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Nanotechnology Strategies to Advance Outcomes in Clinical Cancer Care

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Abstract

Ongoing research into the application of nanotechnology for cancer treatment and diagnosis has demonstrated its advantages within contemporary oncology as well as its intrinsic limitations. The National Cancer Institute publishes the Cancer Nanotechnology Plan every 5 years since 2005. The most recent iteration helped codify the ongoing basic and translational efforts of the field and displayed its breadth with several evolving areas. From merely a technological perspective, this field has seen tremendous growth and success. However, an incomplete understanding of human cancer biology persists relative to the application of nanoscale materials within contemporary oncology. As such, this review presents several evolving areas in cancer nanotechnology in order to identify key clinical and biological challenges that need to be addressed to improve patient outcomes. From this clinical perspective, a sampling of the nano-enabled solutions attempting to overcome barriers faced by traditional therapeutics and diagnostics in the clinical setting, are

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discussed. Finally, a strategic outlook of the future is discussed to highlight the need for next-generation cancer nanotechnology tools designed to address critical gaps in clinical cancer care.

Keywords

nanotechnology; alliance; immunotherapy; radiotherapy; biological barriers; cancer; oncology; national cancer institute; cancer nanotechnology plan; metastasis; image guided surgery

Efforts focused on implementation of nanotechnology into cancer treatment and diagnosis have been substantial in the pre-clinical space, with the field continuing to mature while waiting for its full impact to be realized. The original promise to oncology was predicated on the potential of improved drug delivery to tumor(s) with reduced side effects, as well as the earlier detection of cancer *via* either early-warning *in vitro* diagnostic devices or increased resolution of *in vivo* imaging detection. Clearly not a trivial task, as cancer is not a singular disease, nor does it involve a singular target. To date translation to the clinic of nanotechnology, has advanced at a pace comparable to the undertaking; a nominal improvement to cancer patient outcomes and care. Of course, the first clinical success occurred in 1995 with the approval of Doxil (a liposomal formulation of doxorubicin). This success story was bolstered by understanding of the core *in vivo* characteristics of liposomes that had been studied since the 1960's. Clinically approved nanomedicines have trickled in over the subsequent years and only recently have they begun to have approvals for next generation platforms (*e.g.*, targeted liposomes, combination therapies).[1] Additionally, over 80 clinical trials for cancer nanotechnologies are currently active and / or recruiting.[2] Many of these incorporate next generation nanomaterials platforms (*e.g.,* non-liposomal materials, targeted liposomes and more). Albeit, the field has not been without its share of setbacks and commercial disappointments during this time nor has it plateaued to being standard of care for any cancer indication.[3] As with any technology for human use, much has needed to be discovered, reassessed, and revised – and the process continues to this day.[4] Moreover, the only way by which it will continue to thrive is by way of the return on the ongoing investment; specifically, an improvement in patient outcomes over the current standard of care.[5]

There is no doubt that significant and highly innovative work has been demonstrated in the pre-clinical space, and a limited number of nanoscale products have already been approved for cancer.[6] There has been a rapid expansion in clinically-promising nanoscale systems for drug delivery,[7] including polymeric particles,[8] inorganic particles (*i.e.*, silica),[9] metallic particles (*i.e.*, gold),[10] iron oxide[10] and solid lipid-based materials. In addition to addressing unmet clinical needs, these therapeutic particle systems have evolved to address pivotal challenges associated with our understanding of cancer as a complex, dynamic disease process. Their tunable size, surface chemistry, and architecture confer distinct biological properties and enable transport of diverse payloads with high efficiency.[11] However, based on an examination of the field by common merits, *vis-a-vis* measuring medical technologies from their *impact (e.g.,* increased safety or efficacy*), time to market (e.g.,* cost*), and regulatory approval(s),* there remains a legitimate argument that the field has yet to come of age. As testament to this, the primary advantage provided by currently available

nanomedicines for cancer is associated with a reduction of side effects to the patient when compared to the respective standard of care.[12] This improvement stands behind success of Doxil and Abraxane (albumin formulation of paclitaxel). Currently available nanotherapeutics offer, at best, only a modest increase in survival (*e.g.,* in treatment of pancreatic cancers), relative to their small-molecule drug counterparts. Although, several recent nanotechnology platforms have received approvals (*e.g.*, Vyxeos™, AML and Onivyde™, advanced pancreatic cancer) while displaying a legitimate increase in efficacy and / or for indications that good solutions did not already exist. These platforms played upon strengths of nanotechnology by way of combined delivery of small-molecule drugs (*daunorubicin* and *cytarabine*) or the efficacious delivery of a small-molecule drug (*irinotecan*) at tolerable toxicities. However, it is expected that further increases in efficacy can and will be achieved for non-liposomal platforms. This will only be accomplished by re-envisioning the *fundamental and clinically relevant mechanisms involved in nanoscale delivery* as well as *by incorporating precision medicine steps as part of trial designs to better select, stratify, and treat disease*. Furthermore, recent successful nanodrug approvals and much research, as to *in vivo* delivery mechanisms, have reminded all that % dose delivered to tumor is *not* the only aspect that separates efficacious drug *versus* failed platform.[13-16] Simply stated, tumors are heterogeneous tissues with varying degrees of surface area, vasculature, and local inflammation. All of this will affect delivery on a case-by-case basis, but much can be gleaned from the last century of drug delivery principles coupled to a renewed understanding of the core biology with nano-sized delivery platforms.

Public funding began in 2001 and has been directed towards both therapeutic and diagnostic development of next generation nanotechnology platforms as well as resources to enable their translation. The goal of any publically funded effort in healthcare research is to advance our collective understanding of disease and/or to bolster emerging areas in medicine which need inducement; both of which enable a more successful or rapid transition to the clinic by way of the private sector and ultimately improved outcomes for patients. Publically funded efforts for cancer nanotechnology (*e.g.,* NCI Alliance for Nanotechnology in Cancer begun in 2005) have had the same goals in mind and continue to do so by way of coherent and calculated prospective strategies for team science. The results of which have been a bolus of pre-clinical work (*e.g.,* over 3600 publications cited more than 170K times) and translational output (*e.g.,* over 100 companies, 24 clinical trials and over 220 patent disclosures). Over this time, strategic planning has occurred by way of workshops, reports and much more.[17-19]

The National Cancer Institute publishes the Cancer Nanotechnology Plan (CaNanoPlan) every five years to assess the status of the field and chart strategic directions for the future. The most recent iteration was published in 2015[20] and was written by researchers and clinician scientists who are pioneering nano-enabled solutions for cancer. The CaNanoPlan 2015 is focused on areas that have matured considerably over the last decade and are approaching clinical utility, as well as ones which are beginning to evolve from a comprehensive body of experts in the field. The preparation of the CaNanoPlan revealed that while the field of cancer nanotechnology is maturing and evolving, it is also diverging into multiple sub-disciplines. Beyond the recent nano-enabled success stories of delivering therapeutic combinations, we consider several specific areas, which are described more

broadly herein, as having strong potential for further development and translation by way of nanoscale platforms. They include: *exploring nanotechnology opportunities in cancer immunotherapy*, *tools for interrogation of tumor in vivo,* and *overcoming traditional barriers to cancer therapy*. The underlying principal that motivates this contribution is to discuss these areas from a clinical and biological perspective in order to aid in rationally driving them forward in the near future.

Immunotherapy *via* Nanoscale Solutions

Cancer immunotherapy has emerged as a powerful long-term strategy in cancer treatment.[21,22] Antibodies that block negative immune regulatory pathways (checkpoint inhibitors), including antibodies targeting the CTLA-4 (*cytotoxic T-lymphocyte–associated antigen 4*) and PD-1 (*programmed cell death 1*) receptors, improve survival in several difficult to treat diseases, including melanoma, bladder, renal and non-small-cell lung cancer (*e.g.,* Pembrolizumab, Durvalumab, and Nivolumab). Following upon years of pre-clinical evidence and discovery research dating back to the 1987 discovery by Allison *et al*, checkpoint inhibitor based therapies are currently the basis of over 140 clinical trials.[23-25] In addition to checkpoint blockade agents, dendritic cell therapy and chimeric antigen receptor (CAR) T-cell therapies have also achieved clinical success (*e.g.*, Kymriah, Yescarta). In a similar trend to checkpoint inhibitor therapies, cell-based CAR T-cell therapies, including those pioneered by Rosenberg *et al*, are now part of over 230 clinical trials.[26-29] Lastly, recent clinical data suggest that some cancer vaccines as well as immunotherapies acting as the backbone therapy when coupled in combination with other therapeutic modalities may also provide survival benefit. Such successes have generated high interest in developing strategies to further improve cancer immunotherapy.[30]

The major limitation of checkpoint inhibitors is the low rate of long-term, durable responses. Most patients eventually develop resistance and progressive disease. CAR-T cells are difficult to engineer, maintain, and have high toxicity (frequently fatal) if the targeted antigens are also present on normal cells. As such, this approach is limited hematological cancers of specific cell types which can be augmented and controlled post-therapy. Lastly, current dendritic cell therapy has low potency and the therapeutic benefit is only realized several years after treatment. The primary aspects that separate these approaches for their respective clinical use cases are (*i*) the patient response rate; (*ii*) the ability to be used for solid tumor *versus* hematologic cancers; (*iii*) durability of response; and (*iv*) the range of the toxicological profiles post treatment. Thus, there is ample opportunity for the development of alternative strategies and therapeutics to improve cancer immunotherapy.

Immune activation against tumor cells is a highly complex process. Nanomedicine has a few distinct advantages for improving cancer immunotherapy (Figure 1). Nanoparticles, because of their virus-like size, readily elicit an immune response upon *in vivo* administration. With or without pegylation or other anti-fouling surface modification, nanoparticles are taken up by macrophages and other antigen presenting cells (APCs). In the past, the field has worked diligently to minimize this immune activation. However, in the context of cancer immunotherapy, such immune activation is advantageous and can be utilized for therapeutics development. For example, nanoparticles are being utilized to deliver tumor antigens to

APCs to enhance immune response.[31] Nanoparticle-bound antigens have been shown to elicit greater immune responses than free antigens. In addition, nanoparticles can also act as immune adjuvants, enhancing response when given together with cancer vaccines. Particularly, nanoparticles' ability to deliver therapeutics which are synergistic with other immune applications.

Nanoparticles can be formulated to deliver pro-inflammatory/pro-immune molecules with tumor antigens to enhance immune reactions. Such co-delivery is more likely to activate APCs and thus result in robust immune responses. Another budding application for nanoparticles in immunotherapy includes the development of tumor-targeting T cells as well as CAR-T cell treatments especially in treatment of solid tumor.[26,32] Specifically, nanoparticles' ability to deliver molecular agents, such as CRISPR-CAS, will improve the engineering of CAR-T cells as well as other types of tumor-targeting T cells *in vivo*.[33-35] Furthermore, nanotechnology enabled separation of tumor infiltrating lymphocyte subpopulations *in vivo* could increase throughput for adoptive cell transfer approaches in solid tumor applications. These approaches have had only limited clinical success since their initial application with metastatic melanoma, although have the potential to induce durable and curative responses across patient populations for solid tumor. Thus, are ripe for solutions that can be imbued *via* nanotechnology.[36-39]

Targeting and controlling regulatory T cell (Treg) sub-populations (CD4+, CD25+, and Foxp3+) are another potential avenue by which nanoparticle delivery systems will play a role.[40-42] Treg cells act to maintain immune response homeostasis by way of modulating/ suppressing self-tolerance. Paradoxically, this same mechanism acts to drive tumor progression by way of immune evasion in cancer. Treg cells act by way of several competing mechanisms to downregulate the targeting / activation of APCs by effector T cells and cytokine starvation (*e.g.,* IL-2). Treg cells are recruited (*e.g.,* biochemically attracted) to tumor sites and ultimately accumulate in both peripheral sites (peripheral blood, spleen) as well as infiltrating the tumor tissue. The degree by which this upregulation of Treg occurs often correlates to increased tumor burden and negative patient outcomes. It is an interplay between this primary component of immune evasion in cancer and other regulatory pathways that reduce the efficacy of checkpoint inhibitors. As such, much biological research has occurred in the last decade to elucidate the subpopulations of T cells and their mechanisms of activation. Furthermore, targeting of cancer specific Treg cells is an active area of clinical research for both the peripheral and local populations either for therapeutic regulation/reprogramming or diagnostic assessment for downstream prognostication.[43,44] Nanotechnology has the ability to target the cancer derived subpopulation by way of its commonly upregulated surface markers. Further silencing of the genes specific to this set of Treg cells, reprogramming, or labeling for diagnostic imaging of tumor burden are all possible routes nanoparticle solutions. Alternatively, reprogramming phenotypic aspects of tumor-associated macrophages (TAMs) is an exciting therapeutic route being investigated. Many nanomaterial types preferentially accumulate in these cell types located within the tumor microenvironment already. Thus, this offers a passive approach that expands upon current nanomaterials PK/PD properties to selectively deliver immunotherapeutics or disrupt/change phenotype *via* colony-stimulating factor 1 receptor (CSF1R), for example.

Given the exciting clinical data with checkpoint blockade inhibitors, approaches that combine nanomedicine and checkpoint blockade inhibitors are most likely to have immediate clinical impact. Future studies should focus on which checkpoint blockade agents and regimens are synergistic with nanoparticles and how nanoparticle-based agents can be integrated into checkpoint blockade treatments and in combination with multiple therapeutic modalities capable of imbuing durable response rates beyond primary tumor to metastatic sites (*e.g.*, timing of nanoparticle administration, localized radiotherapy to induce cancer cell antigen presentation and its downstream abscopal effects, *etc.*).[45-47] Once more alternative routes (*i.e.,* beyond CTLA-4 and PD-1) for checkpoint inhibitors are displayed (*e.g.,* glucocorticoid-induced TNFR-related protein, KIR2DL1/2L3, T-cell membrane protein 3, OX40, and more) to be successful in clinical trials, these will offer legitimate pathways for nano-enabled delivery vehicles as well.[48] More importantly, given that local immune reaction can translate into systemic immune response, nanotherapeutics can be given locally as vaccines or devices in clinical application.[49,50] Nanoparticle systems can also been used to target lymphatics and induce compliment activation to induce an increased systemic response. Thus, further development in nano-scale systems that can either hijack other cells or are biomimetic will continue to play a role in cancer immunotherapy.

Measuring patient response to immunotherapy (*e.g.,* cytokine release syndrome) and/or its side effects as well as preemptive establishment of responders and non-responders remain as other crucial areas[51] of which nanoscale platforms will have impact.[52-55] Tools for these applications include both *in vivo* imaging agents and *in vitro* diagnostics. Specifically, nanoenabled tools that measure T-cell response, tumor specific subpopulations of *via* peripheral T cells or cytokines, can significantly improve prognostication and cancer management.[56] These tools (*e.g.*, as companion diagnostics) simultaneously help reduce the burden of clinical translation in the approval process of experimental immunotherapeutics, especially when combined with other therapeutic regimens. Regulatory hurdles will necessitate the separation of efficacy from each regimen, and secondary endpoints validated by way of specific, sensitive nanoparticle systems could increase the chance of success.

Interrogating Cancers: *During Surgical Resection*

Detecting, staging, and treating cancer at earlier stages is paramount to reducing cancer mortality. While surgical management serves as a cornerstone of clinical cancer care, intervention is mainly limited to early stage tumors. Outcomes are often dramatically poorer when surgery is no longer a viable option. A key goal of cancer surgery is to reliably distinguish cancer from normal tissues to effect surgical cures while concomitantly preserving cosmesis, limiting risk of injury to soft tissues, and increasing throughput. However, this goal remains technically challenging. *Current resection techniques use palpation and visual inspection to detect subtle textural differences, rather than elucidate molecular signatures defining a given disease stage*. This significantly limits the ability of the operating surgeon to accurately identify the extent of malignancy, microscopic tumor burden, and remnant tissue, particularly at surgical margins, raising the risk of incomplete resection. As these factors collectively affect therapeutic outcomes, prognosis, and treatment management, *targeted optically-driven visualization tools and/or sensors are critically needed in the intraoperative suite to guide minimally invasive procedures with cellular-level*

precision, enhance surgical vision, and provide real-time structural-functional imaging assessments of normal and diseased tissues. Such tools are expected to significantly impact oncologic outcomes by playing an increasingly pivotal role in facilitating surgical diagnosis and management which, in turn, could change surgery on-the-fly.

While technical advances in large-scale imaging devices (PET-CT, MRI) have led to improvements in preoperative cancer diagnostics and staging, their utility has been limited by achievable spatial resolution, sensitivity, and/or practical difficulties of intraoperative implementation. Further, image-guided optical imaging approaches have traditionally been hampered in surgical settings by the small number of available targeted near-infrared (NIR) agents, superficial tissue penetration, limited contrast resolution, rapid photobleaching, and an inability to detect multiple cancer and/or normal tissue targets (multiplexing) known to control different biological processes. Significant progress, however, has been made to overcome these drawbacks, with considerable efforts directed towards developing an increasing number of optically-active and targeted imaging agents, including particle-based probes, for clinical intraoperative use. Designed to offer enhanced sensitivity, specificity, and depth penetration, these probes promise to dramatically improve real-time early-stage molecular detection and treatment of disease in conjunction with state-of-the-art multichannel fluorescence camera systems with clinical grade accuracy (Figure 2). Unlike other imaging modalities, this combination also enables real-time interrogation of biological processes and/or concomitantly identifying one or more tissue-specific biomarkers to facilitate (*i*) accurate identification of cancerous nodes, margins, and adjacent nerves; (*ii*) reliable staging; and (*iii*) precision-based treatments. Such markers can be further validated in the clinical trials setting. Current high resolution intraoperative camera systems permit lesion detection down to sizes smaller than 60 μm, dramatically improving detection sensitivity and specificity over human vision, and truly revolutionizing imaging capabilities. Such tools are being seamlessly integrated with minimally-invasive, robotic-assisted surgical equipment to enable targeted navigation to sites deep within the body. *Collectively, the potential of these technologies to improve outcomes, minimize risk, promote clinical throughput, and lower health care costs represents a significant clinical advance, and promises to transform the current practice of surgical oncology*.

While a significant volume of work has been performed utilizing endogenous tissue contrast or non-specific optical agents, such as indocyanine green (ICG), an FDA-approved NIR fluorescent dye for selected clinical indications, the lack of selective targeting limits their utility for applications aimed at direct cancer cell detection. To impart labeling specificity, a number of targeted and optically-active imaging products, including antibody- and peptide-dye conjugates, have served as visualization tools for improving assessments of surgical margins and metastatic nodes by attaching to upregulated cancer receptors at these sites. Although not yet reaching its full potential in surgical practice, early potential benefits of optical imaging have been shown in clinical studies utilizing targeted molecular probes.[57] In some cases, conjugation to hydrophobic, visible dyes have limited probe bioactivity, tissue contrast, and depth penetration.

More recently, the emergence of diverse classes of NIR optically-active nanomaterials designed to improve sensitivity, accuracy, and reliability of detection of cancerous tissues

over that of organic dyes, offers exciting possibilities for probing and characterizing molecular targets within human subjects.[11] Relative to simple molecular agents, particle-based physicochemical properties are tuned in a controlled manner to achieve more desirable imaging, pharmacokinetic (PK) and tumor-targeting properties. For instance, dye encapsulation within silica nanoparticles[58], rather than their surface-conjugation, have led to exquisite photophysical features offering enhanced depth penetration (*i.e.*, 10^{-2} m). Collectively, these adaptations serve to maximize tumor-to-background ratios and *in vivo* detection sensitivity (*i.e.*, at least nanomolar), with the ultimate goal being to identify and remove all cancer cells.

Using such diverse, highly versatile, and integrated particle surface designs, key surgical indications have the potential to be performed more reliably and accurately. The focus of ongoing particle-driven applications includes (*i*) image-guided treatment of cancer-bearing lymph nodes,[59,60] (*ii*) precise mapping of surgical margins,[61] (*iii*) detection/treatment of remnant disease, and (*iv*) reliable assessment of tissue function (*i.e.*, perfusion). For improved mapping of sentinel and higher-tier nodes, the ability to conduct fluorescence-based multiplexing studies in order to highlight multiple predictive biomarkers on heterogeneous cancer-bearing nodes using spectrally-distinct cocktails of particles,[62] will have implications for systemic treatment planning. Improvements in false negative rates, staging accuracy, and surgical risks can also be expected. For instance, particle probes, optimally-sized to 5–10 nm for nodal mapping, will ensure adequate lymphatic transport and nodal retention, thereby overcoming extravasation and less favorable contrast and PK associated with organic dyes. One such ultrasmall, molecularly-targeted, and optically-active silica nanoparticle probe[63], Cornell dots (or C dots), is in Phase 1/2 clinical trials (Figure 3).

A second indication, surgical margin mapping, involves precise delineation of tumor extent. Positive margins serve as a negative prognostic indicator for many solid cancers. The presence or absence of tumor cells at the site of resection- a key determinant of treatment success or failure- is used for therapeutic decision-making. Particle technologies such as MR-photoacoustic-Raman imaging nanoparticles,[61] are used to overcome these limitations and more accurately delineate tumor margins. Another higher resolution whole-body optical imaging strategy, multispectral optoacoustic tomography,[64] detects optical absorption by means of ultrasound, and performed at depths greater than those typically achievable with fluorescence imaging. This method can detect a broad range of light-absorbing nanoparticles (gold nanorods)[64] and endogenous chromophores to yield high-resolution optical assessments of targets deep to the tissue surface, as well as provide functional measures of viability and/or perfusion.

Although significant data has been generated to support the translation of optically-active particle probes for intraoperative cancer treatment, advancing such agents into the clinic has been challenging, particularly those exhibiting molecular specificity. Importantly, FDA IND/IDE approvals have been issued for both targeted particle drug[63] and device[65] technologies, respectively; such developments are paving the way for translating additional targeted optical technologies to the clinic for use in image-guided surgeries. Thus, rather than relying on visual and tactile cues for guiding disease assessment and therapeutic management, the surgeon will utilize a growing array of dedicated intraoperative treatment

tools. It is anticipated that fluorescence-enhanced surgical vision, will significantly impact and likely transform conventional surgical practice in oncology over the next 5 to 15 years by increasing the sensitivity and accuracy of surgical procedures that map surgical margins, loco-regional cancer-bearing nodes, and micrometastases with single-cell precision. Acquired intraoperative structural imaging information will be acquired alongside functional optical imaging evaluations to enable real-time treatment management decisions based on (*i*) three-dimensional (3D) maps of tissue function (*i.e.*, oxygenation status, perfusion, and tissue viability); (*ii*) deep tissue imaging assessments, and (*iii*) volumetric images of *in situ* lesions for surgical navigation. This body of structural and functional data can then be seamlessly co-registered with augmented-reality displays and/or pre-treatment planning volumes for enhanced identification of normal (or pathologic) tissue structures prior to implementation of perioperative high-precision therapies.

Nanoparticle surface versatility and their distinct physicochemical and biological properties will play a key role in precision surgery and pathology, providing future opportunities to probe critical cancer targets and identify potential imaging biomarkers reflecting an array of biological processes – all of which can be validated in clinical trials. Although in its infancy, a variety of particle therapeutic strategies are currently under development for effectively treating disease in the intraoperative setting alongside standard of care therapies. For instance, targeted particle radiotherapies and/or the use of radiosensitizing nanomaterials with high atomic number (Z), will be used to significantly enhance radiation dose within the tumor tissue and, thereby, therapeutic efficacy, with relative sparing of normal surrounding tissues. The future implementation of such transformative tools in clinical practice should accelerate the growth of minimally invasive surgical procedures with the intent of limiting treatment-related morbidity and dramatically improving outcomes.

Interrogating Cancers: *Stimuli-sensitive Self-assembly and Disassembly to Measure Therapeutic Response, Treat, or Both*

Clinically approved and translatable drug delivery systems have been designed to overcome a number of technical hurdles limiting the therapeutic benefits of earlier generation cancer nanomedicines. As the vast majority of these nano-formulated therapeutic platforms are larger than ~10 nanometers (nm) in diameter, their successful non-specific delivery relies upon the EPR effect. Primary obstacles limiting translation have included inefficient delivery to target sites, lack of uniform intratumoral distributions, unfavorable pharmacokinetic (PK) profiles, dose-limiting toxicity, and narrow therapeutic indices.[17] In addition, opsonization and uptake in resident phagocytic cells of the reticuloendothelial system (*i.e.*, liver, spleen, bone marrow, blood) continue as hurdles in rational design of nanoparticle size, shape and more. Few of the current drug delivery vehicles adapted with surface-targeting ligands, which should improve specificity, are in clinical trials perhaps related to chemical and/or pharmacokinetic complexities. Therefore, although a large number of nanoparticle imaging probes have been developed in research laboratories, only a small subset will be suitable for clinical translation.

In addition to addressing the aforementioned challenges, it is clear that given the complexity of cancer, along with tumor heterogeneity and drug resistance, the use of single-agent therapeutic platforms will not be sufficient. To increase the effectiveness of cancer treatments, delivery strategies have progressively shifted towards “multi-pronged” combination approaches. Towards this end, substantial efforts have recently been directed towards developing (*i*) nanocarriers functionalized with multiple drugs that modulate and/or act through different pathways and diverse mechanisms;[66] (*ii*) therapies incorporating biological therapeutics, such as genes, antibodies or siRNAs, in combination with other drugs,[66,67] or (*iii*) “smart” nanocarriers, or stimuli-sensitive platforms,[7,66] that may be used singly or in combination for active targeted cancer therapy. Stimuli-sensitive platforms, can offer better spatiotemporal control of cargo release (*e.g.*, drugs) in response to external cues or internal cues present in the tumor microenvironment. Internal cues triggering release include over-expressed enzymes, low pH, and elevated redox potential, while externally applied stimuli facilitating “on-demand” drug release include magnetic fields, ultrasound, and near-infrared light. Upon exposure to such triggers, stimuli-responsive systems change in composition or conformation, and are accompanied by changes in physicochemical properties and/or drug release.

A number of groups have utilized such stimuli-sensitive strategies (*vide infra*) for overcoming size limitations and/or associated key challenges of larger formulations. These strategies have enabled the scaling down of particle size to create renally-clearable platforms for both diagnostic and therapeutic applications, including (*i*) sub-10 nm enzymatically-cleavable silica nanoparticle drug conjugates (NDCs)[68] that target genetic mutations expressed by different cancer types and (*ii*) enzymatically-triggered nano-aggregated constructs[69] for monitoring treatment response which rely on the self-assembly of endogenous biochemical species and/or utilization of biological pathways.

For the latter class of agents, one precision medicine approach that has permitted monitoring of tumor progression, *vis-a-vis* measuring tumor response to therapeutics in the clinical setting, is termed Target-Enabled *in situ* Ligand Assembly (TESLA).[70] As early assessment of drug-induced tumor cell death is of great prognostic value – enabling rapid selection of the most efficacious treatment and / or precise delivery of therapeutics – such agents could provide a promising solution to challenges encountered in nanotechnology-based cancer imaging of treatment response. This approach is conceptually different from that generally adopted by nanoscale platforms in that particles are built *in situ* at tumor sites from small molar mass building blocks taken up by target cells, as outlined in Figure 4A. Their much smaller size and well-controlled chemical structures enable these probes to readily cross the vasculature into tumors and diffuse into the interstitial space. When TESLA probes meet the tumor-specific target (*i.e.*, enzyme, other endogenous biomolecules), *they are ‘activated’ to form nanoparticles at the tumor site*, affording prolonged retention and enhanced imaging contrast.[71] A variety of tumor-specific biomarkers may be imaged using this strategy to interrogate cancer biology *in vivo*, for example, redox potential in cancer cells and caspase-3/7 in tumor apoptosis. One such agent – an [^{18}F]-labeled, *caspase 3/7*-sensitive, and nanoaggregation PET probe, [^{18}F]-C-SNAT – has been validated for direct PET imaging of *caspase-3* activity with a doxorubicin-induced tumor apoptosis model in nude mice bearing HeLa tumor xenografts (Figure 4B).[72] A direct comparison of its performance to

established radiotracers, ^{18}F-FDG, ^{99m}Tc-Annexin V and ^{18}F-ML-10, for the non-invasive detection of cell death has displayed favorable image contrast and better ability to sensitively and specifically detect tumor cell death in the lymphoma model.[73,74]

Another recent example of a translatable “smart” particle-based system exhibiting anti-cancer activity with internally-triggered cues involves an ultrasmall nanoparticle drug conjugate (NDC), adapted from a clinically translated sub-10 nm fluorescent core-shell silica nanoparticle, C dots.[9,63] NDCs incorporate tyrosine kinase inhibitors, which are attached to the particle surface through a protease sensitive, self-immolative linker responsive to lysosomal cysteine proteases (*i.e., cathepsin B*)[68] for site-specific tumor therapy at doses that are nearly 1000-fold lower than the free drug. In addition, only a few “smart” platforms, responsive to external triggering, have recently made it to the clinical trial stage and/or have been approved for treating cancer:[7,10] thermosensitive liposomes (ThermoDox) and magnetic iron oxide particles (NanoTherm AS1, MagForce Nanotechnologies).[10]

Further, multi stimuli-sensitive nanopreparations bearing different classes of drugs could serve as a broad platform for enhanced delivery of various poorly-soluble anti-cancer drugs into many tumors and individual tumor cells in response to locally up-regulated enzymatic activities. Further research into matrix metalloproteinases (MMPs) and glutathione (GSH), for instance, as additional endogenous stimuli are being explored. Over-expression of MMPs in certain tumor types are the stimulus for either targeted delivery of *in vivo* contrast or therapeutic agents.[75] These, and many other potential endogenous stimuli exist that will prove useful for self-assembly and/or subsequent biodegradation of nanoscale diagnostic[76] and therapeutic delivery vehicles. Distinctive to all of these systems is the ability to directly measure real-time biological processes, specific to the developing tumor and its supportive microenvironment. Advantages of these classes of nanotechnology platforms are that they overcome common limitations in both small-molecule and nanoscale materials delivery; specifically, low bioavailabilty inefficient cellular uptake, and / or poor solubility. Additionally, their respective translational paths will prove less complicated, as they are delivered as small-/medium-size molecules or macromolecular agents, reducing both manufacturing and regulatory burdens.

Overcoming Traditional Barriers to Cancer Therapy: *Crossing Biological Barriers*

Low efficiency in drug delivery into solid tumor has been the major challenge in cancer therapy. Following systemic delivery, chemotherapy drugs are rapidly cleared out from blood circulation, resulting in a very low level of drug delivery in tumors (0.001% to 0.05% of total injected dose (ID)/gram).[77,78] Non-targeted or targeted nanoparticle drug carriers, as well as more recent “self-therapeutic” particles[79,80] that can modulate metabolic and/or cell signaling pathways[80,81] in the absence of cytotoxic drugs, are highly promising therapeutic vehicles, as they can prolong blood residence half-times, increase intratumoral uptake (0.5 to 10 % of ID/gram), and/or enhance treatment response at overall lower effective doses when compared to the free drug, leading to reduced systemic toxicity.[82-84] Importantly, self-

therapeutic particles can act as “stand-alone” therapeutics[85] - an recent treatment strategy for inducing cell death programs and/or modulating the tumor microenvironment. They can potentially enhance treatment efficacy or overcome drug resistance in certain tumor types by (*i*) reducing barriers to transport (*i.e.*, fibrotic stroma), (*ii*) enhancing vascularity, and/or (*iii*) diminishing tumor growth.

It is well known that human tumors impose a number of physical barriers that collectively limit drug delivery into the tumor.[86,87] Abnormal tumor blood vessels that are unevenly distributed, lack a mature vascular structure, and demonstrate high permeability represent initial barriers to drug delivery.[86] The well-vascularized peripheral margins of tumors are comprised of leaky blood vessels that lead to extravasation of therapeutic agents, including nanoparticles, into the perivascular space by an enhanced permeability and retention effect. However, the tumor center and proliferating nests of tumor cells, which typically lack vascular function and / or blood vessels, present more challenging barriers to delivery. Moreover, areas of tumor perfused by relatively normal blood vessels may also slow delivery. Finally, high intratumoral pressures, induced by progressive interstitial fluid accumulation and poor lymphatic drainage, creates a positive pressure gradient that retards intratumoral drug transport and distribution.[86,87] Thus, the development of particle probes capable of navigating biological barriers is of *paramount importance* for ensuring successful particle therapeutic strategies for clinical care.[88]

In addition to crossing vascular barriers, therapeutic agents must navigate tumor stromal barriers by passive diffusion in order to reach tumor cells. Many solid tumors have a dense fibrotic stroma that significantly restricts intratumoral transport of therapeutic agents. It has been shown that the majority of nanoparticle systems are trapped in perivascular networks.[86,87] Thus, to achieve a significant treatment response, further approaches are needed to overcome stromal barriers in order to enhance delivery of high therapeutic particle concentrations to tumor cells. The tumor stromal barrier (Figure 5) has two components: the stromal cell barrier, which mainly includes fibroblasts and macrophages and the extracellular matrix barrier, which consists of collagen, fibronectin, hyaluronan, fibrin, and proteoglycan. Moreover, extensive infiltration of tumor associated macrophages into the stroma not only creates a physical barrier, but reduces bioavailability by non-specific uptake of nanoparticles in tumor tissues.[89]

By comparison with other drug delivery vehicles, therapeutic particle-based systems offer an opportunity to overcome these delivery barriers. For example, human serum albumin-paclitaxel nanoparticles could disrupt pancreatic tumor stroma and increase drug delivery.[90] Theranostic nanoparticles targeting cell receptors highly expressed on tumor stromal and cancer cells, such as *insulin-like growth factor 1* (IGF-1) receptor, were able to disrupt the tumor stromal-cellular barrier and significantly inhibit tumor growth in a human pancreatic cancer patient tissue-derived xenograft model.[91] In addition, iRGD peptide-conjugated nanoparticles that target $\alpha_V\beta_3$ *integrin* have also been shown to transport particles among different cells to facilitate penetration into the center of the tumor.[92]

Such receptor-targeted theranostic nanoparticles also have potential to overcome other cancer cell-specific delivery barriers involving the cellular membrane and multi-drug

resistance (MDR) mechanisms.[3,93] For instance, receptor-mediated internalization of theranostic nanoparticles *via* the endolysosomal pathway leads to efficient intracellular drug delivery while bypassing MDR (p-glycoprotein) on the cellular membrane. Currently, investigations have focused on the development of nanoparticle drug carriers that can overcome barriers in human tumors. Examples include protease-active particle probes and targeted theranostics able to bind to a range of intratumoral cells (*i.e.*, endothelial cells, fibroblasts, macrophages, and cancer cells). Furthermore, theranostic nanoparticles can also be modified as imaging agents to monitor drug delivery in highly heterogeneous human tumors.

Other traditional biological barriers also remain a challenge to drug delivery. One barrier, the blood-brain barrier (BBB), remains a significant obstacle to the delivery of drugs to primary gliomas and central nervous system (CNS) metastases arising from breast and lung carcinomas.[94,95] Collectively, these tumor types are among the most aggressive and deadly cancers known with 5-year survival rates of < 5%. As a result of an inability to efficiently deliver chemotherapeutics across this barrier following systemic injection, surgical resection followed by radiotherapy remain the principal standard of care treatment options- both of which are highly invasive and destructive to neural tissue. Recently developed delivery strategies (*e.g.,* intracerebral implantation, local disruption, convection-enhanced) of chemotherapeutics remain either too invasive, toxic, or lack efficacy.[96] Beyond surgical resection, which will remain the standard of care due to the late-stage in which diagnosis typically occurs, nanotechnology offers a viable alternative potential as a delivery vehicle for this and many other CNS diseases.

Much has been discovered in the last 15 years as to the functional biology and transport physiology of the BBB (Figure 6) and its respective neurovascular unit (*i.e.,* endothelium, pericytes, astrocytic feet, neurons, and microglia).[97] From this core understanding of the brain's very efficient filtration system, it is clear that there are a few access routes to traverse and deliver to tumor, which play to the strengths of nanotechnology platforms.[98] Relevant design rules required to enhance delivery across the BBB include the following considerations:

(i) Targeting of the blood-brain/blood-tumor barriers and / or achieving sufficient local therapeutic accumulations to ensure adequate treatment efficacy.

For systemically delivered platforms, the eventual therapeutic endpoint must be determined carefully.[94] If the platform is created to treat brain metastases or early stage lesions, the assumption that the BBB is intact and functional should be the driving force underlying such a development. Alternatively, if tasked to target late-stage glioma (most patients are diagnosed at this stage), then assumptions that the barrier will be locally heterogeneous (*e.g.,* varying degrees of disruption, inflammation, *etc.*) and display tumor-specific targets, need to be considered.

(ii) Choice of the optimum biological route to navigate the barrier.

Although, many possible routes have been envisioned,[99,100] receptor-/cell-mediated and absorptive transcytosis play to the strengths of nanoscale delivery systems. Other known

routes are either not accessible *via* nanomaterials (*e.g.,* paracellular or transcellular) or increase complexity and / or risk of interruption to essential CNS functions.

(iii) Cargo retention across the barrier and subsequent release to the abluminal side.

Many of the problems often realized during *in vivo* assessment of delivery and treatment efficacy rest with this challenge alone. The transcellular transport process involves capture and transport of cargo across cell membranes within vesicles that have different internal properties than the cell itself, as well as different effects upon the circulating delivery system. Even when utilizing the optimum biological route across the BBB, the process often leaves the drug or nanocarrier immobilized or degraded in the epithelium. Thus, delivery of the drug to tumor ceases or is greatly reduced, resulting in suboptimal efficacy relative to the systemically delivered dosage.

(iv) Transport across neural tissue to ensure therapeutic penetration and uniform drug delivery.

Even with the precision surgical resection of tumor, small lesions, cell clusters, and micro-metastases may not be detected and remain locally aggressive. In order for a drug or its delivery platform to have real impact, it must be able to penetrate deeply into neural tissue, and exhibit rapid diffusion within the matrix. Neural tissue is highly hydrophobic, and delivery to distant undetectable sites, coupled with the need to navigate the BBB, adds to these challenges.

Ultimately, efforts in the development of future delivery systems need to ensure that delivery vehicles not only crosses the BBB, but can penetrate tumor tissue to deliver therapeutic / diagnostic cargos. Combinatorial approaches designed to address one or more of the aforementioned challenges have begun to show success in pre-clinical studies,[61,101-104] although future platforms will undoubtedly need to incorporate several delivery strategies in a single platform to have real impact in the clinic. Beyond delivery of diagnostics and therapeutics to CNS tumors, platforms that can monitor therapeutic trafficking across the BBB, therapeutic response, or act as companion diagnostics will continue to be of need in the foreseeable future.[105,106]

Overcoming Traditional Barriers to Cancer Therapy: *Targeting Metastatic Disease*

Development of cancer metastasis treatment regimens is an iterative process, implemented over the years by taking advantage of important cancer biology insights and incorporation of patient outcomes, all the while, developing complementary targeted therapies. For example, thirty percent of women with stage I to III breast cancer have silent bone marrow (BM) micro-metastases that increase their likelihood of cancer recurrence[107] as well as complications, such as pathological fractures, related to the osteolytic nature of the disease.[108] Patients with limited early-stage disease, who responded well to chemo- or hormonal therapy at the primary site, may relapse years later when dormant bone marrow micro-metastases, previously protected within the bone marrow niche, reemerge.[109-112]

Metastasizing epithelial tumor cells including breast cancer (95%) undergo a phenotypic change referred to as epithelial-mesenchymal transition (EMT).[113] While the phenotypic plasticity of the carcinoma cells transitioning between *E-cadherin-positive* epithelial phenotype and lethal mesenchymal metastatic outgrowths are known to be linked to the complex interactions of the tumor and metastatic microenvironment, the network of signaling cross-talk between cells and the stroma is poorly resolved. Nevertheless, at the tumor level, mounting evidence suggests that macrophages impact tumor progression and may influence EMT. Closely intermixed within primary and metastatic breast cancers are tumor-associated macrophages (TAMs). TAMs are present in high density and are generally correlated with poor clinical outcomes.[114,115] TAMs are categorized into tumoricidal, inflammatory (M1) or tumor-promoting, immune suppressive (M2) subpopulations in accord with the local polarization signal balance.[114,116] M2 macrophages suppress the cytotoxic immune response to cancer, which is reflected as diminished CD8+ T-cell number and function. Indeed, M2 macrophages may significantly contribute to EMT through a direct cell-cell contact mechanism inducing cancer cell migration and invasion; conversely, M1 macrophages tend to inhibit EMT and metastases. Consequently, anti-M2 macrophage therapy, particularly if initiated early may markedly diminish the incidence of occult micro-metastases into the protective bone marrow niche and reduce the risk disease relapse.

Disseminated tumor cells (DTCs) typically migrate into the axial skeleton, including the spine, ribs, and pelvic bone,[117] and there become relatively resistant to neoadjuvant therapies. Micro-metastases embed within an environment-rich in hematopoietic cells, extracellular matrix, and nutrition surrounded by bone stromal elements including osteoblasts, osteoclasts, mesenchymal stem/ stromal cells (MSCs). There the interaction of trophic factors, cytokines, and chemokines modulate the progression or prolonged dormancy cancer metastases.[118,119] Stromal adherence of DTCs within the bone marrow leads to systemic chemotherapeutic resistance. However, the ultrastructural architecture of bone marrow characterized by vascular and extravascular sinus compartments is favorable towards ligand directed nanomedicines. The sinus walls have an inner layer of flattened endothelial cells and outer discontinuous layer of advential cells that sandwich a loosely dispersed basal lamina. The endothelium is interrupted by three types of fenestrations. One type, 1 μm to 3 μm in diameter is associated with migratory blood cells. A second type has numerous pores (~100 nm) forming sieve plates. A third type has fewer, but larger fenestrations (> 3 μm) unassociated with cellular migration. In contradistinction to other parts of the body, the marrow endothelial cells juxtapose edge to edge with few tight junctions.

Many investigators have focused on delivering drug into the bone marrow compartment, for instance bound to hydroxyapatite through bisphosphonates.[120] While effective for global osteoclast-targeting, these approaches are less beneficial against tumor targets within the bone microenvironment. The key to addressing protected solid cancer cells metastatic to the bone marrow hinges upon the identification and targeting of lynch pin biomarkers shared among metastatic cancer cells, immune-phagocytic cells, and cancer stem cells.

Metastatic cancers to bone, of course, are not the only type (*e.g.,* metastases to brain, lung, and liver) of interest to oncologists and nanoscale delivery systems for metastases have had a growing body of researcher attention.[121-123] Much of the very complex cancer biology of

metastatic tumors, as well as contemporary methods to treat and determine clinical trial endpoints, have recently received considerable attention and even revision.[124] A few of these include: (*i*) challenges to the importance of the epithelial-to-mesenchymal transition in the metastatic processes;[125] (*ii*) emerging evidence against acquired somatic coding mutations as metastatic drivers; (*iii*) insights into the differing mutational profiles and immunological microenvironment of primary *versus* metastatic lesions;[126,127] (*iv*) mutational profiling of primary tumor and metastases that display varying degrees of intertumoral epi-/genetic heterogeneity;[126,128] and (*v*) multiple studies confirming pre-emptive priming of the metastatic site and more.[129,130] Much can be gleaned from these recent findings to help drive innovative nanoscale delivery platforms as therapeutics or diagnostics of metastases.[45,131-135] Specifically, therapies that rely upon immunoactivation to target downstream metastatic niches or tumor microenvironment (Figure 7), therapies that target transcriptional programing and biological phenotypes, diagnostics that detect metastatic lesions or their primed sites earlier to precisely deliver the proper therapy, nanoscale drug development that includes metastatic lesions as clinically relevant preclinical endpoints *via* recent disease-specific animal models,[136] and redesigned clinical trials specific to metastatic tumor therapies, *de novo*. Ultimately, the ideal nanomedicine approach will require an effective therapy that targets multiple critical cell types (*i.e.,* metastatic cancer cells, M2 macrophages, and cancer stem cells) in a single or multifaceted treatment regimen.

Overcoming Traditional Barriers to Cancer Therapy: *Enhancing or Augmenting Radiotherapy*

Key technological improvements in radiation oncology, including the development of intensity-modulated radiation therapy, have largely been driven by advances in applied physics and computer science.[137] One of the next exciting frontiers currently fueling significant progress in radiation oncology is the field of nanotechnology, which is offering technological capabilities and innovative solutions to improve therapeutic efficacy, reduce off-target toxicity, and enable more personalized cancer care. The application of nanoparticle-based radiotherapies may complement existing standard of care treatments to a variety of tumor types.

Radiotherapy, along with chemotherapy and surgery, is part of the tri-modality treatment option for cancer. More than, 50% of all cancer patients receive radiotherapy during their treatment regimen. Moreover, radiotherapy with or without chemotherapy, is one of the most common and most effective curative regimens for many cancers. Mechanistically, the cellular apoptotic effect is by way of direct DNA damage or indirectly *via* local generation of highly reactive chemical species (*e.g.*, singlet oxygen). Its clinical application, of course, cannot be dispensed with single-cell precision. As a result, this technique relies upon a delicate balance of dosing and spatio-temporal delivery schemes to overcome the heterogeneous biological damage thresholds of the bulk tumor, while avoiding surrounding tissue. Indeed, contemporary radiation oncology has delivered advances in external beam radiation that optimize the delivery of dosages *via* simulation (*i.e.*, treatment planning), beam shaping, and real-time imaging (*i.e.*, image-guided attenuation of therapy)[138] - the culmination of which being stereotactic ablative radiotherapy, commonly used now for early

stage cancers and reoccurrence.[139] Regardless of these advances in the field, radiation oncologists still face hurdles that need solutions. Specifically, non-uniform intra- and inter-tumoral radioresponsiveness and reduction of chemo-/radiotherapy toxicity are constant clinical challenges that are being addressed by nanotechnology.[140]

Nanotechnology-centric efforts in this area have focused on development of radiotherapeutics, or treatments that fall within the larger umbrella of externally applied radiation, and have shown recent promise in both pre-clinical and clinical studies.[141,142] Successful interventions have been aimed at maximizing therapeutic effects within the confines of current dosing regimens; these fall into several specific categories:

(*i*) Platforms which act as radiation broadcasters (*e.g.*, photodynamic therapy (PDT), Cerenkov radiation) or have been developed to increase local radiosensitivity by way of intrinsic materials properties (*e.g.*, high atomic number, Z) combined with local tumor accumulation.[143-146] In the latter case, for example, particle-based agents that can increase the overall effective Z of the target site without altering the Z of surrounding tissues can lead to increased radiotherapeutic doses to tumors with associated higher therapeutic efficacy.[142] In one such study,[147] hafnium oxide nanoparticles (*i.e.*, NBTXR3), serving as radiosensitizers and measuring about 50 nm i.d., were administered intratumorally into both murine sarcoma and colorectal xenograft models. Radiosensitizing effects upon tumor cells were observed, along with enhanced growth suppression; no significant radiotoxicity was noted.

(*ii*) Platforms which attenuate the local biological environment for tumor to be more susceptible or sensitized to the radiotherapy and / or delivery of chemotherapeutics (Figure 8), and that are, in turn, known to pre-sensitize tumor cells for radiation-induced cellular apoptosis.[148-151] Cancers, such as glioblastoma multiforme and pancreatic adenocarcinomas, are traditionally resilient to radiotherapy, and radiosensitization offers enhanced efficacy.

(*iii*) Enhanced and more effective delivery of current and future radioisotopes (*e.g.*, beta- and alpha-emitters).[152] Traditionally, molecular radiotherapies are cleared rapidly by the renal system and suffer short circulation times. However, the observed *in vivo* biodistribution of such radiolabeled molecular constructs (*i.e.*, peptido-probes) will be critically dependent on the sequence, composition, and surface charge of its constituents. As such, radiolabeled peptido-probes have suffered in a number of instances from high accumulations[153,154] in non-target organs, such as the kidneys, which are very radiosensitive, thus limiting applied radiotherapeutic doses and leading to reductions in therapeutic efficacy. Furthermore, radioimmunoconjugates[155] (>10-nm diameter) have been limited by their larger size (*i.e.*, >10-nm), prolonged kinetics, and higher background signal. To address these technical challenges,[156] nanoscale approaches using smaller-size molecularly-targeted particle probes[63] carrying currently approved radioisotopes are being advanced to increase tumor target accumulations and maximize tumor cell kill while, at the same time, reducing off-target toxicities.

At the present time, a number of particle-based radiotherapies are currently in human clinical trials, and many additional clinically-promising radiotherapeutic platforms are being tested in small animal models for delivering potent radiosensitizers[150] and improving delivery to tumors with radiation guidance.[157] Two active Phase 1 clinical trials are administering NBTXR3 as an inorganic particle radiosensitizer for treating extremity soft tissue sarcomas (NCT01433068) and locally advanced squamous cell carcinoma of the oral cavity or oropharynx (NCT01946867).[142] Nanotherapeutics are also being used clinically to improve therapeutic indices of chemoradiotherapy. One such platform, nanoparticle (NP) albumin-bound (nab) paclitaxel, a recently approved particle-based chemotherapeutic, is currently being assessed following its concurrent administration with radiotherapy in several Phase III chemoradiotherapy clinical trials in lung, esophageal, head and neck, endometrial, and cervical cancer.

Although nano-based approaches have been showing positive results in these therapeutic areas, other areas will remain critical needs for radiation oncologists. Parallel modalities to reduce the tumor adaptation of a radiotherapy resistant cell niche, enhanced chemo-radiotherapeutics, enhanced PET-CT contrast for planning / measuring response, and *in vivo* image contrast platforms to aid image guided therapies, remain critical needs.[142,158,159] Finally, one area that has begun to receive increasing attention due to its potentially curative effects is immunoradiotherapy.[160-162] In this approach, local abscopal effects imparted to the primary tumor site can aid the adaptation of current immunotherapy non-responders to a state of immunotherapeutic responsiveness. Combined immunotherapy with radiotherapy platforms are promising.[45,47,163] Whether by utilizing the aforementioned nano approaches with current immunotherapeutics, traditional radiotherapy with nano-immunotherapies, or enhancing both with nanoscale systems, these will be areas of much effort. Furthermore, it has been shown in several pre-clinical trials that response is durable, and will allow administered immunotherapeutics to stimulate eradication of local metastatic tumor sites.[164]

Outlook and Future Tasks for the Field

The therapeutic delivery and anticancer effects *via* nanocarriers are driven by enhanced permeability and retention, active targeting, and / or levels of release of the active drug from the nanocarrier. Much has been discovered to date as to the correlations of nanomaterial delivery and efficacy relative to drug loading, molecular weight, size, shape, and the protein corona. Although, intratumoral heterogeneity and tumor microenvironment variability, patient-to-patient, greatly affect efficacy, and need to be more than just a transient consideration or to rely on dogmatic approaches. This remains the largest contributor to low clinical success stories to date. We view two primary aspects that should guide the field over the near term to more successful outcomes:

(*i*) Overcoming tumor heterogeneity hurdles will rely on an enhanced understanding of nano–bio interactions, particle transport to tumor cells, and targeting of TME or the pre-metastatic niche to further enhance treatment response. Thus, there is much to be gained in our knowledge of the fundamentals of this multi-step *in vivo* delivery process in humans, by way of an approach that begins with more accurate measures and standards of biologically (*e.g.,* clinically) relevant metrics. For example, this could be driven by refining our approach

to identifying patients / patient cohorts whom could have a positive response to nanotherapies *via* standardized and biologically relevant companion diagnostics.[165] Likewise, in the pre-clinical arena, similar approaches need to be exploited to preemptively decipher a companion diagnostic's relevance to a particular nanocarrier while utilizing *in vivo* imaging techniques (*e.g.,* intravital microscopy, *etc.*) to track carrier and drug distribution within heterogeneous tumor tissue and its associated microenvironment. For example, recent studies developed a technique based on FDA approved nanoscale imaging agents to predict accumulation of model therapeutic nanoparticles in tumor.[166] Furthermore, another study utilized FDA approved nanoscale imaging agents to measure co-localization of model therapeutic nanoparticles in order to stratify mouse models relative to local accumulation.[167] This method was used in clinical pilot studies to test similar approach in metastatic breast cancer patients (Merrimack). Further development and habitual use of disease accurate animal models, staged use of models (*e.g.,* xenograft to GEM models and beyond) to decipher results at multiple levels, *ex vivo* biomimetic models of tissue barriers and organs, and more nanocarrier diagnostic standards will collectively aid in reducing the gap of pre-clinical success and clinical failures. Finally, while most approved nanomedicines use existing drugs as payloads, it is expected that next-generation clinical platforms will eventually incorporate additional molecular entities (*i.e.*, small molecule cell cycle[168] and epigenetic inhibitors), biologics (*i.e.*, siRNA, mRNA), and gene-edited therapies. As such, most will need to be integrated into clinical workflows already using the current standard of care during clinical trials—this must drive the development cycle and approach from the beginning (*e.g.*, academic laboratory setting).

(*ii*) One key element often not accounted for in the initial phase of development, is the ultimate marketability that the platform and its target will be judged by. As a guideline, investors will need to be shown a $1 billion market potential for a drug product that is to be invested in and a 7-year investment return schedule is the standard. Furthermore, investors will expect the product to be de-risked by the inventor prior to any investment discussions. This comprises a thorough prior understanding of *in vivo* characteristics, a clear ability to scale up for manufacturing, and often a reduction of complexity in nanoparticle design or active component delivered (*e.g.,* doxorubicin *vs.* biologic inhibitor). In essence, many more nanotechnology-based platforms could be advanced into the clinic, at a higher rate, with rational design methodologies from the beginning. Furthermore, if both the delivery system and drug/diagnostic agent involve higher risk than the clinical target must not be one by which the current standard of care is already successful. As example, clinical efforts need to remain directed to those areas where contemporary approaches have not been or have had limited success and which exploit the advantages of nanotechnologies (*e.g.,* readily functionalized, increased sensitivity, tunable properties). Many of the areas discussed herein fall within this category, such as delivery across barriers (*e.g.*, blood-brain barrier to primary or metastatic lesions), overcoming drug resistance, reprogramming the tumor microenvironment, *in vivo* molecular phenotyping, synergistic enhancement by way of disparate therapeutic/diagnostic modalities, and induction of alternative mechanisms of action (*e.g.,* cell death) using self-therapeutic nanoparticles. Sheer need and basic economics will continue to drive the field in the path of least resistance and must remain at the forefront of investigators minds from the beginning.

As the field was begun with many objectives in mind, not all are outlined in this review yet these should remain in our collective sight moving forward (*e.g.,* the delivery of gene therapy, small-molecule drug combinations, demonstrating access to targets once viewed as undruggable, *in vitro* diagnostics, enhanced multimodality imaging, and more) whether near or far term. In some of these cases, often, there are no viable alternatives outside of nanotechnology, and for drug combination therapies, nanocarriers could offer much more precise delivery of drug ratios, staged delivery of multiple drugs; in essence overcoming critical barriers to drug resistance and transport. A few successes have emerged or are beginning clinical trials within these areas. However, many obstacles still remain that have impeded much of these efforts in clinical translation. For instance, with nanoscale gene delivery systems, progress has been slow due to compliment activation and insufficient release from the endosome post-cellular internalization. Thus, deeper mechanistic understanding to the basic processes of nanomaterials and biology will enable advancement in these, as well. On the diagnostics and imaging front, much less has translated to clinic albeit this much more a function of economics than lack of impact. Beyond intraoperative imaging, companion diagnostics and more discussed herein, a very active area for future clinical efforts is the 'liquid biopsy' and its long held 'holy grail' aim – *earlier disease detection*. This downstream goal will only be realized in near term, as assays for minimal residual disease detection and measuring patient-specific response to therapy.[169-171] *Thus, another area to continue to focus upon will be in vitro diagnostics that can operate under CLIA laboratory standards*. Expanded efforts on diagnostics will be very important for liquid biopsies, again due to nanoscale materials' distinct physical properties. For example, there have been many device demonstrations with clinical samples indicating high sensitivity and multiplexing of measurements;[52,69,172-174] such methods can be superior to and are, in some cases, superseding existing assays.

Ultimately and despite the many research successes to date, *the utility of the field will only be judged by advantages provided to cancer patients and/or patient care outcomes*. We expect that continued research and clinical efforts will identify areas where nanotechnology treatments, diagnostics, and their combination can make most of the difference. We should also remember that several successful therapies took more than 20 years from their initial research discovery to their approval and implementation into standard practice. As such, we look forward to preparing CaNanoPlan 2020 and verifying how future progress aligns with predictions described in its previous iterations.

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VOCABULARY

Antigen presenting cells (APCs):
cells that display surface antigens complexed with major histocompatibility complexes and are vital components of the adaptive immune response (e.g., lymphocytes). In theory, all cells are APCs yet the primary APC cell types are macrophages, dendritic and B cells

Blood-brain Barrier (BBB):
the lining of epithelial cells which form the barrier between all of the vasculature embedded within the central nervous system. The epithelium is distinctive in that its defining characteristic is tight junctions formed between all of its epithelial cells by specific proteins (e.g., occludins, claudins) as well as very tightly controlled mechanisms for passive and active transport. The barrier acts in concert with astrocytes as a living organism to control the rate of flow of biochemical and biological species which can pass. As such, it is the most selective barrier in the human body.

Clinical Laboratory Improvement Amendments (CLIA):
are a set of U.S. federal regulatory standards enacted in 1988 that apply to all clinical laboratory testing of specimens derived from humans for the purpose of providing information for diagnosis, prevention, or treatment of disease. Laboratories that wish to utilize human samples in these ways must be certified by U.S. state and/or federal agencies. The CLIA program is operated by the U.S. Centers for Medicare and Medicaid Services and grades all testing modalities for complexities under seven criteria.

Matrix Metalloproteinases (MMPs):
a multigene family of zinc-dependent enzymatic proteins with a common domain structure mostly responsible for extracellular matrix degradation although also play roles in cleavage of cell surface receptors and other cellular behaviors including apoptosis. MMPs are implicated in many pathological processes including carcinogenesis.

Radiosensitivity:
the biological damage threshold or relative susceptibility of cells or tissue to the effect of ionizing radiation

Tumor microenvironment:
the cellular environment in which the tumor resides (e.g., stromal tissue, etc.) that is directly influenced by tumor signaling, angiogenesis, and induction of peripheral immunotolerence. This environment is directly modified by the tumor and simultaneously can influence and promote tumor growth

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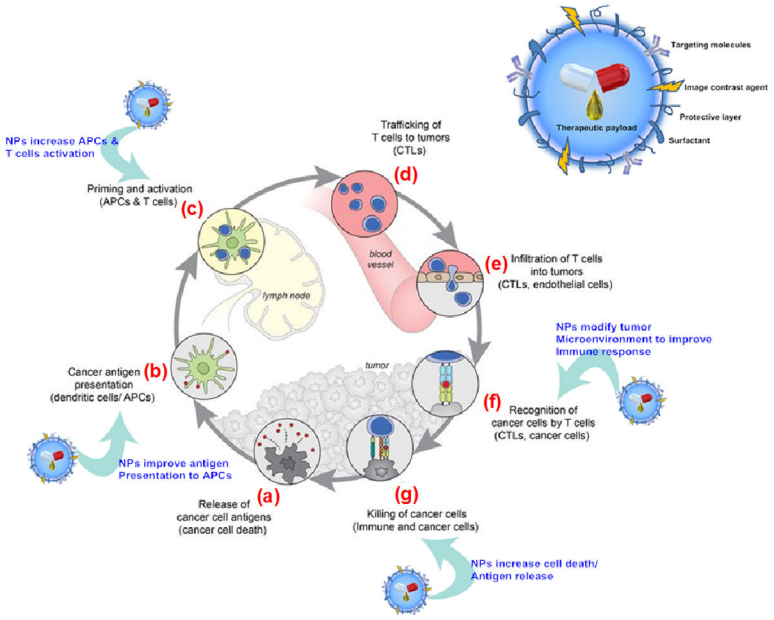

Figure 1. Depiction of the complex pathway involved in cancer immunotherapy.

Nanoparticle delivery vehicles can play a role at multiple points along this pathway. Following cancer cell apoptosis, antigens are released (a) enabling antigen presentation (b). Ultimately, this allows for priming and activation of T cells in lymph nodes (c) that are trafficked back to tumor (d) as cytotoxic T cells (CTL). After tumor infiltration (e), recognition of cancer cell antigens by CTLs (f) follows with CTL killing of specific cancer cells (g). The cancer immune response is an ideal case that does not occur to a large degree due to the ever-evolving immune evasion mechanisms of the cancer. The inherent multicomponent cargo capacity of nanoscale delivery platforms enable alternative approaches in cancer immunotherapy to bolster this response, as depicted in the figure. From the right arrow and moving clockwise, nanoparticles can be designed to help to re-train the tumor microenvironment (TME), increase antigen presenting cells and subsequent T-cell activation, improve antigen presentation, and/or allow increased antigen release from cell death *via* several modalities. Further and/or simultaneous delivery of diagnostics can enable direct measures of T-cell response and more.

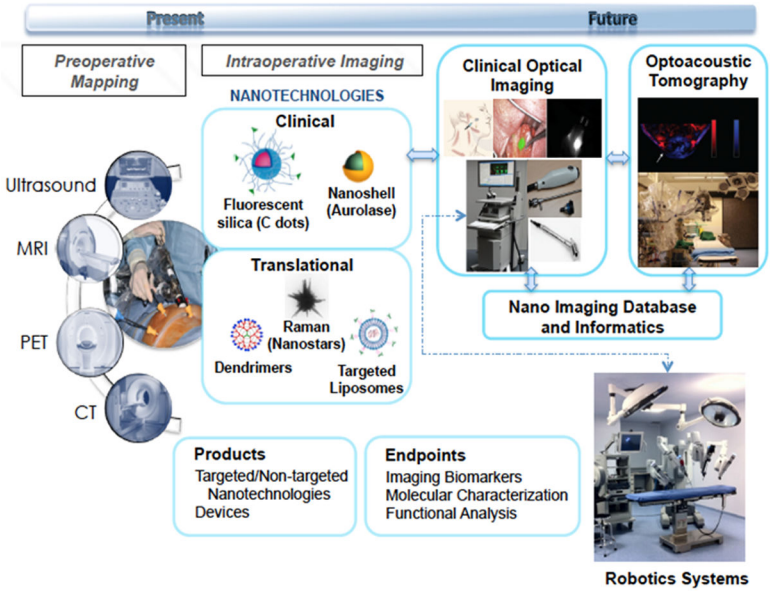

Figure 2. Present and future of NanoOncology Image-guided Surgical Suites.

Preoperative conventional imaging tools are used to screen for disease and inform optically-driven minimally-invasive and open surgical procedures. Clinically available particle platforms are monitored in real-time using portable multichannel camera systems. Representative translational probes and devices for future clinical use are also shown. In the future, the operating surgeon will select suitable probe-device combinations for specific indications, and be provided with structural, functional, and/or molecular-level data regarding tissue status for further treatment management.

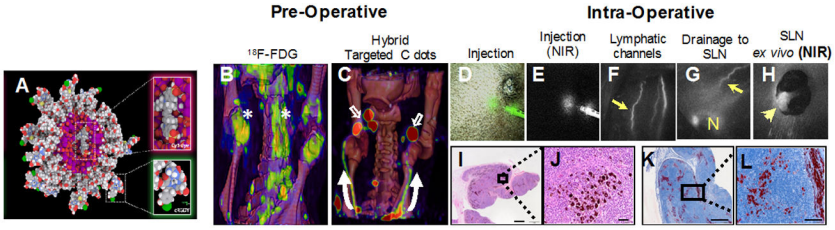

Figure 3. Mapping of Metastatic Lymph Nodes Using a Clinically Translated Hybrid PET-Optical Silica Nanoparticle (C dots).

(A) Schematic illustration of ^{124}I-cRGDY-PEG-C dots. (B) 3D-reconstructed maximum intensity projected image (dorsal view) shows uptake of ^{18}F-FDG in bone but no evident nodal accumulation (asterisks). (C) 3D-reconstructed maximum intensity projected image (dorsal view) of metastatic locoregional nodes (open arrows) and lymphatic channels (curved arrows) within the neck bilaterally following local injection of ^{124}I-cRGDY-PEG-C dots about the primary tumor site (not shown). (D, E) Intraoperative SLN mapping with two-channel NIR optical imaging of the exposed nodal basin. Local injection of fluorescent C dots displayed in dual-channel model (D) RGB color (green) and (E) NIR-fluorescent channels (white). (F, G) Draining lymphatics (arrow) distal to the injection site extending toward the node (N). (H) Image of excised SLN in the NIR channel. (I) Low power view of H&E stained SLN shows a cluster of pigmented cells (black box) (bar=1 mm). (J) Higher magnification of (I) reveals rounded pigmented melanoma cells and melanophages (bar=50 μm). (K) HMB45-stained (red) SLN confirms the presence of metastases (black box, bar = 500 μm). (h) Higher magnification reveals HMB-45+ expressing melanoma cells (bar = 100 μm). Figure adapted with permission from ref 59. Copyright 2013 RSC Publishing.

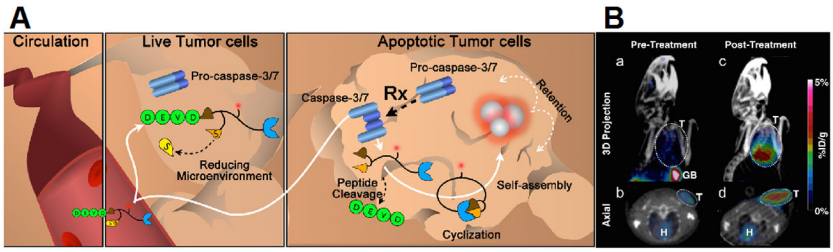

Figure 4. ***In vivo*** **self-assembling nanoparticle strategy for cancer imaging.**

(A) Illustration of a TESLA probe for imaging tumor apoptosis. (B) PET imaging of chemotherapy with C-SNAT; PET/CT images showing HeLa tumor xenografts (white dashed circles) on the right shoulder of mice 60 min after *i.v.* injection of tracer before and after doxorubicin treatment: (**a&b**) before treatment, imaged with C-SNAT (211 μCi) after 3D projection (**a**) and (**b**) in axial view; (**c&d**) the same mouse after treatment imaged with C-SNAT (324 μCi) after 3D projection (**c**) and in axial view (**d**). All images are normalized to the same scale. T: tumor, H: heart, GB: gall bladder. Figure adapted with permission from ref 70. Copyright 2014 Macmillan Publishers Ltd.

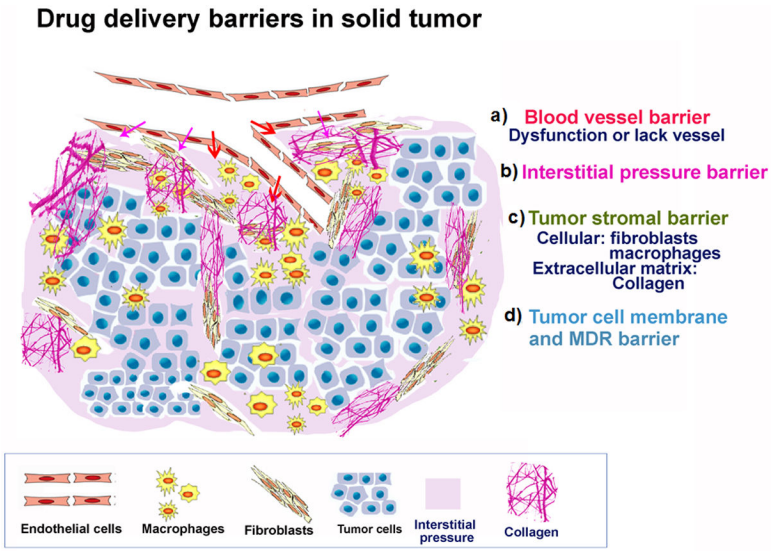

Figure 5. Schematic of Drug Delivery Barriers in Solid Tumor.

Effective delivery of therapeutic agents into human tumor cells requires overcoming the following four biological and physical barriers: a) heterogeneous distribution and lack of functional tumor blood vessels in the tumor tissues, b) high interstitial pressure due to proliferation of tumor cells and accumulation of interstitial fluids; c) dense stroma barrier as the results of proliferation and infiltration of tumor associated stromal cells, such as fibroblasts and macrophages, and increased deposition of extracellular matrix; and d) tumor cell membrane barrier and high levels of multi-drug resistant proteins that pump drugs out of cells.

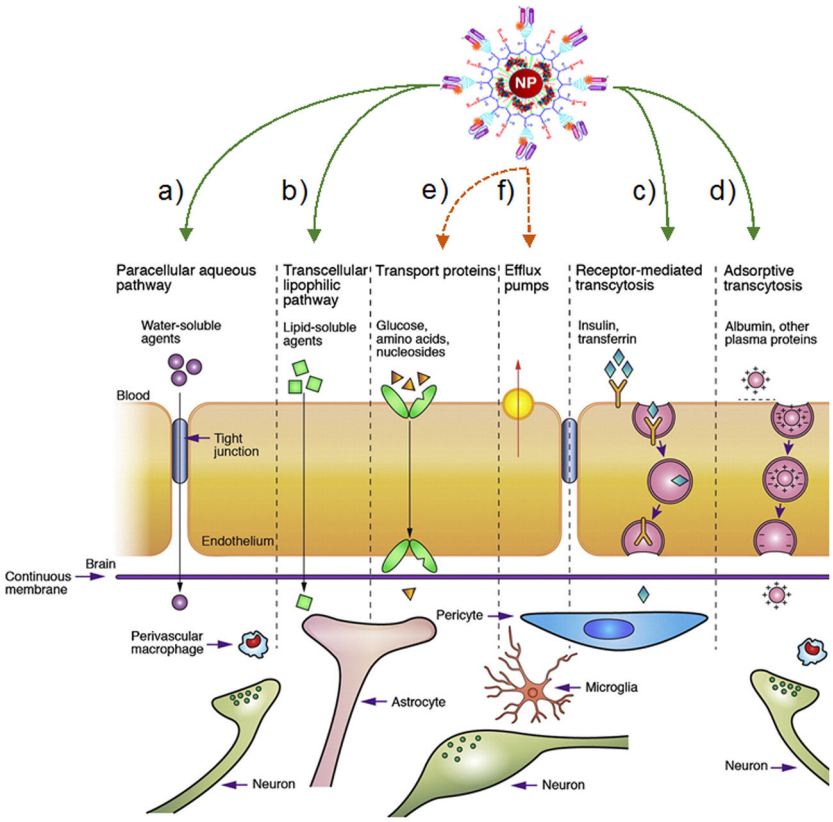

Figure 6. Transport routes to cross blood–brain barrier (BBB).

A schematic representation of potential mechanisms of biomolecular transport, except cell-mediated transcytosis. In healthy BBB, the tight endothelial junctions restrict small-molecule therapeutics by molecular weight, water solubility, and polarity. Limiting therapy to a small subset of potential drugs that are safe and effective. Transcellular lipophilic transport offers another potential route for lipid soluble therapeutics although they must be able to be delivered through circulation. a&b) Nanoscale platforms have utilized both of these pathways by way of targeting vasculature / local drug accumulation as well as local disruption to enable delivery of co-delivered small-molecule drugs. These platforms have relied on varying degrees of blood-tumor barrier inconsistencies of the tight junctions. c&d) The routes of receptor-mediated and adsorptive transcytosis are often used routes for nano carriers by way of transferrin receptor targeting and other passive mechanisms. e&f) Utilization of transport proteins and efflux pumps are not traditionally used in drug delivery schemes although nano carriers offer the opportunity to utilize additional delivery strategies in the future with these two routes. Figure adapted with permission from ref 98. Copyright 2011 Elsevier.

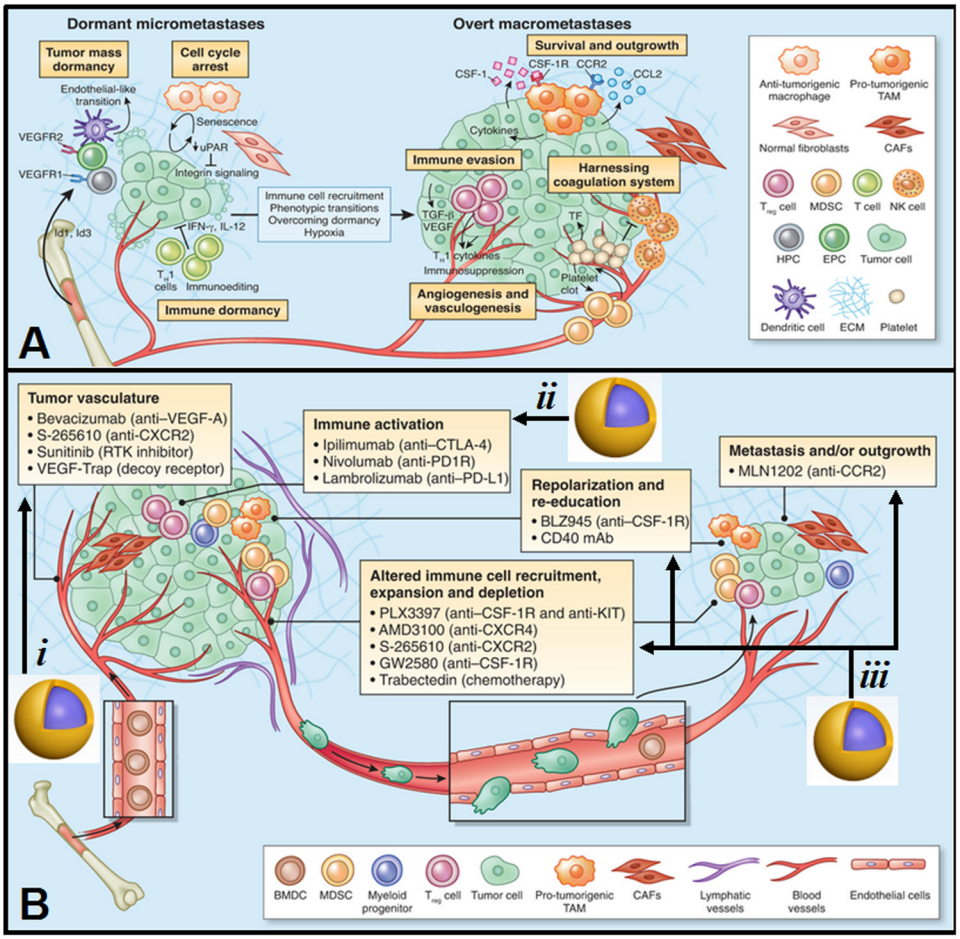

Figure 7. The current understanding of metastasis has evolved greatly over recent years and clinical endpoints are beginning to be reshaped with recent insights.

A) The modern-day view of metastatic dormancy and initiation of secondary outgrowth in metastatic niches. Pre-metastatic seeding with formation of pre-metastatic clusters occurs before tumor cell arrival, which has been shown to be from primary and secondary site communication mechanisms. Once tumor cells arrive they still must compete with the local microenvironment and can exist as asymptomatic dormant micrometastases persisting for years in some examples. Dormancy of these micrometastases are held in check by several mechanisms driven in part by the microenvironment, mass and local angiogenic dynamics. Further driven by cellular dormancy (arrested proliferation and immune-induced), re-establishment at the secondary site will often have been shaped by selection features of the process, ultimately having characteristics of the primary and metastatic sites. Thus, therapeutics require more distinct targets and approaches of relevance to current understanding of metastasis. **B**) Multiple therapeutic approaches to re-train and/or target the tumor microenvironment are either currently in clinical use or development. The figure displays many of these with respect to route of therapeutic intervention and drug target. Vascular targeting has shown to have limited success at reducing metastatic spread. Many nanotechnology platforms already employ this route for other indications in animal models and this will be an area of need (***i***) once the drugs prove to have substantial efficacy in

human trials. Although, many are targeting the primary lesion, several of these will affect downstream metastatic disease as well (*e.g.*, altering immune cell recruitment and repolarization of developing TME). Furthermore, immune cell tumor recognition has recently been shown to be enhanced greatly and durably from co-delivery of radiotherapy and chemotherapeutics *via* nanocarriers (***ii***). These effects are carried downstream towards the nearby lesions and distant metastases. Very few drugs solely target the metastatic lesions to block metastatic seeding and continued growth. Much more emphasis should be placed on nanotechnology co-delivery of chemotherapeutics and inhibitors (***iii***), such as key cytokine axes (*e.g.*, CCR2, CXCR2/4). Figure adapted with permission from ref 135. Copyright 2013 Macmillan Publishers Ltd.

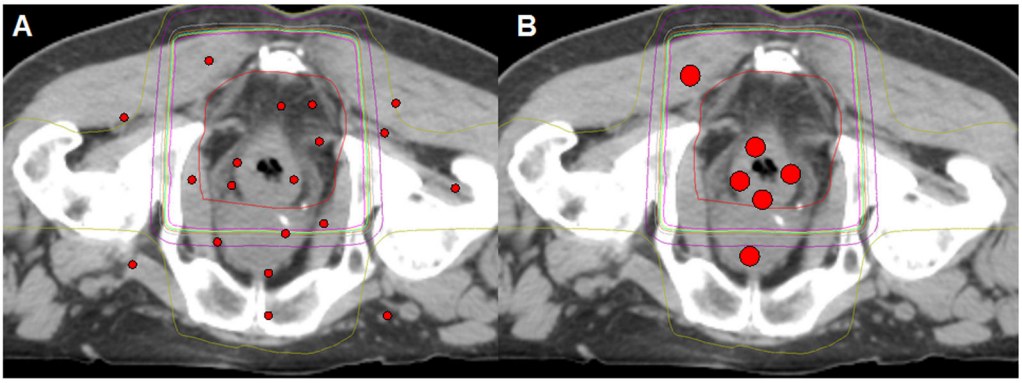

Figure 8. Schematic representation of chemoradiotherapy with small molecule therapeutics (A) or with nanotherapeutics (B).

Axial CT image of patient with rectal cancer is shown. Colored lines represent areas receiving high dose radiotherapy (isodose lines). Small molecule drugs (small red dots) distribute in both tumor as well as normal tissue receiving high dose radiotherapy, thus limiting efficacy and increase toxicity (A). Nanotherapeutics (large red dots) preferentially accumulate in gross tumor, thus improving efficacy and lower toxicity (B).