Review

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Cancer nanomedicine: from targeted delivery to combination therapy

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The advent of nanomedicine marks an unparalleled opportunity to advance the treatment of various diseases, including cancer. The unique properties of nanoparticles (NPs), such as large surface-to-volume ratio, small size, the ability to encapsulate various drugs, and tunable surface chemistry, give them many advantages over their bulk counterparts. This includes multivalent surface modification with targeting ligands, efficient navigation of the complex *in vivo* environment, increased intracellular trafficking, and sustained release of drug payload. These advantages make NPs a mode of treatment potentially superior to conventional cancer therapies. This review highlights the most recent developments in cancer treatment using NPs as drug delivery vehicles, including promising opportunities in targeted and combination therapy.

Nanomedicine in cancer therapy

Nanomedicine (see Glossary) is the design and development of therapeutics and diagnostic tools distinguished by the nanoscopic scale of its delivery vehicles and diagnostic agents [1]. The nanomedical field is rapidly gaining recognition through developing ways of administering treatment, particularly anticancer therapy, with unprecedented safety and efficiency. Researchers have improved on the current standards in drug delivery relating to biodistribution, intracellular uptake, and dosing efficacy by utilizing NPs to encapsulate therapeutic agents and target sites of disease [2]. The successful application of processes to improve the delivery of biomedical entities through functional NPs is a revolutionary approach to disease treatment. Several liposome- and polymer-based therapeutic NPs have been approved by the FDA for clinical use [1]. This review discusses the NPs under investigation with an emphasis on systems that have reached clinical trials (Table 1).

NPs are minute particles, typically less than 200 nm in diameter. Their nanoscopic size facilitates intracellular uptake. NPs have the ability to encapsulate therapeutic agents and release them in a controlled manner to specifically target diseased cells. NP encapsulation also improves the solubility of unmodified drug compounds [3]. Additional advantages of NPs have brought widespread attention to the field of nanomedicine, including their large ratio of volume to surface area, modifiable external shell, biodegradability, and low cytotoxicity [4]. Furthermore, nanomedicine brings us dramatically closer to realizing the full promise of personalized medicine [5].

Engineered therapeutic NPs offer numerous clinical advantages. Surface modification with polyethylene glycol (PEG) protects NPs from clearance from the blood by the mononuclear phagocytic system (MPS), markedly increasing both circulation times and drug uptake by target cells [2,6]. Functionalization of the NP surface with multivalent targeting moieties not only improves drug efficacy but simultaneously reduces the dose, providing a novel method to optimize drug pharmacokinetics [6]. NPs spatially localize through passive/active targeting and are capable of delivering drugs through epi/endothelial barriers [3]. Below we present some examples of engineered NPs and their features

Glossary

Active targeting: the targeted homing of NPs to sites of disease by modifying the surface of the particle with ligands specific to biomarkers overrepresented in target cells.
Amphiphilic: possessing both hydrophilic and hydrophobic parts.
Combinatorial nanodelivery: the delivery of more than a single therapeutic agent in one particle, often in an optimized ratio for synergistic effect. Multiple cancer pathways may be targeted with one particle.
Liposome: a spherical vesicle comprising a lipid bilayer.
Microfluidics: a technology used to quickly fabricate uniform NPs by manipulating minute amounts of liquid via channels on the micrometer scale.
Mononuclear phagocyte system (MPS): the MPS, also called the reticuloendothelial system, comprises the phagocytes located in reticular connective tissue present in the liver, lymph nodes, and spleen that are responsible for the eventual clearance of most NPs.
Nanomedicine: the design and development of therapeutic agents and diagnostic tools distinguished by the nanoscopic scale of its delivery vehicles and diagnostic agents.
Nanoparticle (NP): particles, usually comprising lipid or polymer, typically less than 200 nm in diameter.
Passive targeting/enhanced permeability and retention (EPR) effect: refers to the observation that the permeable vasculature and disordered basement membrane of tumor tissue leads to preferential accumulation of entities of 10–500 nm in size.
Poly(D,L-lactide-co-glycolide) (PLGA): a commonly used polymer for the construction of NPs, usually selected for its controlled release capabilities.
Polyethylene glycol (PEG): a polymer used to modify the NP surface, resulting in the prevention of nonspecific binding to blood components. These 'stealth' particles are better able to evade clearance by cells of the MPS.
RNAi: a pathway in eukaryotic cells where short pieces of RNA are able to induce the breakdown of complementary mRNAs.
Zwitterionic polymer: a polymer that is capable of exhibiting both positive and negative charges and has been shown to resist nonspecific protein adsorption.

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Table 1. Nanomedicines in clinical development

	Targeting ligand	Therapeutic agent encapsulated	Indication	Clinical status
Liposomes				
ALN-TTR02 (NCT01559077)	Passive	siRNA	TTR amyloidosis	Phase II
CALAA-01 (NCT00689065)	Tf	siRNA	Solid tumors	Phase I
CPX-351 (NCT00822094)	Passive	Cytarabine and daunorubicin	Acute myeloid leukemia	Phase III
MBP-426 (NCT00964080)	Tf	Oxaliplatin	Gastroesophageal adenocarcinoma	Phase II
SGT53-01 (NCT00470613)	Antibody fragment	p53 gene	Solid tumors	Phase I
TKM-PLK1 (NCT01262235)	Passive	siRNA	Solid tumors	Phase II
Polymeric NPs				
BIND-014 (NCT01300533)	Small molecule	Docetaxel	Solid tumors	Phase II
Atu027 (NCT01808638)	Protein kinase N3	siRNA	Solid tumors	Phase II
CRLX-101 (NCT01380769)/ (NCT00333502)/(NCT02010567)	Passive	CPT	Non-small cell lung cancer/rectal cancer/renal cell carcinoma	Phase II

that have been designed to address existing challenges in drug delivery, with a specific focus on cancer therapy.

NPs increase drug solubility, mitigate cytotoxicity, and improve drug pharmacokinetic profiles, as exemplified by nanomedicines such as Doxil® and Genexol-PM®. The past decade has witnessed numerous new biotechnological approaches to the treatment of cancer. For example, the 2006 Nobel Prize in Physiology or Medicine brought renewed focus on gene silencing, and the therapeutic opportunities offered by precise regulation of gene expression have fostered the interest of medical stakeholders in siRNA and miRNA technologies [7]. Nevertheless, delivering nucleic acids into cells is challenging to say the least: nucleic acids are vulnerable to nucleases ubiquitous in the blood and their dense negative charges hinder cell internalization. Furthermore, the nonspecific interferon response triggered by the presence of foreign nucleic acids in the cytoplasm is a major impediment to clinical translation [7–9]. To avoid these drawbacks, the ideal siRNA delivery system should efficiently encapsulate the negatively charged siRNA molecule, prevent degradation by endogenous enzymes, and facilitate cellular uptake and intracellular release.

Technologies already in clinical trials addressing the delivery of RNAi therapeutic agents are presented in the following sections. The last section highlights examples of current trends and novel applications of nanomedicine in the field of combination therapy.

Methods of NP preparation

Their nanoscale size means that NPs require a very specialized formulation method. The most common methods employ self-assembly processes to amphiphilic lipid, polymer, or polymer–drug conjugates. Such processes include nanoprecipitation, oil-in-water (O/W) single emulsion, and water-in-oil-in-water (W/O/W) double emulsification [10–12]. The most recent development in the synthesis of NPs involves the discipline of microfluidics, which is capable of manipulating nanoscale volumes in microscale fluidic channels [13]. Microfluidic reactors offer precise control and manipulation of the fluids used to create NPs. Microscale channels offer the advantage of a very large surface-to-volume ratio and controllable mixing time, which promotes higher NP yield and uniform size [14,15]. Through multi-inlet mixing at different ratios and hydrodynamic flow focusing, the NPs self-assemble through diffusive mass transfer at the interface of miscible liquids (Figure 1) [12]. Other significant advantages of microfluidics include the reproduciblility of device fabrication and rapid, consistent NP synthesis with narrow size distributions [14]. Microfluidic devices are tunable and can use 3D hydrodynamic focusing to create NPs of different sizes and targeting ligand densities with multiple polymers, which can in turn produce diverse NP libraries (Figure 1) [16,17]. In addition, microfluidics provides a means of rapidly and continuously forming consistent nano- and microstructures while simultaneously encapsulating drugs, which is not readily feasible with conventional approaches [18,19]. However, to take full advantage of the benefits of microfluidic nanoformulation, the challenges associated with the high costs of glass/silicon fabrication and large-scale production for clinical use remain to be addressed [14].

'Stealth' modification of NPs

Modification with PEG is currently the gold standard for NP coating [10,20,21]. PEG surface functionalization has been shown to dramatically reduce protein adsorption, particularly of apolipoprotein J and complement protein C3, through hydrophilicity and steric repulsion effects, with the effect of extending circulation time in blood [22–24]. This has allowed 'stealth' NP carriers to persist in the bloodstream long enough to reach or recognize their therapeutic site of action [25]. Examples of stealth nanocarriers include PEGylated liposomal doxorubicin (Doxil®) and the polylactic acid (PLA)–PEG micelle form of paclitaxel (Genexol-PM®). Since the first PEGylated nanomedicine, Doxil®, was approved in 1995, many of the current FDA-approved NPs and NPs in clinical trials have begun to carry the PEG modification. In addition to PEGylation, new biomaterials and delivery strategies have been developed to prolong the circulation time of NPs [26–29]. For example, zwitterionic polymer-based NPs are resistant to nonspecific protein adsorption due to electrostatically induced hydration [30,31]. Modification of the zwitterionic polymer with a pH-switchable moiety allows the NP

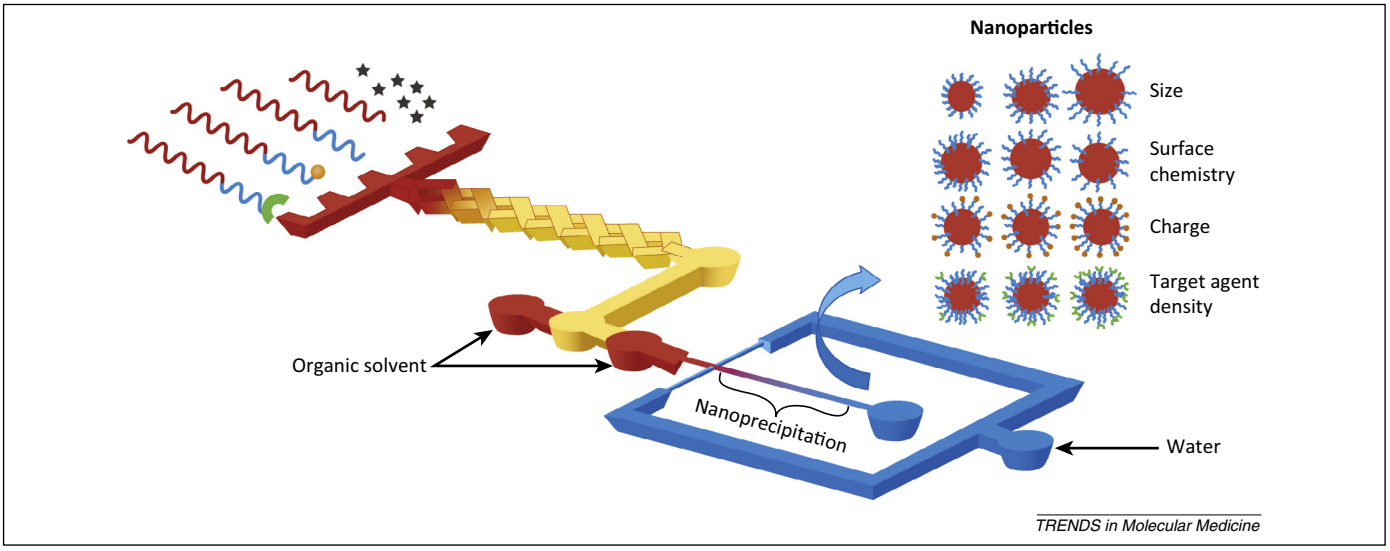

Figure 1. Schematic of a system in which nanoparticle (NP) precursors enter a multi-inlet mixer at different ratios to self-assemble a library of NPs. Programmable mixing of polymer precursors allows the synthesis of NPs with a wide range of sizes, surface chemistry, charge, and targeting agent densities. Adapted, with permission, from [16].

surface charge to be altered and recognizable by tumor cells, based on pH differences between normal tissue and the tumor microenvironment [31]. The switchable surface charge of these particles allows more efficient cellular uptake than the highly hydrophilic PEG NP [26,27,31].

A concept prevalent in scientific innovation is the notion of borrowing from nature. The longest circulation time achieved by synthetic particles in clinical trials is under 300 h, whereas the human red blood cell circulates for 100–120 days in the body [32]. This is mainly because the membrane protein CD47, a 'self-marker' on cell membranes (including the red blood cells of humans, mice, and other mammals), signals the phagocyte receptor CD172a, preventing cells from being phagocytosed [33]. NPs modified with a synthetic minimal 'self' peptide that was computationally designed based on human CD47 showed prolonged circulation half-life in a mouse model [33]. Further research extended this concept to prolong the residence time of NPs *in vivo* by coating poly(D,L-lactide-co-glycolide) (PLGA) NPs with erythrocyte membranes that incorporated the mouse's own membrane lipids and membrane proteins (Figure 2). Preliminary preclinical tests showed that these novel NPs had a longer circulation half-life than PEG-coated NPs [34]. Although more research is necessary, zwitterionic and erythrocyte-coated NPs may become viable PEG substitutes, as zwitterions offer increased uptake and erythrocytes pose little risk of immunogenicity from the patients' own somatic cells.

Nanoformulation and controlled release

Nanoformulation is an important opportunity to revisit promising molecular entities that failed in the development process due to poor pharmaceutical properties such as high cytotoxicity or poor cellular uptake. A recent example is CRLX101, which is a polymer-based NP containing camptothecin (CPT) conjugated to a cyclodextrin-containing polymer (CDP) for the treatment of solid tumors [35]. Unfavorable cytotoxic effects led to the shelving of development of CPT despite clear efficacy in tumor suppression. The CRLX101 NP displays a sustained intracellular release profile that lowers systemic exposure and significantly decreases CPT toxicity [35]. The Phase I/IIa study shows low levels of toxicity and promising antitumor activity [35]. CRLX101 partly solves the decades-long problem of CPT toxicity by using NPs to release a controlled amount of CPT over a longer period of time. Many promising drugs such as CPT and wortmannin failed clinical development because they did not meet toxicity, stability, or solubility requirements. Nanomedicine has the potential to solve these problems and revive abandoned cancer drugs for clinical use [36].

Passive targeting

A major benefit of nanomedicine is the improved biodistribution of therapeutic agents through passive targeting, a defining feature of first-generation NPs. The enhanced permeability and retention (EPR) effect refers to the fact that tumors retain more polymeric NPs, proteins, liposomes, and micelles than other tissues [10,37,38]. Most tumors have an abnormally dense and permeable vasculature created through stimulation by vascular endothelial growth factor (VEGF). Tight junctions in normal vasculature prevent particles larger than 2 nm from crossing between endothelial cells. However, the tight junctions and basement membrane of tumor vasculature are disordered, allowing entities from 10–500 nm in size to extravasate and accumulate within the tumor interstitium [39,40]. The lymphatic drainage system is also impaired in tumors, further entrapping macromolecular particles and delaying their clearance [41,42]. Passive targeting is based on both the minute size of drug carriers and the leaky neovasculature of the tumor (Figure 3). With the longer blood circulation time achieved by stealth modification (e.g., PEGylation), increased accumulation of NPs is possible through the EPR effect [39].

Although the notion of utilizing NPs for therapeutic purposes has existed for decades, nanotherapeutic agents that have reached the market have had varying degrees of

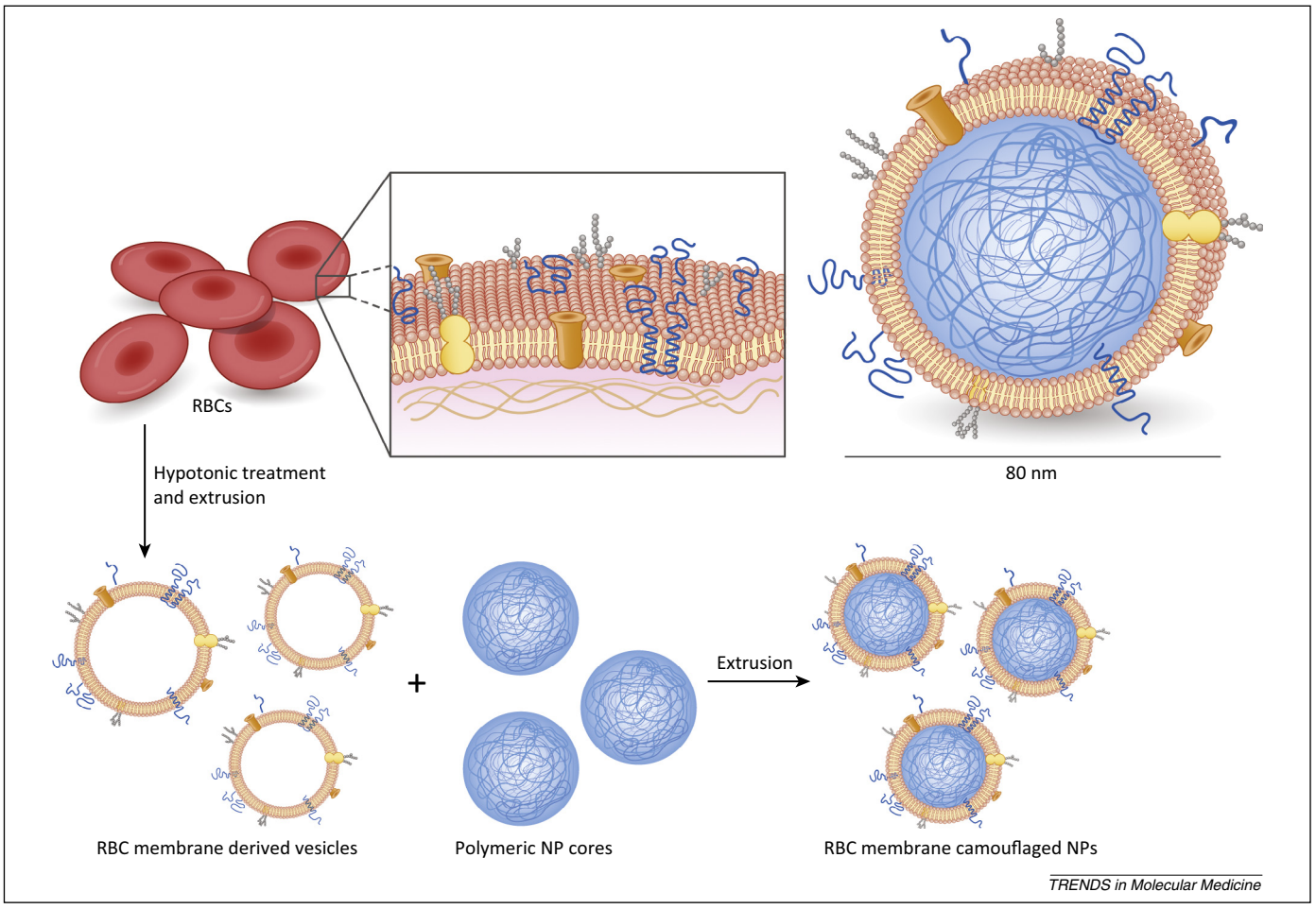

Figure 2. Red blood cell (RBC) membrane-coated poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles (NPs). Cellular membranes provide a robust natural functionality to the particle. In comparative studies with polyethylene glycol (PEG)-coated NPs, RBC membrane-coated NPs exhibited a 39.6-h half-life compared with 15.8 h for PEG NPs. Adapted, with permission, from [34].

success [43]. Doxil® was approved for clinical use in 1995 to treat AIDS-related Kaposi's sarcoma, ovarian cancer, and other cancers [44]. Encapsulating doxorubicin within PEGylated NPs allows extended circulation half-life and higher tumor concentration of the drug. Homing to the disease site is driven solely by the particles' nano-dimensions through the EPR effect [45] rather than any specific recognition of the target. Another example of a passive-targeting nanomedicine is Genexol-PM®, a polymeric micelle delivery system whose formulation includes poly(D,L-lactide), which allows controlled release of the therapeutic agent (Genexol-PM® was approved in Korea in 2007).

ALN-TTR02 is a lipid NP (LNP) that encapsulates siRNA targeting a conserved sequence in the 3′ untranslated region of the transthyretin (TTR) gene. The NP structure comprises a neutral lipid, a PEG lipid, and an ionizable cationic lipid to facilitate encapsulation of negatively charged siRNA through electrostatic interactions [46]. It is used to treat TTR amyloidosis, a condition produced by a mutant TTR gene that causes the accumulation of TTR amyloid in peripheral nerves and the heart [47]. Phase II trials showed greater knock down and continuing suppression of TTR with varying single doses compared with placebo. The therapy seems to be generally safe, with no serious adverse events yet reported [47]. However, ALN-TTR02 targets delivery to the liver, which is already a proven site of NP accumulation due to reticuloendothelial system uptake. There remains progress to be made to treat diseases requiring differential biodistribution of therapeutic agents.

TKM-PLK1 is another LNP, similar in structure to ALN-TTR02, encapsulating siRNA that inhibits the protein product polo-like kinase 1 (PLK1). PLK1 phosphorylates Cdc25C, regulates DNA damage checkpoints, microtubule nucleation, chromosomal condensation and segregation, and is an important target for therapeutic treatment [48]. This nanoformulation is significant because it does not rely on accumulation in the organs of the reticuloendothelial system (such as the liver) to deliver its payload. TKM-PLK1 relies mainly on the EPR effect to localize NPs in solid tumors, with encouraging results [49]. Phase I trials measured the effects of dose escalation on solid tumors in advanced cancer patients with promising safety and efficacy results, culminating in an ongoing Phase II clinical trial for patients with advanced gastrointestinal neuroendocrine tumors (GI-NETs) or adrenocortical carcinoma (ACC).

The NP therapeutic agents in clinical trials are clearly an improvement over current treatments. ALN-TTR02 uses gene silencing to knock out mutant TTR production.

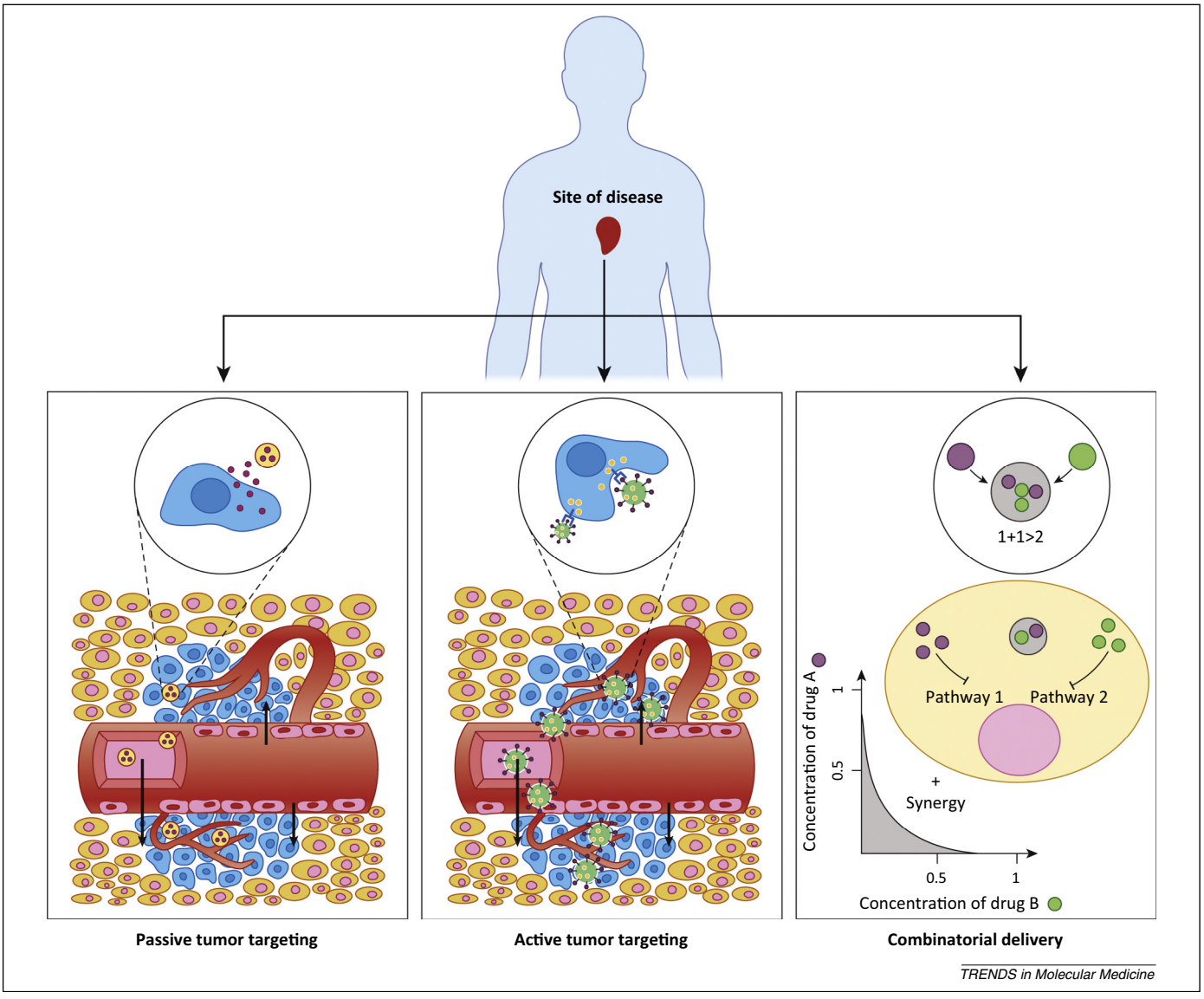

Figure 3. Passive targeting, active targeting, and combinatorial delivery. In passive targeting (left), nanoparticles (NPs) passively extravasate though the leaky vasculature via the enhanced permeability and retention (EPR) effect and preferentially accumulate in tumors. In active targeting (middle), targeting ligands on the surface of the NP trigger receptor-mediated endocytosis for enhanced cellular uptake. In combinatorial delivery (right), two or more therapeutic agents inhibit different or identical disease pathways for a synergistic effect.

This shows promise as a viable alternative to invasive procedures for TTR-mediated amyloidosis, such as liver transplantation, as well as offering a possible cotreatment with TTR stabilizers such as diflunisal. TKM-PLK1 is delivered systemically to solid tumors and Phase I trials targeting PLK-1 for tumor proliferation have had generally positive results. Due to the specific sizes of the NPs described above, the EPR effect combined with hemodynamic and diffusive mechanisms contributes to the longer blood circulation time and accumulation of NPs in the tumor. However, passive targeting has several drawbacks. Suboptimal biodistribution, with particles being trapped mainly in the liver and spleen due to reticuloendothelial function, is a major impediment to efficient delivery. In addition, the extent of the EPR effect varies between tumors and even intratumorally due to heterogeneity and to vascular permeability differences within an individual tumor [40,50]. Furthermore, the higher interstitial pressure within the tumor core causes the NPs to flow from the inner regions to the outer regions, further exacerbating this issue [10]. Two major challenges must be resolved in targeted delivery: further extending blood circulation time and homing the NPs toward specific sites of targeting for intracellular delivery. Therefore, efforts are needed to synergize passive targeting with a more dynamic method capable of further improving the accumulation of NPs at disease sites.

Active targeting nanomedicine

Even with the improvements in biodistribution offered by the EPR effect and PEGylation, most of a therapeutic agent (90% or more) will inevitably be concentrated in the reticuloendothelial organs such as the liver and spleen due to clearance by mononuclear phagocytes [51]. Active targeting is being explored as a method to achieve spatial localization by intentionally homing NPs to active diseased sites

while eliminating off-target adverse effects in normal tissue. Polyvalent decoration of a NP's surface with a ligand can facilitate binding to a biomarker that is specifically overrepresented in targeted cells and trigger receptor-mediated endocytosis (Figure 3), a process that has considerable implications for targeted delivery [5]. The ligands used to modify NPs include antibodies (Figure 4), engineered antibody fragments, proteins, peptides, small molecules, and aptamers [52]. The specific ligand–receptor interaction can be utilized to concentrate a therapeutic nanomedicine at a diseased tissue *in vivo*, producing a preferred distribution profile [3,53]. The ligand density can be fine-tuned in the formulation process to optimize avidity [10,54].

A few active-targeting nanoplatforms utilizing ligand–receptor interactions have entered clinical trials. The first targeted NP delivery system to feature siRNA was CALAA-01, which comprises a cyclodextrin-containing cationic polymer, a PEG corona, and human transferrin (Tf) as a targeting ligand [55]. The Tf on the NP surface binds to overexpressed Tf receptors (TfRs) on cancer cells and the NPs are then internalized via receptor-mediated endocytosis. When these siRNA-containing targeted NPs were administered intravenously to melanoma patients, they circulated in the body and localized in tumors [55,56]. Tumor biopsies showed a correlation between the dose administered and the amount of intracellularly localized NPs. Furthermore, levels of both the specific mRNA and the protein were lower after injection of the targeted NPs. In this study, the TfR was used as a potent target; it is typically upregulated on cancer cells and triggers cellular uptake via clathrin-coated pits. It is also ubiquitously expressed in all types of tissue to satisfy the iron requirements of dividing cells. Other examples of ubiquitous receptors, including folate receptors and the receptor tyrosine kinase epidermal growth factor receptor (EGFR), have been explored for the active-targeting delivery of nanotherapeutic agents to tumor cells [57–59].

Biomarkers distinctively expressed by certain organs offer the possibility of further improving the specificity of nanomedicine treatments. Prostate-specific membrane antigen (PSMA) is a good example of such a tissue-specific receptor. BIND-014, a targeted nanomedicine functionalized with the PSMA-specific ligand, is currently in human clinical trials. It is a polymeric NP encapsulating docetaxel (DTXL) for solid tumor treatment. This NP is modified with a PSMA substrate analog inhibitor, S,S-2-[3-[5-amino-1-carboxypentyl]-ureido]-pentanedioic acid (ACUPA), specific to PSMA, that is upregulated on prostate cancer cells. In Phase I clinical trials, BIND-014 displays promising results

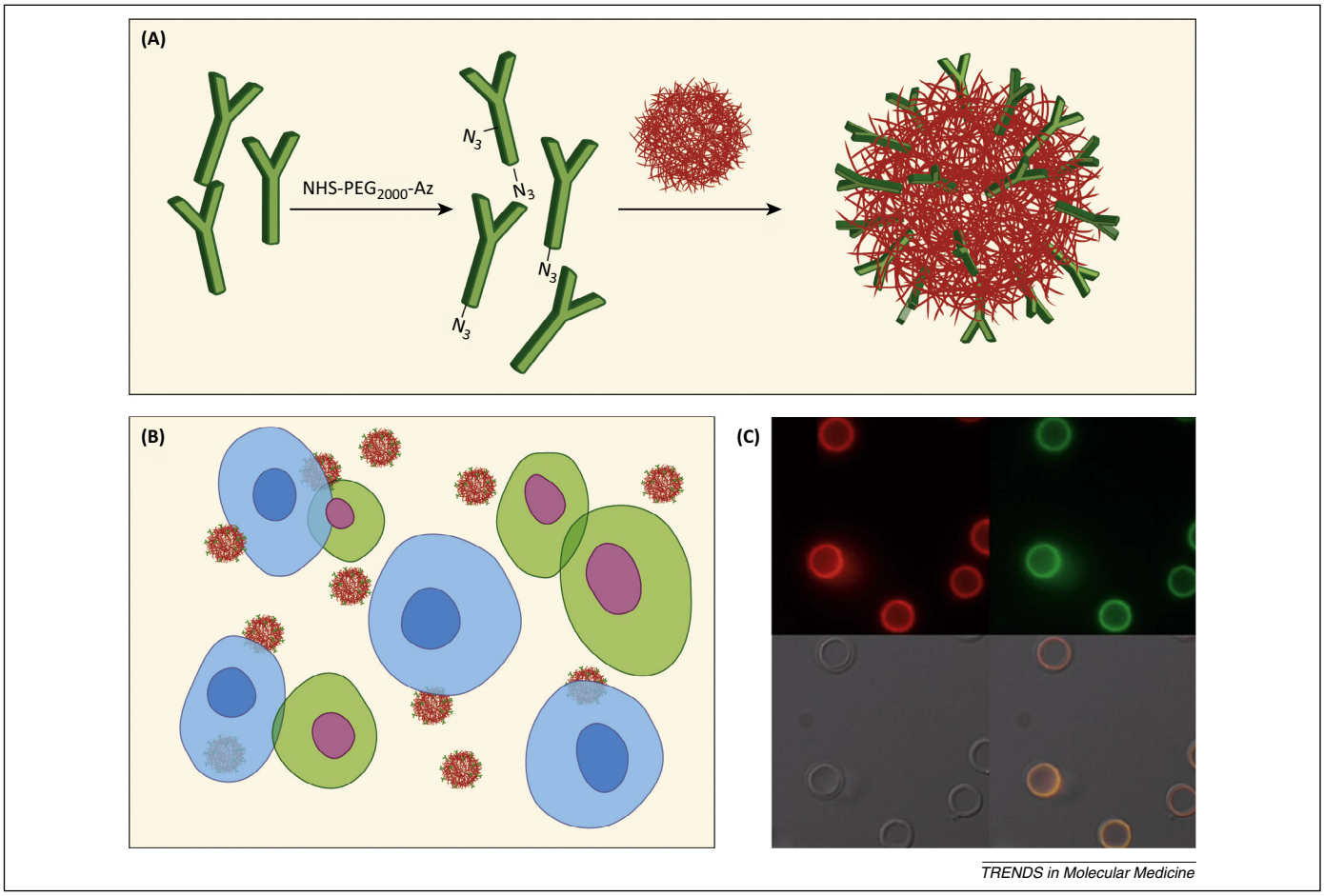

Figure 4. Antibody functionalization and visualization. **(A)** Antibodies conjugated to the nanoparticle (NP) surface through 'click' chemistry. **(B)** Cells that express the complementary antigen are blue and show antibody (Ab)-facilitated binding of targeted NPs. Cells that do not express the complementary antigen are green with no NP binding. **(C)** Fluorescence microscopy images of huA33 mAbAz-functionalized nanocapsules with: (i) antibody labeled with AF647 (red); (ii) antibody labeled with AF488 (green); (iii) bright field; and (iv) overlay images. Adapted, with permission, from [61].

in patients with advanced or metastatic large tumors [1]. Encouragingly, tumor recession has been observed in patients with cancer unresponsive to other treatments [60]. The enhanced therapeutic index of DTXL was mainly attributed to PSMA targeting, which is consistent with preclinical results. Phase II clinical trials of BIND-014 are under way for the treatment of metastatic drug-resistant prostate cancer and non-small cell lung cancer. Other specific targets have been investigated for targeted drug delivery. For example, the increasing availability of monoclonal antibodies has fostered interest in antibody-functionalized nanomedicines over many years [61,62].

Active targeting with NPs has yielded promising findings in preclinical studies and, in some cases, early clinical trials. However, some studies involving NP targeting have been inconclusive and therapeutic efficacy in humans has not yet been convincingly demonstrated overall [3,51,63]. The protein corona that forms around a NP as it interacts with physiological proteins in the body, as well as factors that interrupt the orientation and proper display of the targeting ligand, highlight the need for further studies of the clinical relevance of actively targeted nanomedicine [3,63].

NP-based combination therapy

Cancers are complex diseases involving multiple pathways and their progression is marked by many successive mutations in a line of cells. In addition, since mutations favorable to the survival of tumor cells are selected as chemotherapy progresses [64], tumors often present challenges such as intrinsic and acquired resistance to chemotherapeutic agents. Therefore, inhibition of a pathway by a single drug may be insufficient to achieve tumor recession. In combination chemotherapy, the synergistic effect of two (or more) agents targeting different disease pathways, genes, or cell-cycle checkpoints in the cancer process is utilized to raise the chances of eliminating the cancer (Figure 3). Combination of chemotherapeutic medications allows oncologists to use drugs at lower doses, reducing cytotoxic effects but increasing efficacy, and therefore present a promising approach for cancer research [10]. In practice, combination chemotherapy results in a better response and improved survival compared with single-agent therapy; recent examples include the combination of Platinol (cisplatin) and Navelbine (vinorelbine) to treat non-small cell lung cancer and TCH (Taxol, carboplatin, and Herceptin) for the treatment of HER2/neu-positive tumors [65,66].

Nevertheless, the effective administration of multiple drugs at an optimized dose ratio is complicated by dissimilar pharmacokinetics and biodistribution due to different rates of metabolism within the body [67]. Nanoformulations can help avoid such limitations by carrying (in one NP) multiple therapeutic agents with different physicochemical properties and pharmacological behaviors. In addition, NPs are able to maintain the optimized synergistic drug ratio in a single carrier to the point of intracellular uptake into the target cancer cell. This ratio may not be maintained by the use of separate carriers that each encapsulate a different drug. Currently, this novel 'two-in-one' approach is under clinical and preclinical investigation.

CPX-351 is a liposomal NP for the treatment of acute myeloid leukemia designed to incorporate the chemotherapeutic drugs cytarabine and daunorubicin in an optimized 5:1 molar ratio. Such a combination has previously been used clinically with small-molecule drugs but the efficacy was limited by unsuitable pharmacokinetics and poor solubility, requiring coadministration with toxic solvents [68]. In Phase I and II trials, CPX-351 increased overall survival in first-relapse patients [69] and it is currently in Phase III clinical trials. Other combinations, such as CPX-1 (irinotecan/floxuridine) and paclitaxel/tanespimycin, are under preclinical/clinical investigation [68,70,71].

Combinatorial therapies involving both siRNA and miRNA have the potential benefits of dual inhibition of a target gene product as well as modulation of oncogenes within the same pathway. A team used univariate Cox regression analysis and multiple miRNA target-prediction programs on a dataset from a previous ovarian cancer study to identify miRNA candidates likely to improve antitumor potency when combined with EphA2-targeting siRNA [72]. EphA2 is overexpressed in ovarian cancer and is associated with shorter median survival [73]. In a mouse model, the chosen miRNA:siRNA combination was delivered via a neutral liposomal nanocarrier comprising 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) and demonstrated a tumor-suppressive effect superior to that of either miRNA or siRNA alone [72]. In addition, a multiple siRNA combination therapy recently demonstrated increased efficacy in Phase I clinical trials [74]. The combination of siRNA and miRNA technologies is a significant step toward realizing the full potential of RNAi therapies.

The combination of chemotherapy with RNAi is also a promising synergistic strategy for cancer treatment. Recently, a polymeric NP platform comprising an aqueous inner core, a cationic and hydrophobic PLGA layer, and a hydrophilic PEG corona was developed to circumvent acquired chemoresistance by simultaneously delivering a cisplatin prodrug and REV1/REV3L-specific siRNAs, which suppress gene targets crucial to translesion synthesis (TLS) pathways in tumors [75]. Most mutations that result from DNA damage are a consequence of error-prone TLS DNA synthesis, which plays a significant role in cisplatin-induced mutations. This eventually results in acquired chemoresistance by improving the capacity of tumor cells to either repair or tolerate DNA damage [76,77]. NPs were shown to synergistically suppress the target genes involved in TLS, resulting in tumor cell sensitization to chemotherapy and tumor inhibition in a mouse model that was more effective than with cisplatin monotherapy. Although this small-molecule drug/siRNA approach remains far from clinical evaluation, it presents a robust platform that not only screens and validates target pathways involved in drug resistance, but also achieves an efficacy that may not be possible with dual-drug or RNAi combinations alone.

Concluding remarks and future perspectives

In conventional oral or intravenous drug delivery of small-molecule drugs, the medicine is distributed indiscriminately throughout the body, with arbitrary concentrations reaching both the disease site and healthy tissue. Chemotherapeutic agents in general cause unintended adverse

effects on healthy tissue and require a trade-off between optimal disease treatment and patient quality of life. NP-based drug delivery systems offer revolutionary opportunities to develop highly effective targeted therapeutic agents with improved circulation half-life, bioavailability, biodistribution, pharmacokinetics, and safety profiles. In addition, NPs are indispensable in maintaining synergistic drug ratios in combination therapy and offer the first possibility of delivering therapeutic agents such as nucleic acids and unstable proteins. The co-delivery of adjuvants with antigens to tumors promotes antigen-specific immune responses against the cancer and is yet another facet of the numerous NP anticancer therapies in development [78]. However, there remains much to be learned in the emerging field of nanomedicine. We have yet to develop a carrier that can effectively deliver a payload intratumorally with clinically validated results. Extending circulation closer to the timescale of red blood cells and retention of particles at the disease site rather than in the reticuloendothelial organs remain significant challenges.

Advances in nanomedicine occur through the development of novel nanocarriers and technologies for drug delivery. An ideal nanocarrier should fit the following profile: (i) biodegradable and biocompatible; (ii) capable of effective homing, with most of the therapeutic agent localized within the target site; (iii) designed with optimal biophysicochemical properties for superior drug loading, circulation half-life, and sustained drug release across infrequent administration times; and (iv) amenable to cost-effective scale up for commercialization. The refinement and incorporation of these qualities in one nanocarrier is the 'Holy Grail' of nanomedicine, synthesizing cutting-edge knowledge and technologies from the disciplines of medicine, chemistry, engineering, and physics.

Besides the complications in the experimental design of NPs, there exist multiple challenges in the manufacturing, regulation, and approval of NPs for clinical use. Compliance with quality-control guidelines such as Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP), as well as passing the three phases of FDA Investigational New Drug trials, will be challenging in bringing new nanoformulations to the market [6]. Moreover, patent disputes are becoming more common as more companies acquire broad patent rights on a wide range of NP compositions and usage methods [79].

However, these challenges in nanomedicine are accompanied by new opportunities. The field of cancer nanomedicine has begun to experience success in clinical applications and remains to reach its full potential. There is mounting evidence that effective encapsulation of small-molecule drugs, nucleic acids, or other compounds may be capable of mediating comprehensive cancer management or even achieving a potential cure [6]. The virtually limitless modular possibilities for different ligands, materials, and therapeutic nanoformulations coupled with improved treatment efficacies allow us to consider NPs not just as drug delivery vehicles but as an entirely new class of therapeutic agent [10]. The broad range of diseases that NPs are capable of treating, the considerable amount of important research yet to be conducted, and the potential to commercialize novel formulations are undoubtedly important 'draws' for the brightest minds in research. There remain 'plenty of room at the bottom'. The era of nanomedicine is poised to mature in the next few decades; incorporating elements of personalized medicine, it will affect the therapeutic world in a powerful and permanent way.

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