The Challenges of Developing Targeted Theranostic Nanoparticles and Potential Solutions That Are on the Horizon

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It has been nearly 50 years since President Richard Nixon declared “War on Cancer” with the enactment of the National Cancer Act of 1971. Yet, according to the CDC the cancer death rate has decreased by only ~20% over that time, which pales in comparison to the >65% reduction seen in the death rate for diseases of the heart and stroke.(Sun, Ojha, Kiessling, Lammers, & Shi, 2017) The vast majority of cancer chemotherapeutics, which primarily consist of small-molecule drugs, have failed to make a major impact on the death rate for most cancer types. This can at least partially be attributed to the substantial risk of systemic toxicity, which limits the dose that can be administered. Systemic toxicity stems from the rapid clearance of small-molecule drugs, which requires the use of high doses to achieve appreciable serum levels, the ability of the drug to perfuse both healthy and diseased tissue, which can lead to undesirable effects in healthy organs, and broad mechanisms of action, which can lead to the disruption of unintended cellular pathways. Initially, it was thought that nanoparticles would provide an immediate solution to all of these problems.

Nanoparticles that are used in therapeutic applications are typically produced with sizes between 10 and 150 nm, to bestow long circulation times following intravenous administration. In general, drugs below 10nm are rapidly cleared by the kidneys, while nanoparticles larger than 150 nm are more efficiently cleared by phagocytic Kupffer cells in the liver. Nanoparticles are also designed to be biocompatible, so they do not elicit an significant immune response, and biodegradable to ensure eventual excretion.

There were many reasons for the initial excitement surrounding nanoparticles as drug delivery vehicles. Firstly, their circulation half-life in serum can be 10 to 100-fold longer than the small-molecule drugs that they carry.(O’Brien et al., 2004) This allows drugs more time to find their targets and generally allows for the use of lower doses. Longer circulation times are also generally associated with reduced toxicity to organs involved in drug excretion (e.g. kidney and liver), due to a slower accumulation in these organs and a lower maximum drug

concentration at any particular time. A second advantage of nanoparticles, compared with small-molecule drugs, is they do not freely perfuse through all tissues. Nanoparticles are confined to blood vessels and tissues that possess highly permeable vasculature, which is essentially just the liver, spleen, and tumor. This results in a lower chance of toxicity to healthy organs. The best known example of this is with the drug Doxorubicin, whereby its cardiotoxicity is reduced 7-fold when packaged into a nanoparticle.(O'Brien et al., 2004) Third, nanoparticles can be used to solubilize drugs that are highly hydrophobic and otherwise cannot be administered to patients, thus increasing the number of drug candidates.

Despite the clear advantages of nanoparticles, nanoparticle-based drug formulations have not consistently led to a significant improvement in patient survival compared with free drug. As a result, there are currently only 6 nanoparticles that have been FDA-approved for the treatment of cancer.(Ventola, 2017) There seem to be two primary (and related) reasons for the limited efficacy of nanoparticles, low levels of accumulation within tumors and limited penetration into tumor tissues. A recent survey of the literature from the past 10 years found that only 0.7% (median) of the administered nanoparticle dose is found in solid tumors, despite their extended circulation time.(Wilhelm et al., 2016) The accumulation that is seen is primarily driven by the enhanced permeability and retention (EPR) effect, which is a consequence of the increased vascular permeability in tumors and the poor lymphatic drainage. Once nanoparticles cross the vascular wall, they still need to penetrate a dense extracellular matrix in order to reach tumor cells. Unfortunately, because of their relatively large size, nanoparticles typically travel just tens of microns over the course of days.(Sykes, Chen, Zheng, & Chan, 2014; Wang et al., 2015) This has led to exploration of new strategies to improve both the accumulation and penetration of nanoparticles into tumors.

To facilitate the study and evaluation of nanoparticle pharmacokinetics, they are often prepared with both imaging agents and therapeutic agents. Nanoparticles that include both a "therapeutic" agent and "diagnostic" imaging agent are often referred to as a 'theranostic'.

The most common approach that is taken to improve the accumulation of nanoparticles within tumors involves functionalizing the nanoparticle surface with targeting ligands specific for a tumor biomarker. While targeting

alone does not address the issue of tumor penetration, some improvement in tumor penetration has been shown to increase with repeated dosing. The benefits that can result from targeting likely stem from improved retention of the nanoparticles within the tumor - as opposed to an increase in the amount of nanoparticle that reaches the tumor. Targeting is also likely to improve the probability of nanoparticle binding and internalization by cancer cells, in relation to the surrounding stromal cells, which can improve drug efficacy. Moreover, the targeting agent could exhibit an additive, or even synergistic, therapeutic effect on target cells when combined with the chemotherapeutic payload in the nanoparticle.(Yang et al., 2007)

While targeting is widely considered to be beneficial, numerous studies have shown that receptor targeting does not always result in a selective increase in the efficacy of therapeutic nanoparticles. It is now understood that many complicating factors can limit the success of targeted nanoparticles. Not surprisingly, poor tissue penetration remains a significant problem; however, heterogeneous antigen expression and/or the loss of cell surface antigen expression during disease progression are also problematic. One strategy that is being tested to overcome the high variability and instability of cancer cells involves taking advantage of cues in the tumor microenvironment to promote nanoparticle retention within tumors. For example, numerous nanoparticles have now been developed to be specifically retained in tumors in response to the acidic tumor microenvironment, matrix-metalloproteinases, hypoxia, binding of stromal cells and other factors that are commonly seen across most tumor types.(Du, Lane, & Nie, 2015) A slight deviation of this approach involves using biological cues to induce a change in the size of the nanoparticle, such that smaller nanoparticles are generated in the tumor environment and can diffuse more readily through the interstitium.(Li et al., 2016; Wong et al., 2011) While the use of targeting strategies that take advantage of the tumor microenvironment have looked encouraging in preclinical studies, the general consensus is there is still no single approach that can be used in all patients, due to patient-to-patient variability, and that there must be a move toward personalized medicine to determine which targeting strategies will be effective in individual patients.

As an alternative to using molecular and environmental signatures for targeting, externally administered stimuli have also been used to improve the accumulation and penetration of nanoparticles. Pharmacological stimuli have included the use of enzymes capable of degrading the extracellular matrix,(Parodi et al., 2014) inhibitors

capable of limiting matrix generation,(Diop-Frimpong, Chauhan, Krane, Boucher, & Jain, 2011) or drugs that can alter the vascular permeability or blood flow.(Chauhan et al., 2012) Physical triggers have also been used and include radiation,(Baumann et al., 2013; Koukourakis Sofia Koukouraki, Alexa, 2000) which is already part of the standard of care, and ultrasound,(Mullick Chowdhury, Lee, & Willmann, 2017) typically in combination with microbubbles. Both approaches can dramatically improve nanoparticle delivery when timed appropriately by increasing vascular and tumor permeability. Magnetic forces can also be used to enhance the accumulation and penetration of nanoparticles within tumors. While this has previously been limited to superficial tissues,(Al-Jamal et al., 2016; Schleich et al., 2014) due to the rapid drop-off of the magnetic field gradient with distance from the magnet, proper configuration of multiple magnets can be used to enhance the delivery of magnetic nanoparticles into deep (permeable) tissues.

Physical triggers can also be used to promote the release of drugs from nanoparticles. The hypothesis here is that once a drug is released it can more readily perfuse through the tumor tissue. A second possibility is that the rapid release of drug from intratumoral nanoparticles can lead to a higher effective dose. Importantly, for this approach, drug release must be limited to the tumor and is not triggered in healthy organs. The most common physical trigger that has been used to date is light irradiation, which can promote drug release from light- or thermally-responsive nanoparticles.(Linsley & Wu, 2017) However, the critical drawback of light is that it is limited to superficial tumors. However, recently it has been shown that it is possible to spatially target the heating of magnetic nanoparticles with alternating magnetic fields. In this case, heat is used to trigger drug release from thermally-responsive nanoparticles.(Tay et al., 2018) Nonetheless, a limitation of all physical triggers is that their effect is confined to the primary tumor. Therefore, it is envisioned that the use of external triggers will need to be complemented with biological targeting strategies in order to ensure the elimination of metastatic niches.

As advances continue to be made in nanoparticle design to overcome the many challenges of treating cancer, there has been a corresponding increase in nanoparticle complexity and cost, and yet there are still very few examples of clinical benefit. Notably, many of the failures along the way have stemmed from the inability to produce complex nanoparticles at large scale. Therefore, there seems to be a gradual movement back towards simplifying nanoparticle designs that can be produced in one or two steps, achieve high drug encapsulation

efficiencies, high drug payloads, and high conjugation efficiencies with little to no purification steps required. While it has been a longer than expected road toward finding effective treatments for cancer, we are slowly beginning to understand the obstacles that have thus far prevented nanoparticles from significantly reducing the cancer death rate and innovative solutions are being identified that will one day allow nanoparticles to live up to their lofty expectations.

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