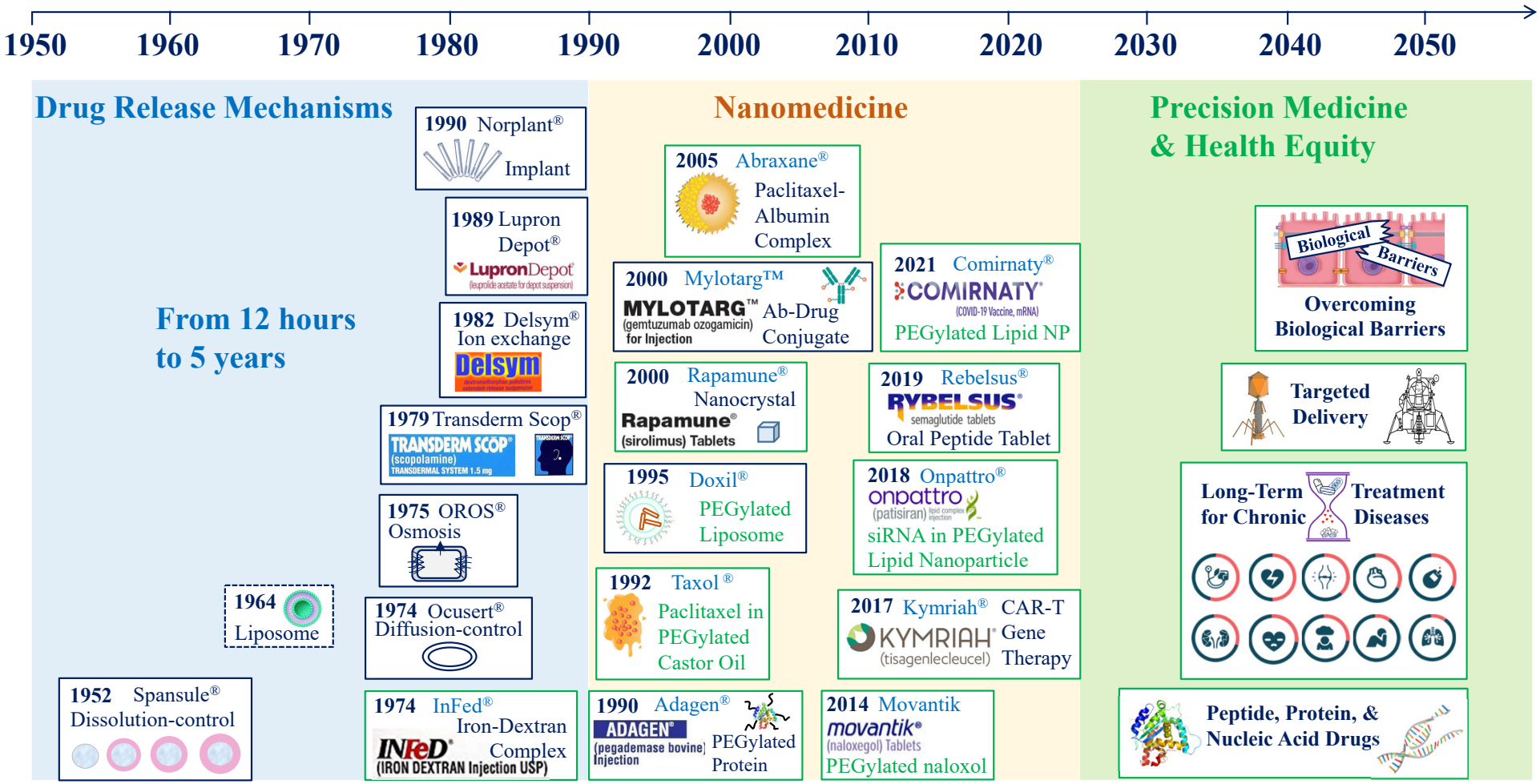
5 Claims. (Cl. 167—82)

2

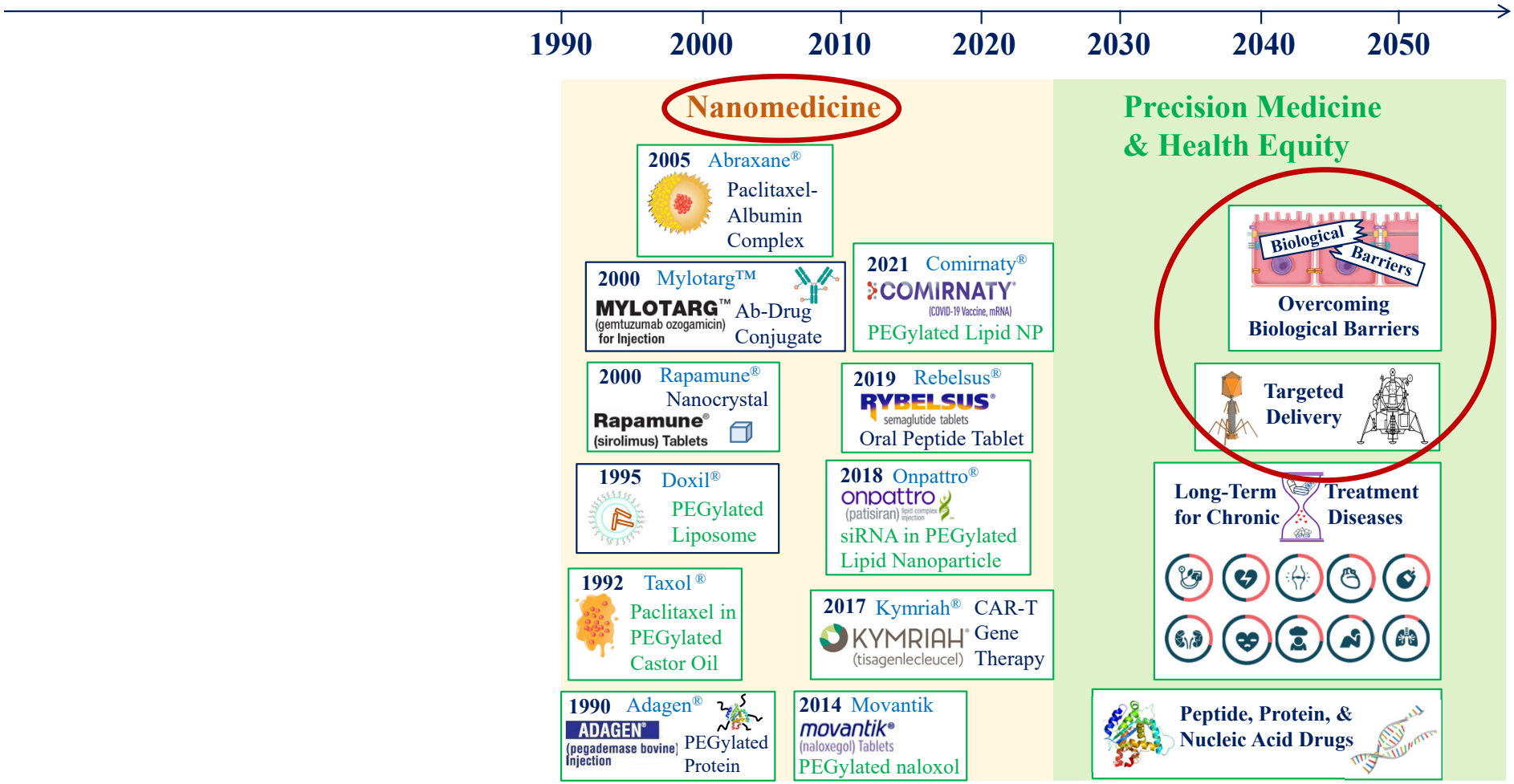
optimum effective range. The normal single dosage is larger than the initial dose of this invention in order to achieve long action and results in numerous side effects at peak body levels, particularly jitteriness, subsequent let-down and depression of mood.

In the case of a dextro-amphetamine salt this initial dosage preferably should be from 3 to 5 mg. In the case of a racemic amphetamine salt this initial dosage preferably should be from 5 to 10 mg. In the case of a dextro-desoxyephedrine salt this initial dosage preferably should be from 2 to 5 mg. In the case of a racemic desoxyephedrine salt this initial dosage preferably should be from 4 to 10 mg.

Evolution of Controlled Drug Delivery Systems



Two Issues to Clarify for Future Progress



What is Nanomedicine?

No clear definition: Different definitions by NIH, USP, & FDA. It is a matter of interpretation.
Most nanomedicine formulations were tested for tumor-targeted drug delivery.

Arbitrary Classification of Nanomedicine in the Literature

These are NOT considered nanomedicine

Taxol

1992

Polymer micelle (Cremophor EL)

Taxotere

1996

Polymer micelle (Polysorbate 80)

These are considered nanomedicine

Doxil

1995

PEGylated liposome

Abraxane

2005

Albumin-drug conjugate

Genexol

2007

Polymer micelle (PEG-b-PLA)

The formulations are designed to increase the water solubility of poorly soluble drugs.

Meanings of Targeting & Targeted Drug Delivery

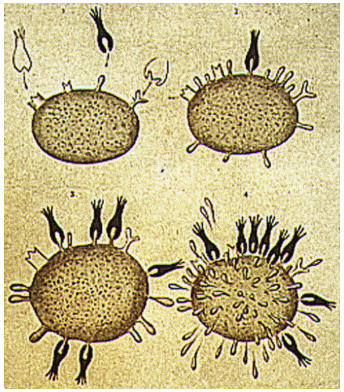
Paul Ehrlich (1854-1915)

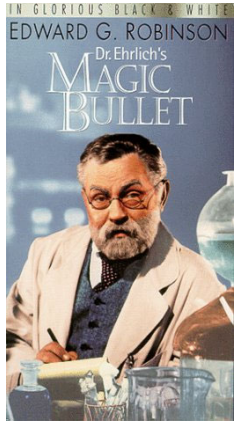
Scientific disciplines of Chemotherapy:
Binding of dyes to certain fabrics & cells.

Many chemical molecules have an affinity to tissues, cells, and cellular components

- Sleeping sickness: trypan red.
- Syphilis: Sarvasan 606

(1890s: Antitoxins = Antibodies)



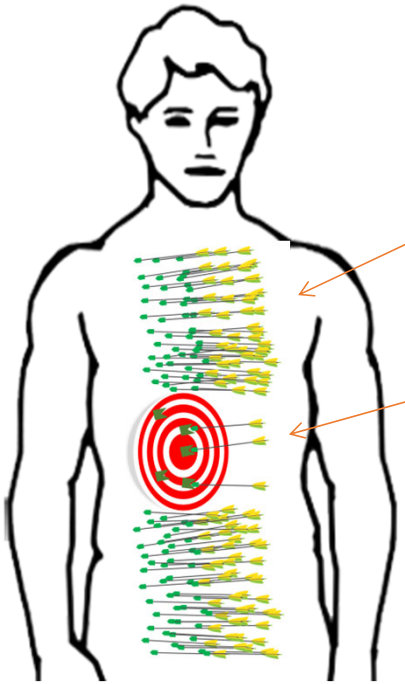


Nobel Prize 1908

Paul Ehrlich's Magic Bullet (Targeted Drug)

A drug specifically targeting a particular pathogen without affecting normal host cells.

= binding to



1. **Random distribution of drugs throughout the body!**
2. **Binding to a particular tissue, cell, and cellular components**

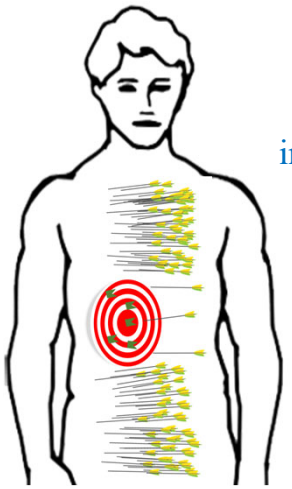
Targeted Delivery

Valent et al., Paul Ehrlich (1854–1915) and his contributions to the foundation and birth of translational medicine, J. Innate Immun. 8:111–120, 2016.

"Targeting" Simply Means "Binding & Retention" If Reaching the Target

Distribution throughout the body after I.V.

The targets selected are present not only in disease cells, but also in healthy cells. **The same drug amount reaches the target for both the control and targeted delivery systems.**

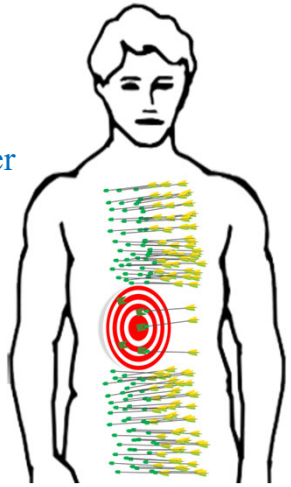


Control:
No ligands or PBS

Description 1:
The drug reaching target increased from 1% to 3%. Better efficacy than current therapy?

1% → 3%

Description 2:
200% Increase in delivery and better than control.



Targeted:
Ligand-attached

The difference in semantics might seem insignificant, but it **completely changes the point of view and scope of the event.**

(Butterfly effect S3E7: Lawrence of Arabia: For a fistful of sand, HBOMax)

The term "**targeted**" is often used to describe various new drugs and therapies with the intention to suggest that they exhibit higher specificity in treating the disease. The reality, however, is that the use of all such recently denoted drugs is associated with a large number of often very serious and **undesirable side effects**. Using the term "targeted" when it relates to the intent of what the drug is to do, and ignoring the fact that **its action is generally distributed throughout the body rather than focused on the locus of the disease**, is misleading.

Karel Petrak, Concepts and Misconceptions of Drug Targeting, Cambridge Scholars Publishing, 2020.

Terminology Matters: There is No Targeting, but Retention

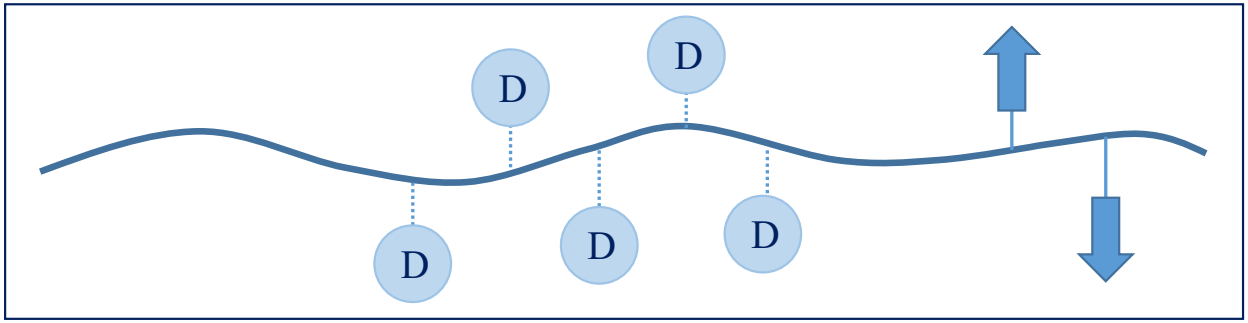
Accurate terminology is critical in advancing the drug delivery field and in understanding barriers and capabilities. The nanoparticles will have the same distribution at the intended target site, while **nanoparticles with "targeting ligands" may have a chance to more strongly interact with the target cells.**

To reiterate, there is no targeting. A danger of using such misnomers is that the researchers who are exposed to this area for the first time will have misconceptions that will probably spill over into their research, making the same mistakes as their predecessors. Additionally, misnomers can result in unintended, and sometimes unrealistic, expectations by individuals outside the drug delivery field. It is time to correct our past mistakes and use the right terminology, such as "**retention ligands**" to replace '**targeting ligands**'.

J. Reineke, Terminology matters: There is no targeting, but retention, J. Control. Release 273: 180-183, 2018.

The Origin of the Misunderstanding of Targeted Drug Delivery

Helmut Ringsdorf, Structure and properties of pharmacologically active polymers. J. Polymer Sci.: Symposium No. 51: 135-153, 1975.



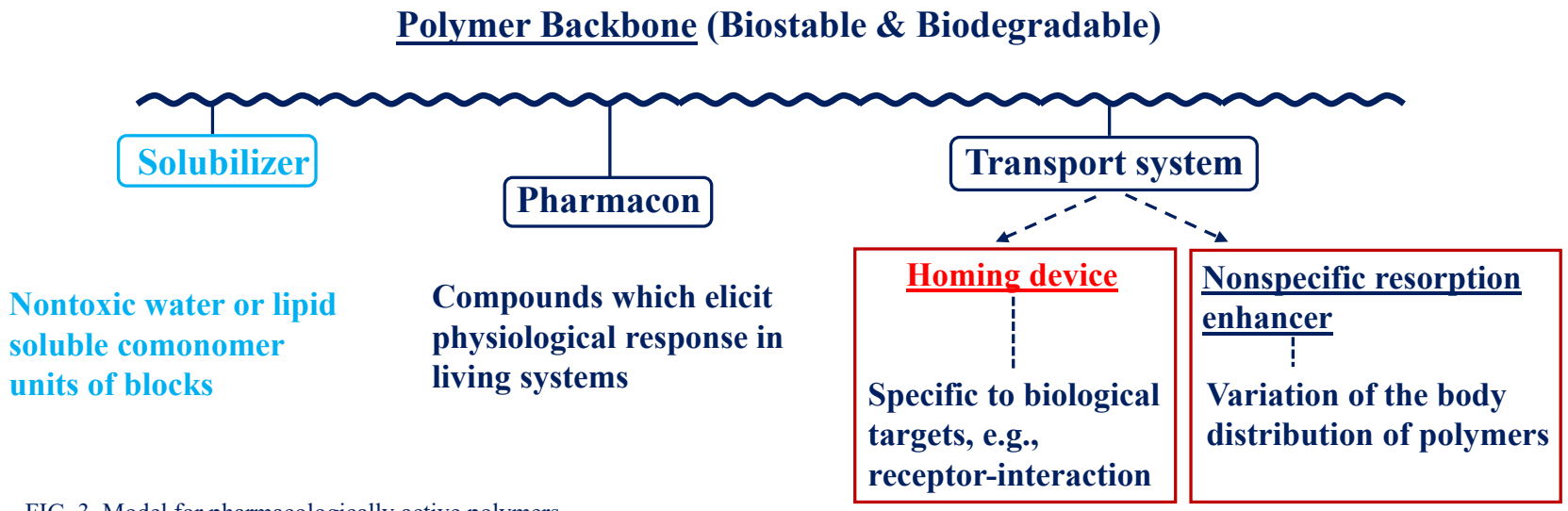


FIG. 3. Model for pharmacologically active polymers.

Targeted Drug Delivery: Biodistribution First Followed by Receptor Binding

Increasing Importance

3. Binding to receptors **2. Reaching target cells** **1. Biodistribution**

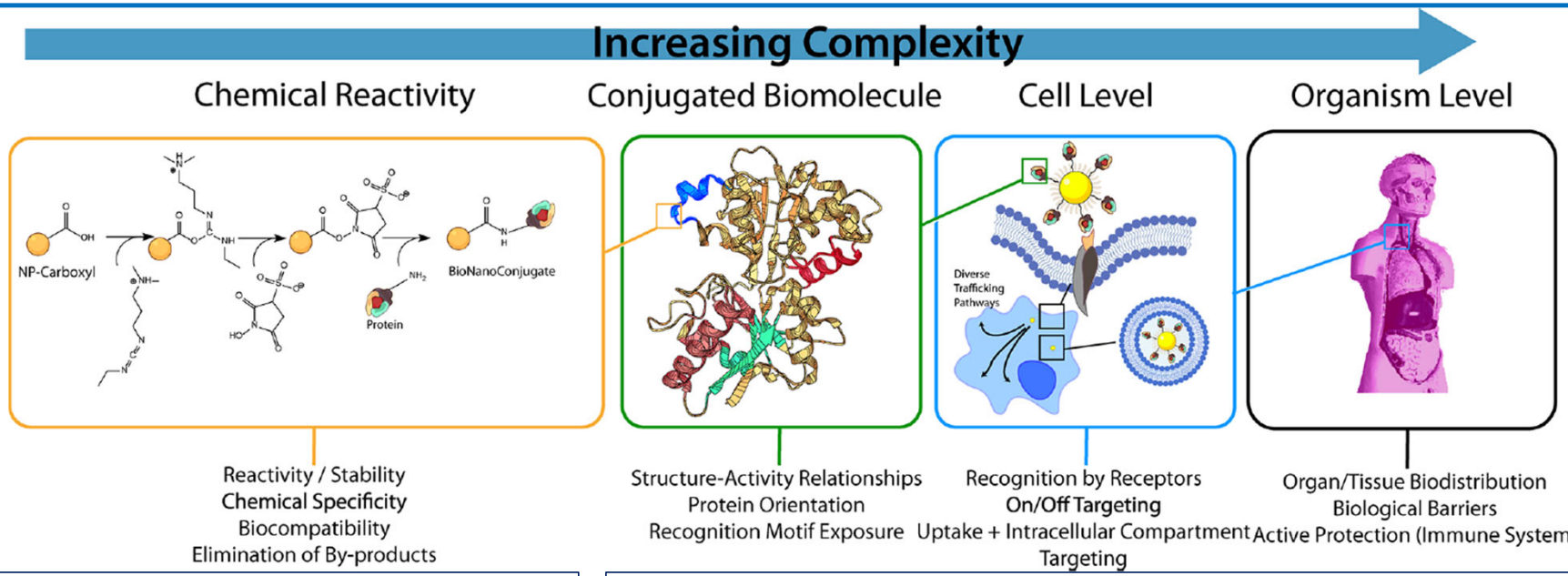


Figure 1. Levels of complexity in bionanoconstruct formation. The chemical reactivity of particular moieties found within biomolecules, as well as their exploitation in the selective formation of stable bionanoconstructs, represents the best-studied and most understood of these levels. At the level of the conjugated biomolecule, consideration must be given to the biomolecule's orientation, conformational structure, and arrangement upon the nanomaterial surface to ensure functionality.

Fleming et al., Designing functional bionanoconstructs for effective in vivo targeting, Bioconjugate Chem. 2022, 33, 429−443.

At the organism level, the outcome of a particular bionanoconstruct targeting experiment in vivo will be examined in terms of the **biodistribution** of these constructs in a given tissue or organ, and at this level, the role of **biological barriers** such as the blood brain barrier, as well as the potential for recognition of the construct as a foreign entity by the organism's immune system, must be considered. **At the cell level**, complexity increases further as cellular mechanisms and interactions may be considered to govern the bionanoconstruct recognition event. Forms of bionanoconstruct uptake and transport through diverse intracellular trafficking pathways must also be considered, and a complex decision-making process undertaken by the cell is expected in response to the recognition event. Though we do not explicitly outline them here, one can consider increasing levels of complexity (at the tissue, organ, and system levels).

Targeted Drug Delivery Systems: Better Retention than the Control!

Professor Agata A. Exner, Departments of Radiology & Biomedical Engineering, Case Western Reserve University, Cleveland, OH.

Nanobubbles (NB) targeted to the prostate-specific membrane antigen (PSMA)

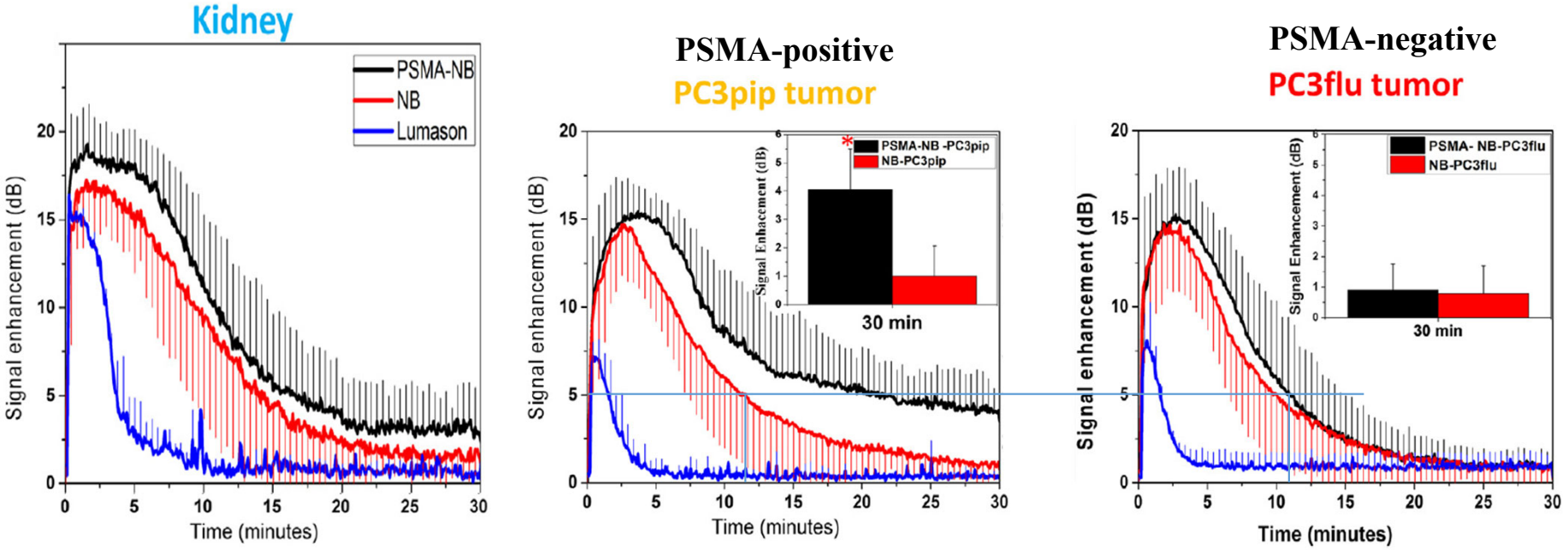


Figure 3. PSMA-NB enabled imaging of prolonged enhanced US signal in PSMA-positive PC3pip tumors. Mean time intensity curves (TIC) of tumors, and kidneys after bubble administration. At the peak intensity, the contrast in both tumors was similar with both NB and PSMA-NB. At later time points PC3pip tumor show high-contrast with PSMA-NB.

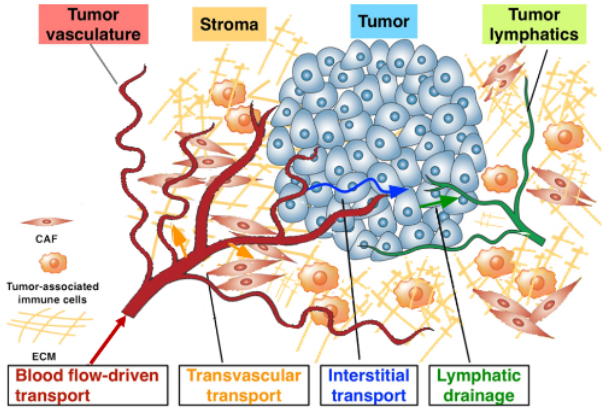
Lumason: Sulfur hexafluoride lipid-type A microspheres

Perera et al., Real time ultrasound molecular imaging of prostate cancer with PSMA-targeted nanobubbles, Nanomedicine: Nanotechnology, Biology, and Medicine 28 (2020) 102213.

Retention on the Cells is Not Enough: Delivery Into the Cells

In vivo whole tumor imaging

Delivery near solid tumor ≠ **Delivery into tumor cells** ∝ **Drug efficacy**

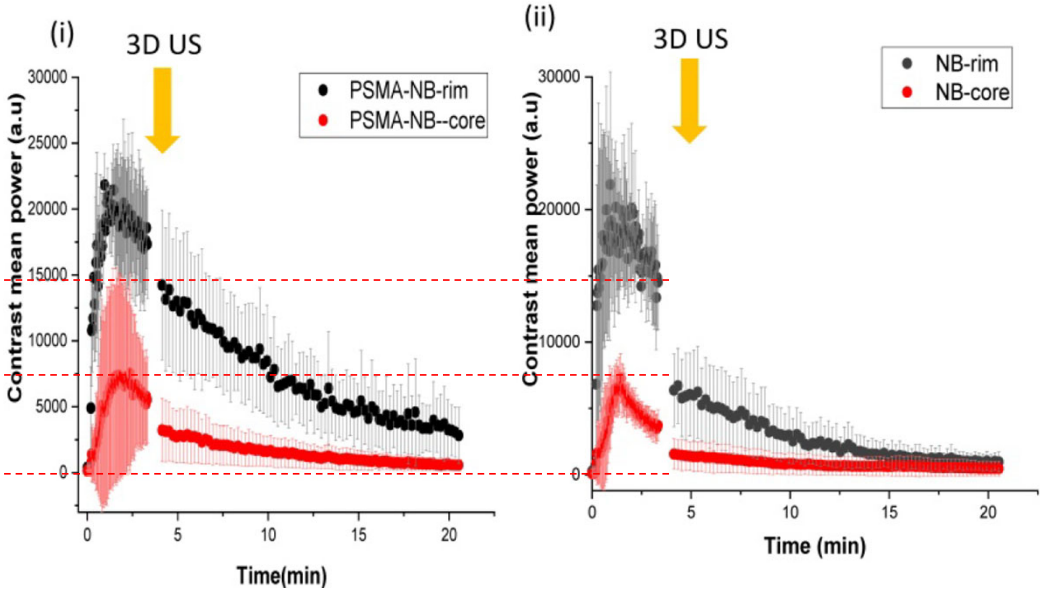


Park 2015, Targeting the tumor microenvironment

Professor Exner

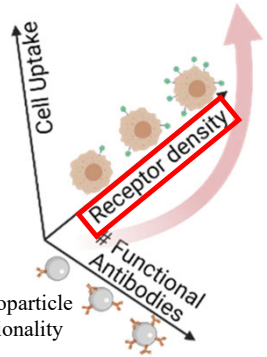
Perera et al., Intracellular vesicle entrapment of nanobubble ultrasound contrast agents targeted to PSMA promotes prolonged enhancement and stability in vivo and in vitro. Nanotheranostics 2022; 6(3): 270-285.

Variability due to the tumor heterogeneity

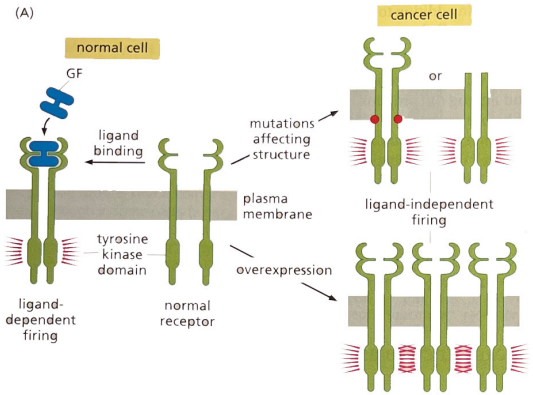


The high rate of cancer heterogeneity poses a major challenge in its utilization of RGD modified liposomes.

Sheikh et al., Recent progress of RGD modified liposomes as multistage rocket against cancer. Frontiers in Pharmacology 12: Article 803304, 2022.

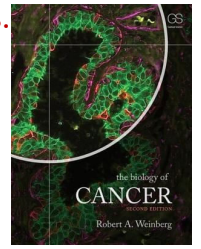


Woythe et al., A single-molecule view at nanoparticle targeting selectivity- Correlating ligand functionality and cell receptor density. ACS Nano 2022



Many receptor molecules are displayed at the surface for **only a limited amount of time** before they are internalized via endocytosis.

Robert A. Weinberg. The Biology of Cancer, 2nd Edn., Garland Science, 2014. pp. 142-143



Overcoming Cellular Barriers

Entering cells & Endosomal escape

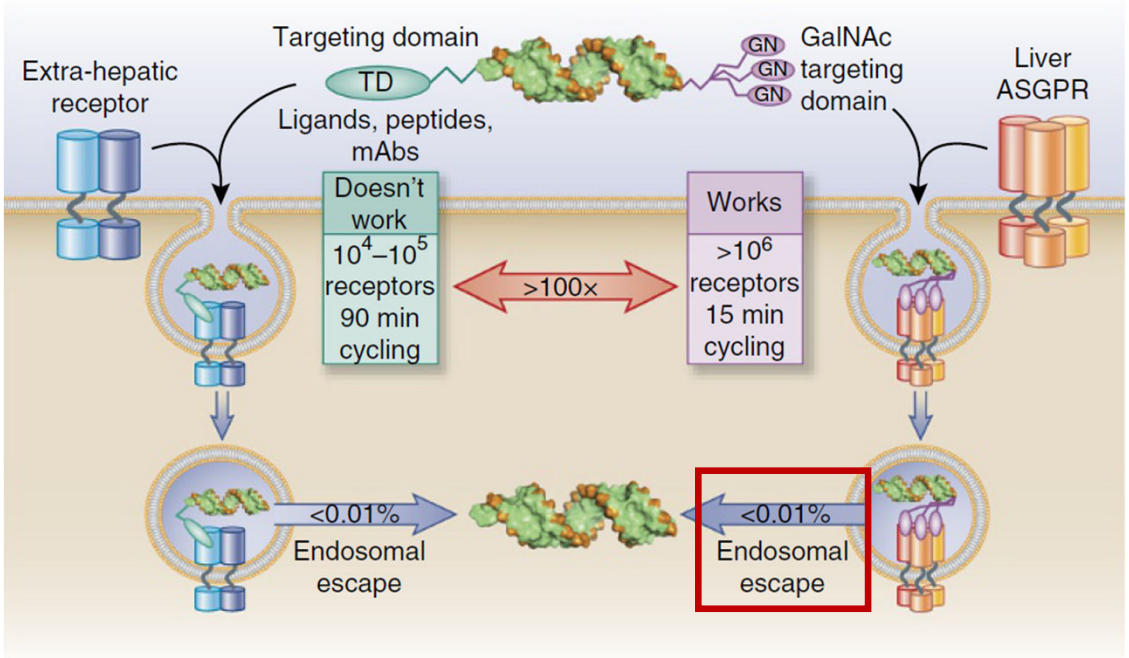


Figure 3. The numerology of endosomal escape. Tris-GalNAc binding to liver asialoglycoprotein receptors (ASGPRs) (~10^6/hepatocyte) induces endocytosis (~15 min) where a small fraction of the siRNA or ASO cargo escapes into the cytoplasm to induce selective RNA drug responses. In contrast, targeting non-hepatic cell surface receptors (10^4–10^5) that have a much slower rate of endocytosis (~90 min) has proven extremely difficult. Assuming there is no endosomal escape advantage in ASGPR endosomes, ASGPR brings in ~100-fold more siRNAs/ASOs into hepatocytes than is mathematically possible in any other ligand–receptor pair. Consequently, development of next-generation RNA-based therapeutics needs to incorporate new chemistries, materials and/or mechanisms of enhancing endosomal escape ~100-fold.

Dowdy 2017, Overcoming cellular barriers for RNA therapeutics

Reaching intracellular organelles

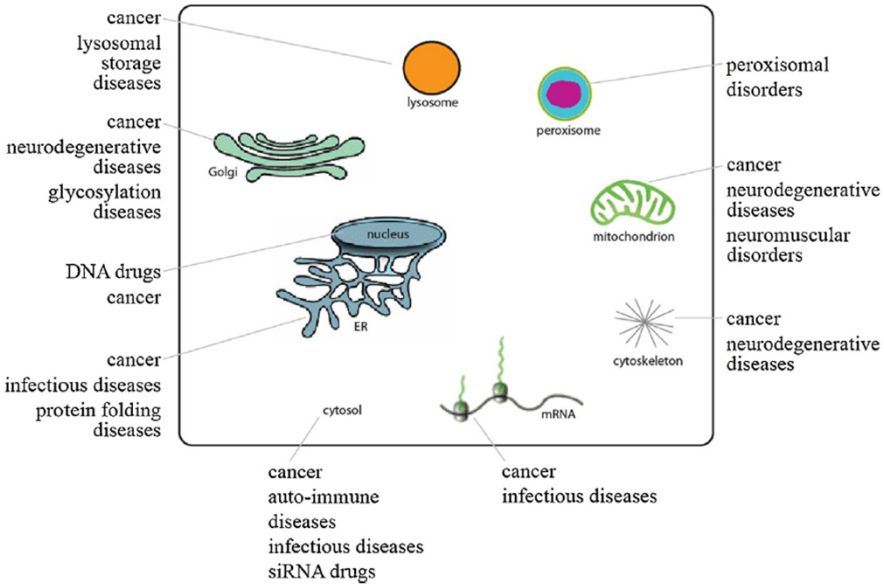
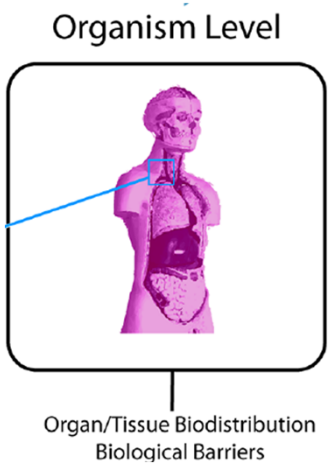


Fig. 1. Examples of target organelles and therapeutic indications for intracellularly-acting drugs. Drugs act in nucleus, mitochondrion, endoplasmic reticulum, Golgi apparatus, cytoskeleton, peroxisome, cytosol, and in other intracellular organelles and localizations.

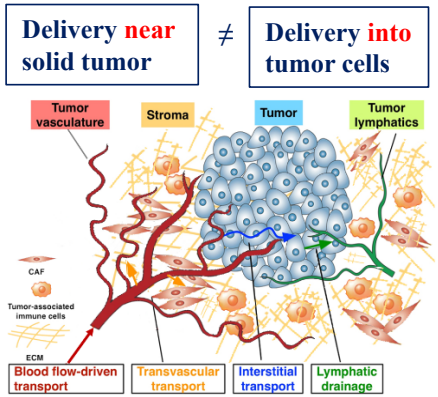
Maity & Stepensky, Delivery of drugs to intracellular organelles using drug delivery systems-Analysis of research trends and targeting efficiencies, Int. J. Pharm. 496 (2015) 268–274

Many Events Before and After Targeted Delivery

1. Biodistribution

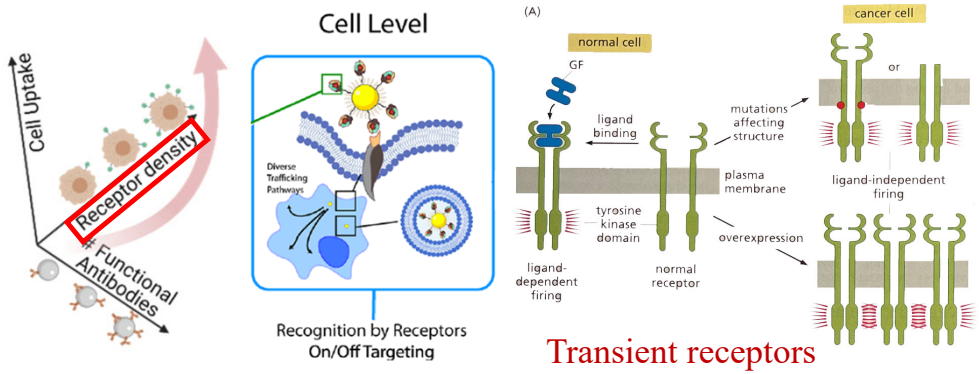


2. Reaching target area

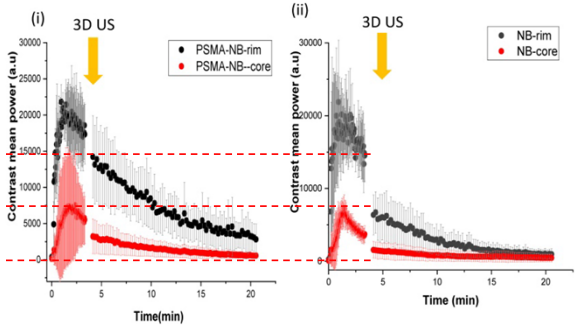


Targeted Delivery

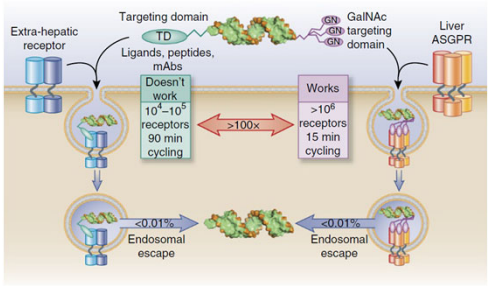
3. Binding to receptors



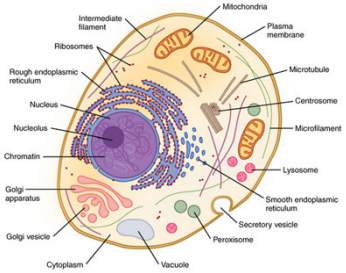
4. Entering cells



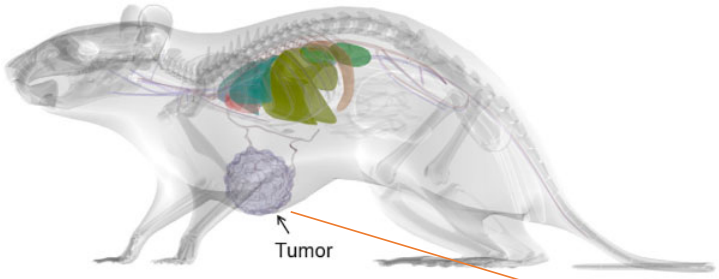
5. Endosomal escape



6. Intracellular organelles

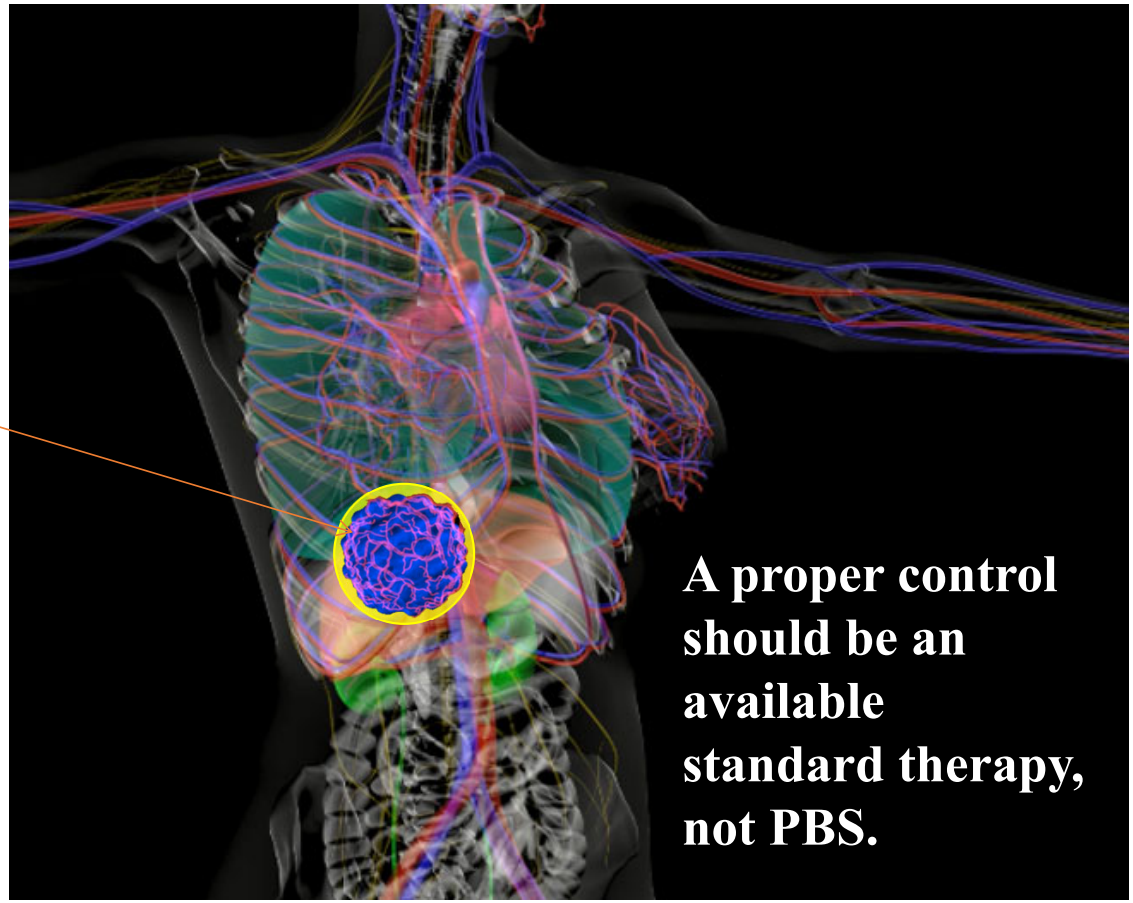


Why Xenograft Mouse Models Fail to Predict Clinical Study Outcomes



Tumor Size: ~ Liver
Tumor Growth: ~ 1 month
Blood Volume: ~ 2 mL
Control: PBS or Delivery Vehicle

Mouse data have a reproducibility crisis in humans

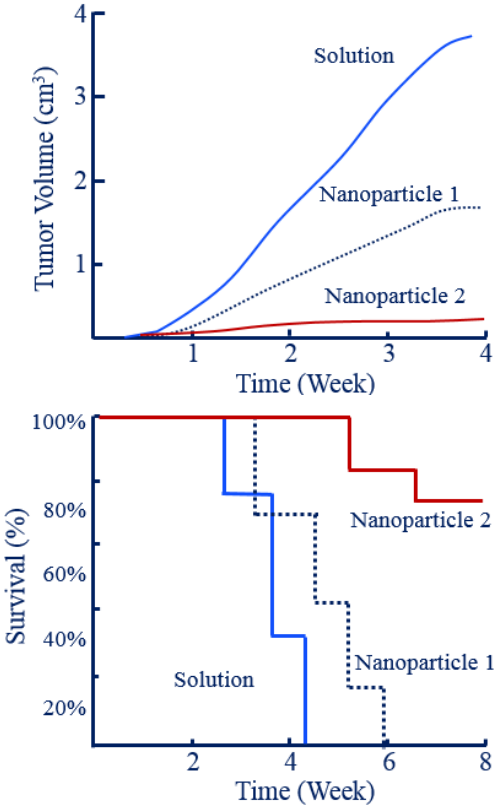


Digital Human by Professor Tonglei Li, Purdue University.

A Proper Control: Mouse Studies vs. Clinical Studies

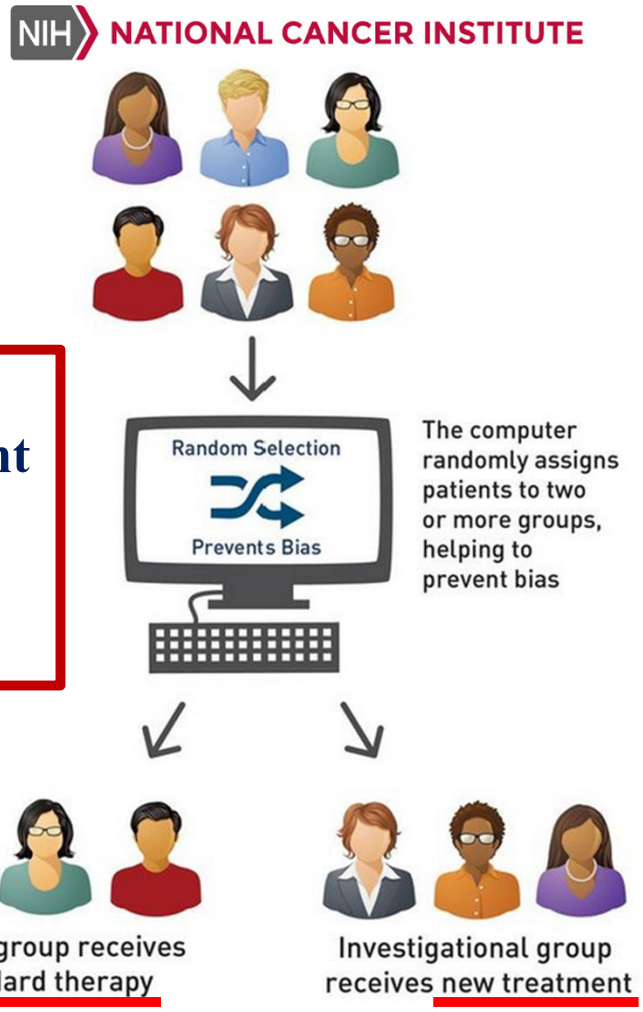
Typical results after i.v. administration of nanoparticle formulations

The control in mice studies is usually a saline solution, and not a standard therapy.



K. Park, Y.H. Bae, R. Mrsny. The missing components today and the new treatments tomorrow, in: Y.H. Bae, R. Mrsny, K. Park (Eds.) Cancer Targeted Drug Delivery: An Elusive Dream, Springer, New York. 2013, pp. 689-707.

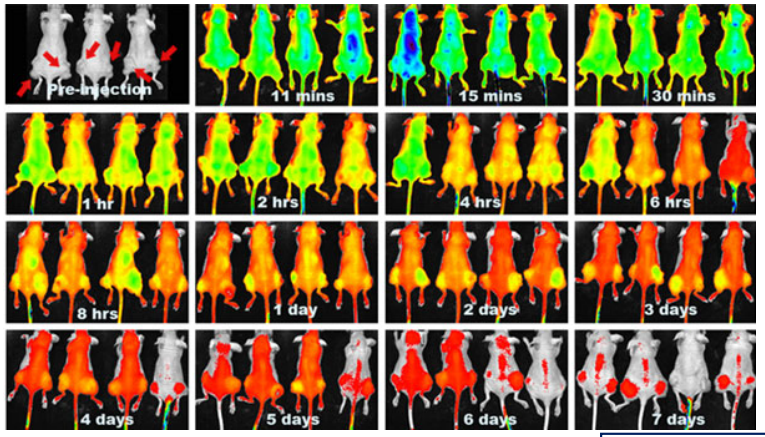
Clinical studies are designed to evaluate whether a new treatment is "superior", "equivalent", or "non-inferior" to an available standard therapy.

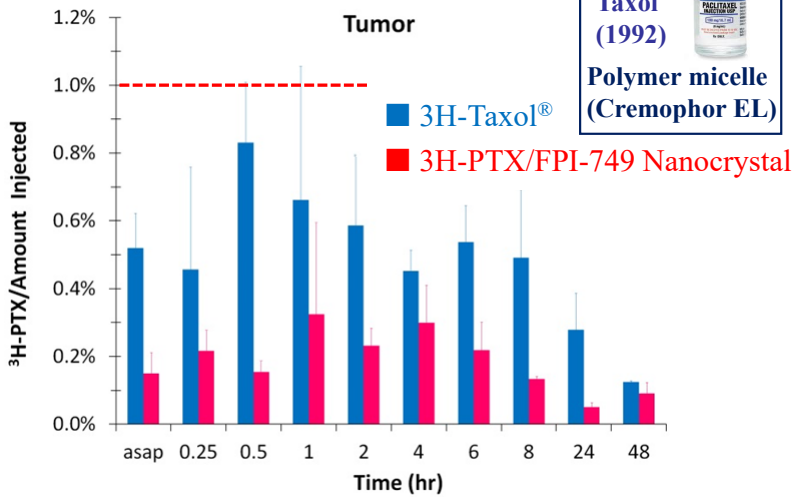


https://www.cancer.gov/about-cancer/treatment/clinical-trials/what-are-trials/randomization/clinical-trial-randomization-infographic

Taxol® (the Standard Therapy Control) is Better than Nanomedicine

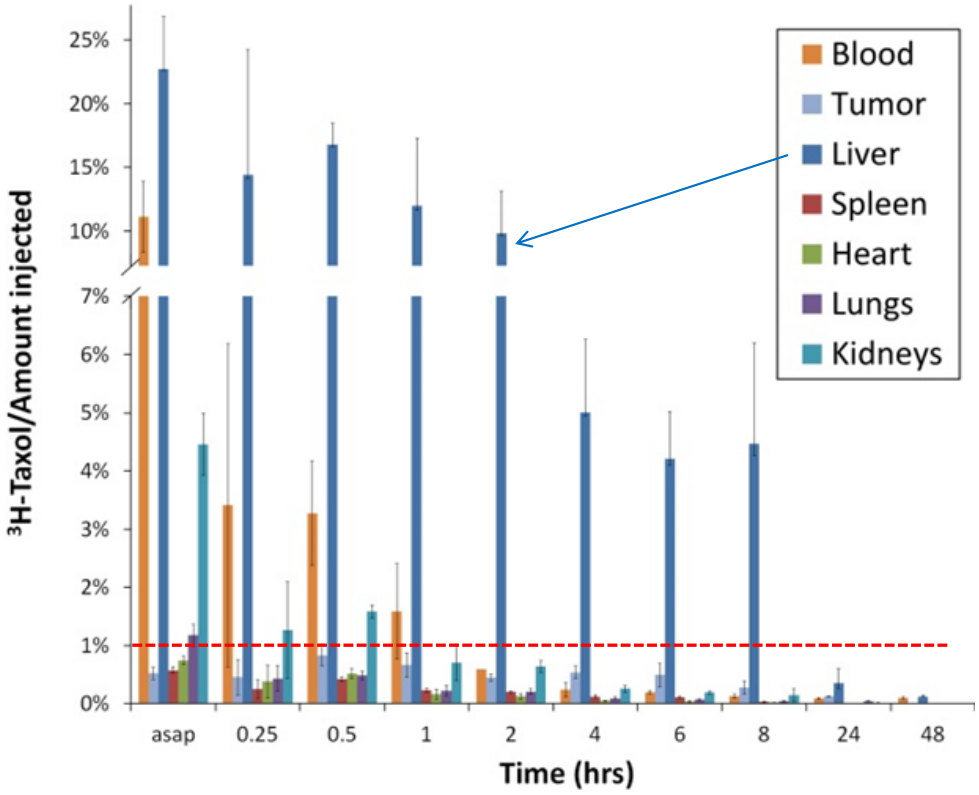
Biodistribution and bioimaging studies of hybrid paclitaxel nanocrystals vs. Taxol® (Standard therapy)





Taxol (1992)

Polymer micelle (Cremophor EL)



C.P. Hollis, H.L. Weiss, M. Leggas, B.M. Evers, R.A. Gemeinhart, T. Li. Biodistribution and bioimaging studies of hybrid paclitaxel nanocrystals: Lessons learned of the EPR effect and image-guided drug delivery. J. Control. Release, 172 (2013) 12-21.

The Standard Therapy Control (Taxol®) is Better than Nanomedicine

Taxol® (Standard therapy) is better than Transferrin-coated paclitaxel nanocrystals

Lu et al., Development and evaluation of transferrin-stabilized paclitaxel nanocrystal formulation. J. Control. Release, 176 (2014) 76-85.

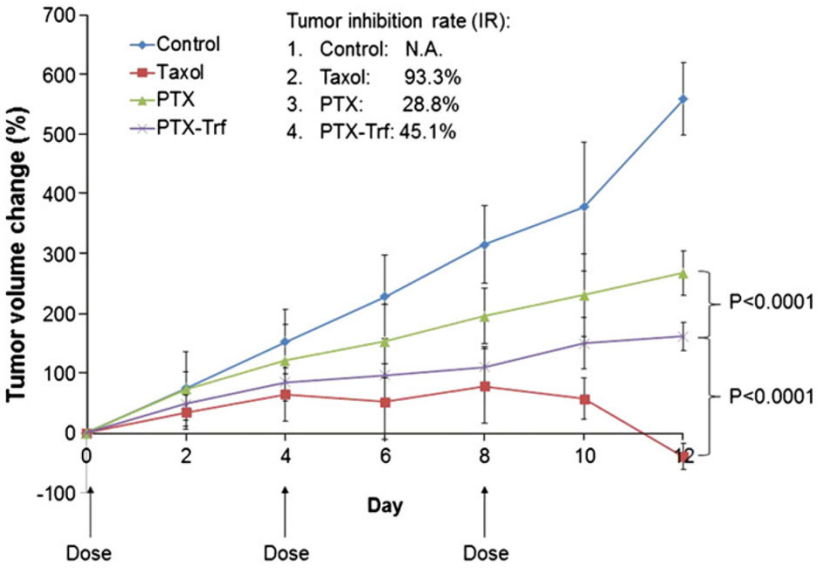


Fig. 7. *In vivo* antitumor efficacy of PTX formulations in mice.

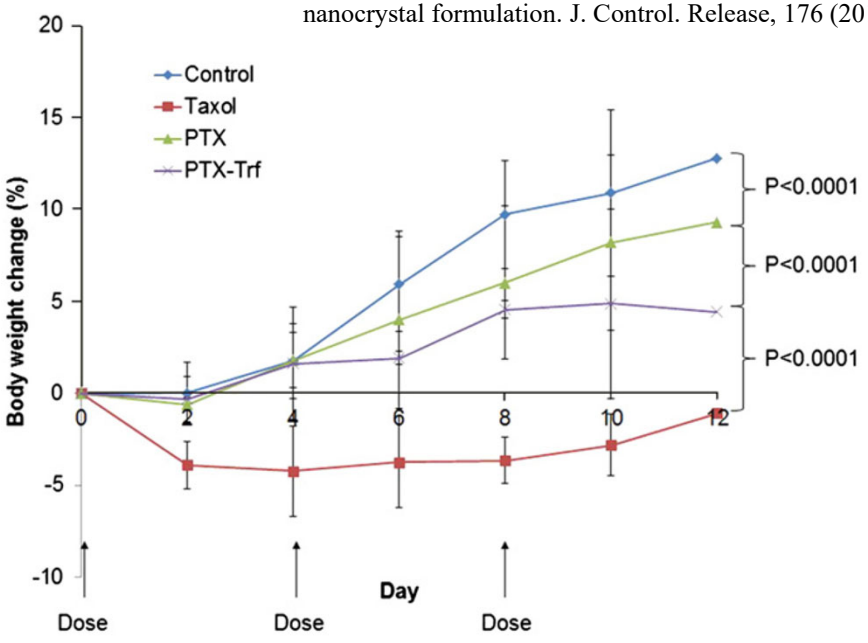
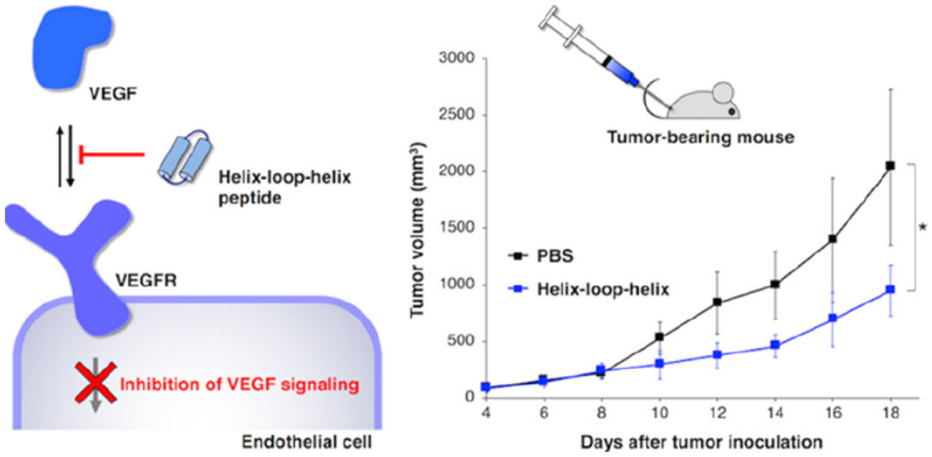


Fig. 8. Body weight change of mice after administration of various PTX formulations.

Interestingly, paclitaxel (PTX) solution in a 50:50 mixture of Cremophor EL and ethanol (Taxol®) showed significantly better tumor volume inhibition compared to both positive treatment groups, PTX–Trf and PTX nanocrystals. However, while the antitumor efficacy of Taxol® was higher, its toxicity was also significantly higher compared to the control and PTX nanocrystal formulations. This superior efficacy has been attributed to the presence of Cremophor that forms micelles small enough to penetrate deeper into the tumor mass compared to bigger nanocrystals [53,54]. Studies have observed that Taxol accumulates at a significantly higher extent compared to nanocrystals, which may have led to the higher tumor inhibitory effect seen in the present study [55]. But the quantity is around 1% of the total administered dose [55], and at that level, the anti-tumor effect may be mainly due to PTX that is absorbed into the tumor cells, rather than the amount deposited near the tumor. This makes sense, since Taxol provides better PTX solubility than PTX–Trf, which in turn provides better solubility than PTX nanocrystals which are prone to aggregate.

The Standard Therapy Control (Avastin®) is Better than Nanomedicine

Bevacizumab (Avastin®, Standard therapy) is better than Helix−Loop−Helix (HLH) Peptides



In Vivo Tumor Growth Inhibition.
LS174T cells were expanded in EMEM supplemented 10% FBS. On day 0, LS174T cells (1×10^6) were transplanted subcutaneously into BALB/c nude mice.

The mice were injected i.p. with 5 mg/kg bevacizumab every 3 days for a total of four doses (days 1, 4, 7, and 10) or treated with daily injections of the HLH peptides at 10 mg/kg (i.p. administration: days 1−10). The tumor volume was calculated according to the formula: (longest diameter) × (short diameter)^2 × 0.5. Mice were killed when the tumor volume reached 2000 mm^3.

Michigami et al., New class of drug modalities directed evolution of a de novo designed helix−loop−helix peptide to bind VEGF for tumor growth inhibition, ACS Chem. Biol. 17: 647−653, 2022.

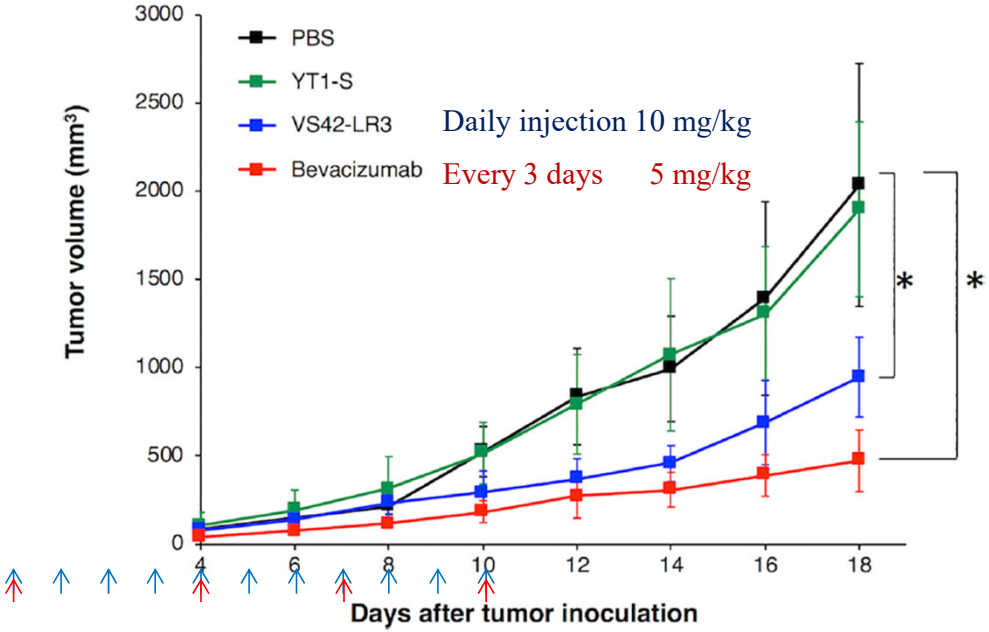
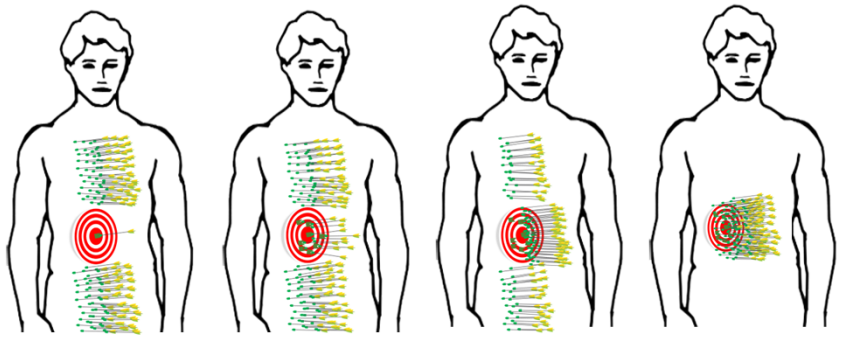


Figure 5. Tumor growth inhibition by the HLH peptides. LS174T cells were inoculated into BALB/c nude mice. The mice were treated with bevacizumab (5 mg/kg on days, 1, 4, 7, and 10) or the HLH peptides (10 mg/kg on days 1−10). (Mean ± standard deviation (n = 5).

As shown in Figure 5, the HLH peptide VS42-LR3 and bevacizumab both inhibited tumor growth. The tumor volumes were significantly smaller than in PBS-treated mice ($p < 0.01$). Although the peptide administration was ended at day 10, VS42-LR3 inhibited tumor growth at the same level as bevacizumab until day 14, and still significantly inhibited it at day 18 compared with PBS and YT1-S as a control peptide.

Key Question to Ask: Does It Deliver Enough Drug without Side Effect?

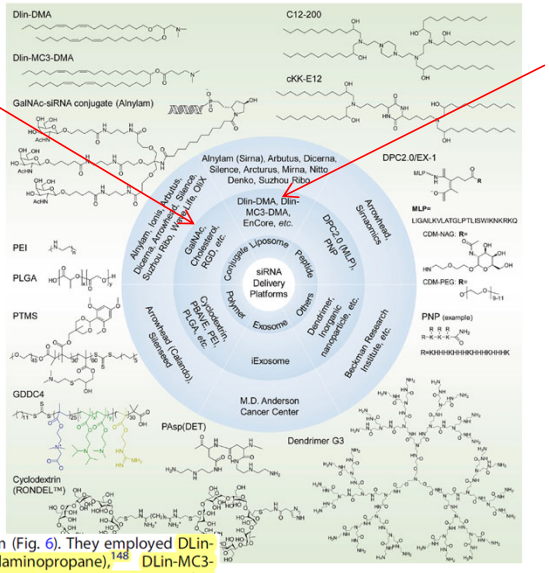
How much drug delivery is enough to be effective?

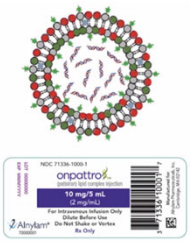


The quantity of the drug necessary to produce a given effect.

The criteria of a new therapy is whether it is effective and safe.

- siRNA covalently linked to a ligand (GalNac) targeting hepatocytes
- Acute hepatic porphyria
- FDA approval in 2019





- siRNA encapsulated in a lipid nanoparticle
- Hereditary transthyretin-mediated amyloidosis
- FDA approval in 2018

developed by Arbutus and Alnylam (Fig. 6). They employed DLin-DMA (1,2-dilinoleyloxy-3-dimethylaminopropane),[148] DLin-MC3-DMA ((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate)[107] and L319 (di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino) butanoyl) oxy) heptadecanedioate)[149] as the

effect' or 'colloid osmotic pressure effect' results in membrane destabilization[116–119] or membrane swelling,[120,121] respectively. However, the underlying mechanism of endosomal release remains to be further illuminated. Only 1–2% of internalized LNP-loaded siRNAs were released into the cytoplasm, and this only occurred within a limited time frame after internalization.[122,123] Hence, further understanding the escape mechanism and how to enhance the escape efficiency is of great importance for siRNA drug development. Recently, Wang and colleagues[124] developed novel endoplasmic reticulum (ER) membrane-modified hybrid nanoplexes (EhCv/siRNA NPs). Compared with unmodified nanoplexes, they showed much higher RNAi activity in vitro and in vivo. The functional proteins on the ER membrane have an important role in intracellular trafficking of siRNA, helping siRNA reach the cytoplasm through the endosome–Golgi–ER pathway instead of the endosome–lysosome pathway, thereby avoiding the lysosomal degradation of siRNA. In addition, electroporation enables siRNA to directly cross the cell membrane, which also constitutes an effective approach to circumvent the endosomal escape issue.[125–131]

Hu 2020, Therapeutic siRNA- State of the art

Chemosaturation: Delivery of Sufficient Amounts of the Drug

Modi et al., Chemosaturation with percutaneous hepatic perfusion of melphalan for metastatic uveal melanoma. Melanoma Research 2022, 32(2): 103-111, 2022.

Chemosaturation with percutaneous hepatic perfusion of melphalan for metastatic uveal melanoma

Sachin Modi[a], Tom Gibson[a], Ganesh Vigneswaran[a,b], Shian Patel[a], Matthew Wheater[c], Ioannis Karydis[b,c], Sanjay Gupta[d], Arjun Takhar[e], Neil Pearce[e], Christian Ottensmeier[f] and Brian Stedman[a]

Uveal melanoma, the most common primary ocular malignancy in adults, carries a poor prognosis: 50% of patients develop the metastatic disease with a 10–25% 1-year survival and no established standard of care treatment. Prior studies of melphalan percutaneous hepatic perfusion (M-PHP) have shown promise in metastatic uveal melanoma (mUM) patients with liver predominant disease but are limited by small sample sizes. We contribute our findings on the safety and efficacy of the procedure in the largest sample population to date. A retrospective analysis of outcome and safety data for all mUM patients receiving M-PHP was performed. Tumour response and treatment toxicity were evaluated using RECIST 1.1 and Common Terminology Criteria for Adverse Events v5.03, respectively. 250 M-PHP procedures were performed in 81 patients (median of three per patient). The analysis demonstrated a hepatic disease control rate of 88.9% (72/81), a hepatic response rate of 66.7% (54/81), and an overall response rate of 60.5% (49/81). After a median follow-up of 12.9 months, median overall progression-free (PFS) and median overall survival (OS) were 8.4 and 14.9 months, respectively. There were no fatal treatment-related adverse events (TRAE). Forty-three grade 3 (29) or 4 (14) TRAE occurred in 23 (27.7%) patients with a significant reduction in such events between procedures performed in 2016–2020 vs. 2012–2016 (0.17 vs. 0.90 per patient, $P < 0.001$). M-PHP provides excellent response rates and PFS compared with other available treatments, with decreasing side effect profile with experience. Combination therapy with systemic agents may be viable to further advance OS. *Melanoma Res* 32: 103–111 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

Melanoma Research 2022, 32:103–111

Keywords: interventional radiology, melanoma, melphalan, outcome assessment

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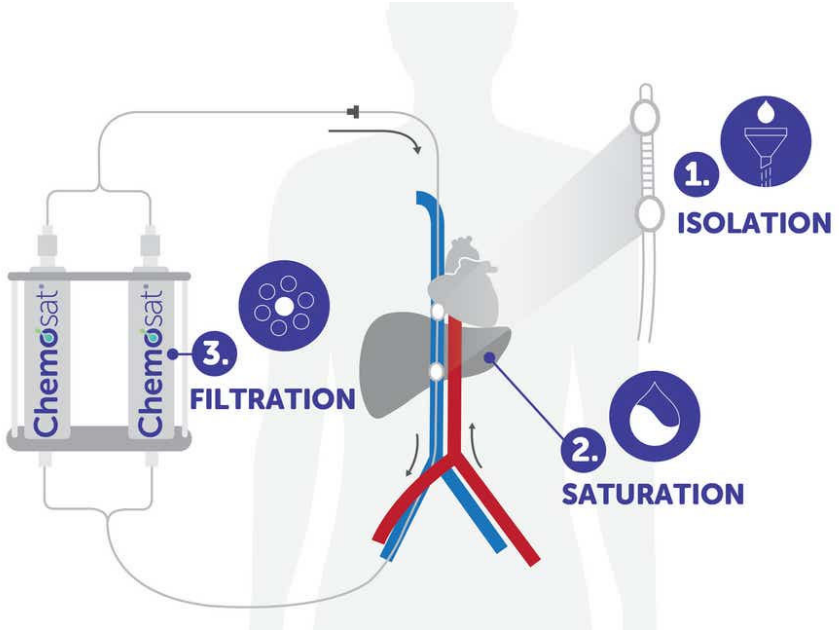
Procedural details

Cases were performed under general anaesthetic with continuous monitoring of the central venous and arterial pressure in a dedicated interventional radiology suite. Patients received melphalan at a dose of 3 mg/kg (ideal body weight) delivered using the Hepatic CHEMOSAT Delivery System (Delcath Systems, Inc., New York, USA) with the GEN 2 filter in line with the manufacturer's recommendations, which have been described in detail previously [13].

13. Meijer TS, Burgmans MC, Fiocco M, de Geus-Oei LF, Kapiteijn E, de Leede EM, et al. Safety of percutaneous hepatic perfusion with melphalan in patients with unresectable liver metastases from ocular melanoma using the Delcath systems' second-generation hemofiltration system: a prospective non-randomized phase II trial. Cardiovasc Intervent Radiol 2019; 42:841–852.

New liver cancer treatment 'effective in 90% of patients'

Chemosaturation allows doctors to administer much larger doses of drug and it does not enter the bloodstream.

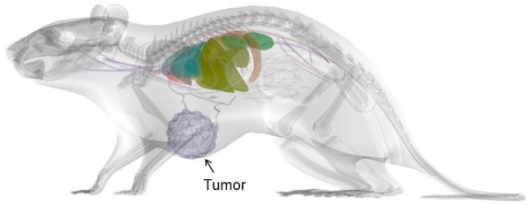


Chemosaturation therapy is being pioneered at University Hospital Southampton to tackle liver cancer (Planets/PA)

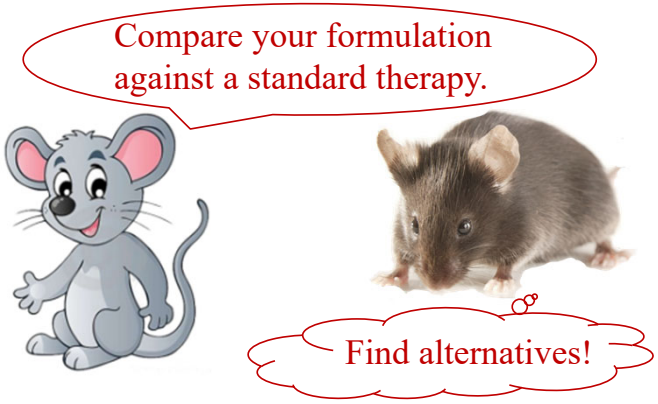
Ben Mitchell: https://www.standard.co.uk/news/uk/university-hospital-southampton-nhs-national-institute-for-health-and-care-excellence-nice-ben-mitchell-b992155.html

Animal Models: Improvements to be Made

Xenograft Mouse Models



Finding relevance to human cancers



Model validity: making sure the mouse is right

It seems an obvious point, but the model used should be appropriate for the question being addressed.

An ideal disease model accurately mimics the human condition, genetically, experimentally and/or physiologically.

Justice & Dhillon, Using the mouse to model human disease increasing validity and reproducibility. Disease Models & Mechanisms (2016) 9, 101-103 doi:10.1242/dmm.024547

Any one model will not be able to represent humans, and each disease requires multiple models.

Pancreatic Cancer Model Mimicking Human Pancreatic Cancer

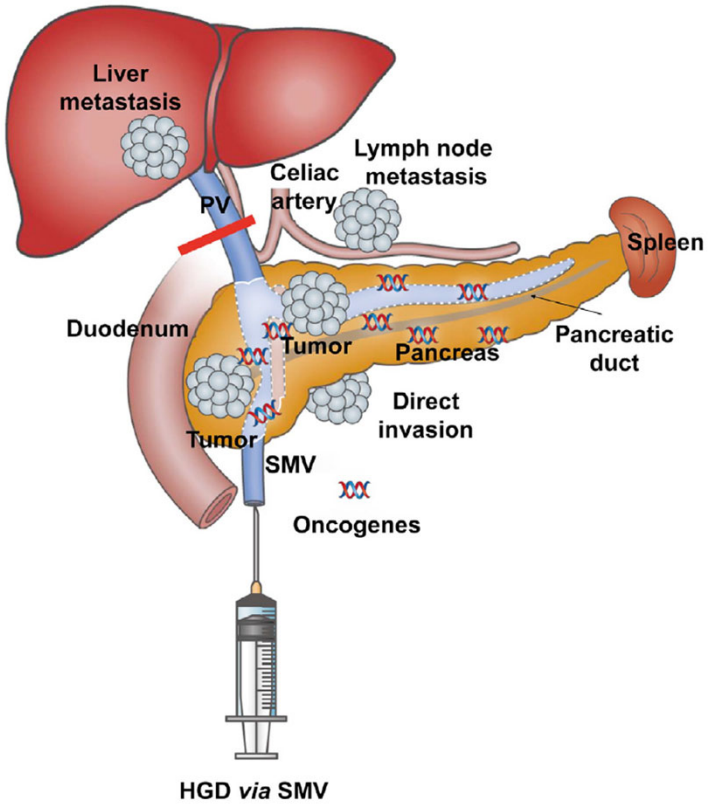


Figure 1. Schematic presentation of pancreas-targeted hydrodynamic gene delivery induced pancreatic cancer model. Schema of pancreas-targeted hydrodynamic gene delivery (HGD) of oncogenes from the superior mesenteric vein (SMV) with a temporary vascular blockade at the portal vein (PV).

The model must mimic human pancreatic cancer in molecular pathogenesis, histological features, and multi-step malignant transformation for tumor marker and therapy development. Novel pancreatic cancer animal models with simple, easy, and reproducible methods are essential.

Pancreas-targeted oncogene HGD induced pancreatic cancer models within 5 weeks in wild-type rats. The tumor occurrence efficacy of this approach depended on the combination and dosage of genes. With molecular signaling activation, the malignant tumor potential increased and exhibited metastatic lesions partly through the cadherin switch. This animal model will speed up pancreatic cancer research for the establishment of the novel treatment strategies and markers for early diagnosis.

Rats were given pancreas-targeted hydrodynamic injections. The portal vein in the hilus and superior mesenteric vein were dissected out and isolated. The catheter was inserted into the superior mesenteric vein with temporary occluding of the blood flow at the portal vein by vessel loops, and the plasmid DNA solution (20 mg plasmid in 4 mL for a 200-g rat) was hydrodynamically injected at a flow rate of 1 mL/s (Figure 1). For the HGD of a combination of plasmids, equal amounts of individual plasmids were prepared in a volume of 2% body weight. Therefore, in a 200-g rat simultaneously receiving two plasmids, 20 mg of each plasmid was diluted in 4 mL of saline solution.

Shibata et al., Establishment of a pancreatic cancer animal model using the pancreas-targeted hydrodynamic gene delivery method. Molecular Therapy: Nucleic Acids V28: 342-352, 2022.

A Human Breast Cancer-derived Xenograft & Organoid Platform

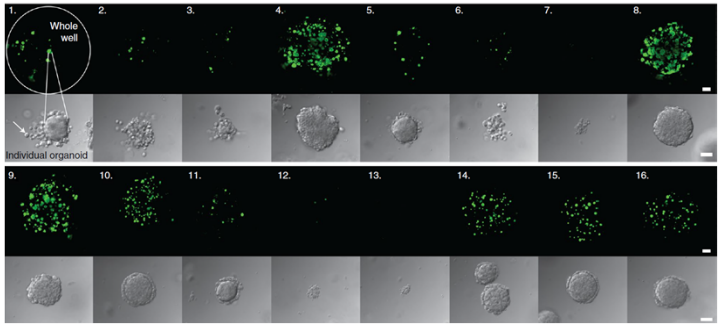


Fig. 2 | Optimization of human patient-derived xenograft-derived organoids (PDxO) culture conditions. a, Live-cell area of entire wells (top) and brightfield images of individual organoids (bottom) representative of PDxO HCI-002 grown under 16 different conditions 15 d after organoid preparation; scale bars, 500 µm (top) and 50 µm (bottom).

PDxO drug response assays are not without limitations. Although we were able to discern cytotoxic effects in our assays, we were unable to reliably detect activity of drugs that convey less potent activity. activity. --- Future work will determine whether longer-term drug exposure, possibly with passaging, will be a better read-out for less potent, yet clinically relevant, drug activity.

In summary, this work provides a large, clinically relevant resource of paired in vivo and in vitro human-derived models of breast cancer, with an emphasis on the most difficult cases for which research advances are urgently needed. We show that these models can be used for drug screening and discovery, and our methods are also conducive to conducting functional **precision medicine** in real time with clinical care.

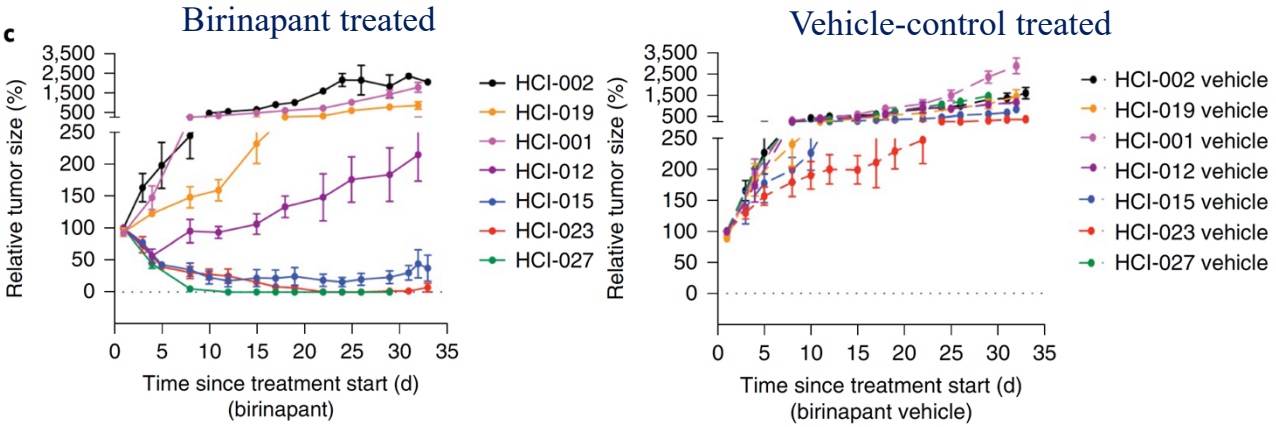


Fig. 7 | Growth rate-adjusted PDxO screening analysis ranks models in concordance with PDX response. c, In vivo drug treatment response to birinapant in various PDX models (left) with matching vehicle controls (right). (Mean ± s.e.m.; n=5).

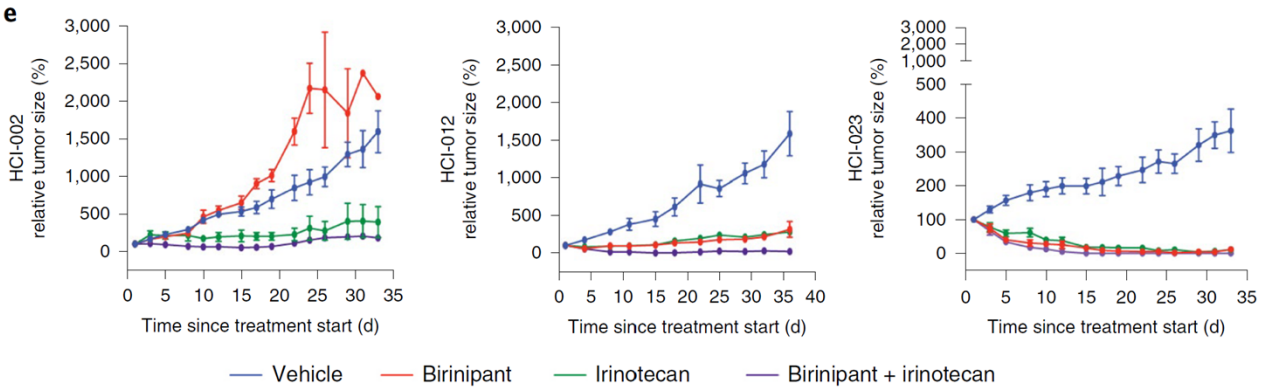
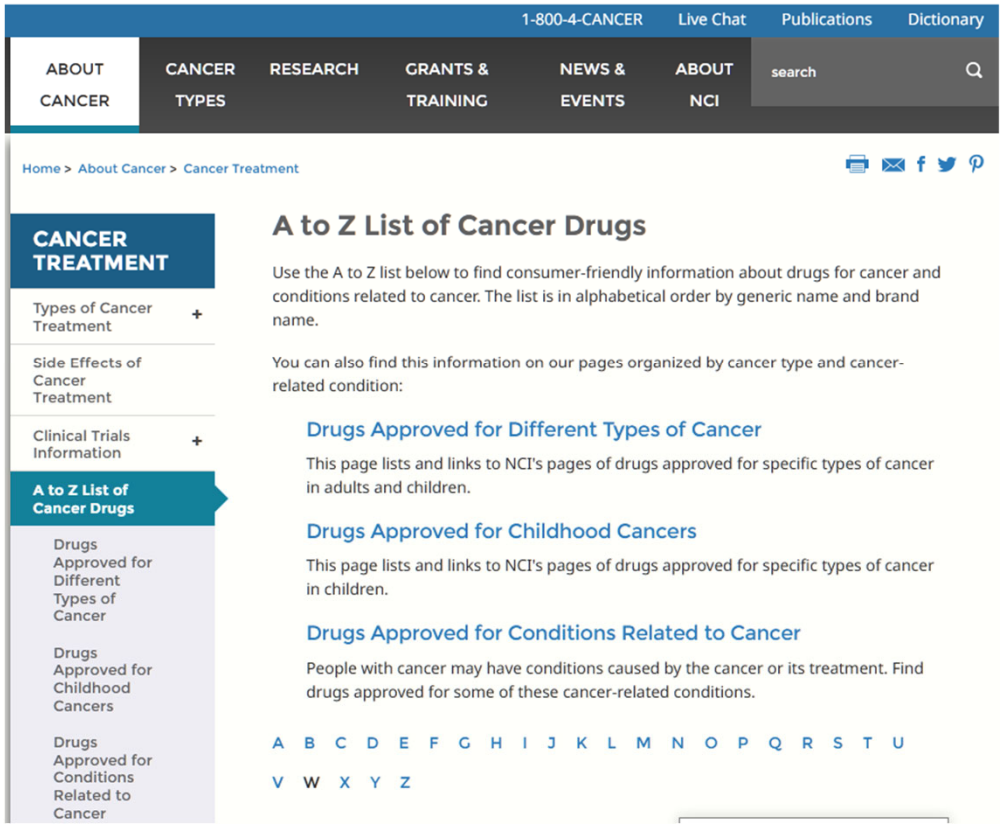


Fig. 7 | e, In vivo drug treatment response to birinapant, irinotecan or a combination in HCI-002 (left), HCI-012 (middle) and HCI-023 (right) PDX models.

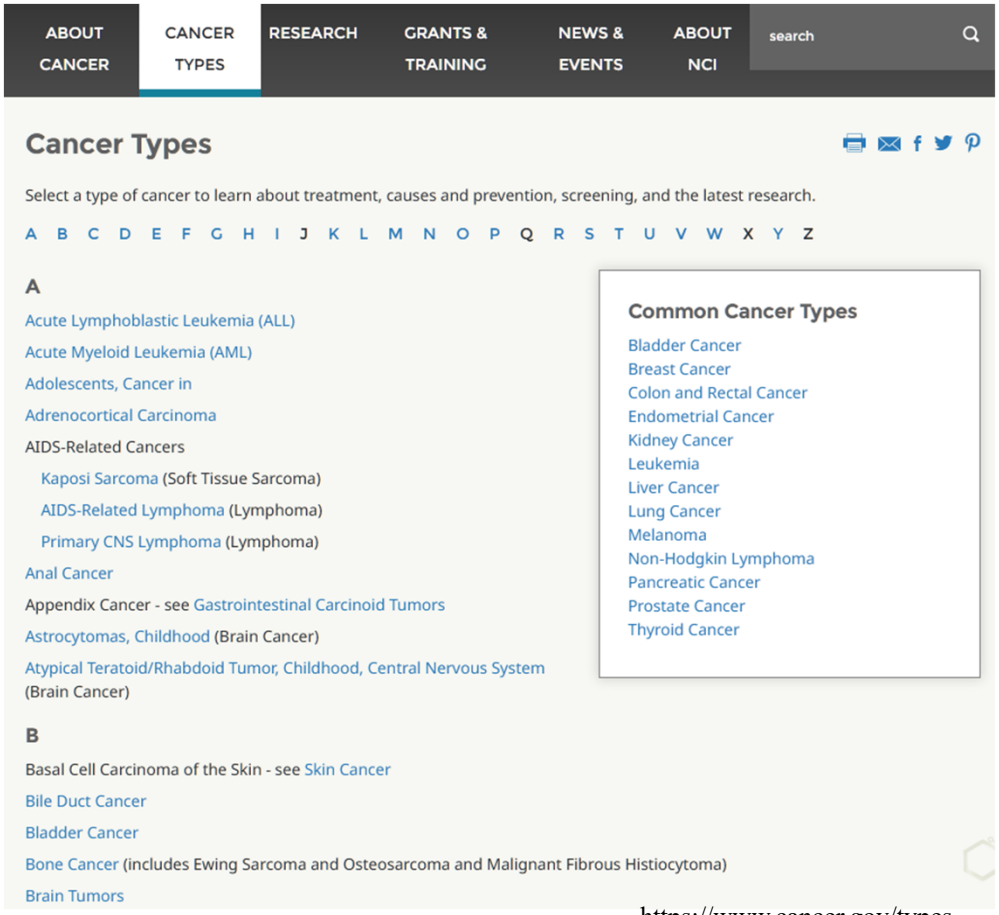
Guillen. ---, Welm. A human breast cancer-derived xenograft and organoid platform for drug discovery and precision oncology. Nature Cancer 3: 232-250, 2022. https://uofuhealth.utah.edu/huntsman/labs/welm-labs/

Standard Therapy as a Control for Tumor-Targeted Nanomedicine



~1,000 drugs for >150 different cancer types

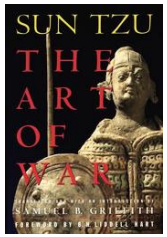
https://www.cancer.gov/about-cancer/treatment/drugs



https://www.cancer.gov/types

How to Win the War against Diseases?

The Art of War (孫子兵法)



"If you know yourself and know the enemy, you need not fear the result of hundred battles.
If you know yourself but not the enemy, for every victory gained you will also suffer a defeat. If you know neither the enemy nor yourself, you will succumb in every battle."

知己　知彼　百戰百勝

We will most likely win (cure).

and know our enemy (our diseases)

If we know ourselves
(our drug delivery technologies)

Knowing our space technology,
Knowing the Moon's orbit.

We do not understand our body

The nanomedicine field is highly developed and has a vast array of sophisticated nano-medicines in its armory. However, if we do not fully understand or oversimplify their behavior in the body and overstate their capability, we will not have the foresight to address many of the barriers in route to a meaningful clinical impact and will continue to have very few nanoparticles successfully make the march to the treatment site.

J. Reineke, Terminology matters: There is no targeting, but retention, J. Control. Release 273: 180-183, 2018

Moving Forward: Goal - Improving the Drug Efficacy and Safety

NIH Targeted Delivery Interest Group (TDIG)

(https://www.niaid.nih.gov/research/nih-targeted-delivery-interest-group)

The Targeted Delivery Interest Group (TDIG) is a NIH multi-institutional team that champions
targeted delivery of therapeutics to different organs
for selective and effective treatments with reduced harmful effects.
The group aims to identify gaps and opportunities to advance basic, translational, and clinical research for multiple delivery platforms applicable for the treatment of multiple diseases including autoimmunity, transplantation, cancers, neurodegeneration, cardiovascular diseases, and infectious diseases.

Treating diseases by improving the drug efficacy and/or safety requires:

- Understanding diseases more
- Building better models representing diseases
- Developing (targeted) delivery systems that can control biodistribution **better than standard therapy**
- Developing (targeted) delivery systems that have efficacy & safety **better than standard therapy**

- New proposal review considerations.

- Innovation should make things simpler, not more complicated.
- Developing clinically effective formulations is very different from publishing papers.
  It requires a re-iterative process solving various practical problems, e.g., scale-up production.
  We all are responsible for developing clinically useful formulations, not just those in the pharma industry.