Harnessing Nanomedicine for Mucosal Theranostics—A Silver Bullet at Last?

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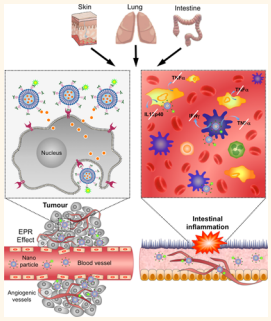
ABSTRACT Inflammatory bowel disease (IBD) has been extensively studied in the last four decades both in animal models and humans. The treatment options remain disappointing, nonspecific, and associated with multiple systemic adverse effects. In this Perspective, we highlight issues related to emerging nanotechnologies designed particularly for treatment and disease management of IBD and discuss potential therapeutic target options with novel molecular imaging modalities.



Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders affecting 0.5% of the Western population, whose incidence is steadily rising. The pathogenesis of this common disorder remains unknown, but it is estimated to result from loss of tolerance of the intestinal immune system in the presence of constant antigenic stimulus in genetically susceptible individuals, mediated by an immense resident microbial ecosystem. The major IBD subsets, Crohn's disease (CD) and ulcerative colitis (UC), feature a wide variety of clinical manifestations. These include chronic inflammation of the gastrointestinal tract that varies in uniformity (patchy disease characteristic in CD *vs* continuous disease in UC) and biogeographical distribution (disease isolated to the large intestine [UC] *vs* an inflammatory process that may involve the tract in its entirety, from mouth to anus [CD]).[1,2] Inflammatory bowel disease is also a systemic inflammatory disease with multiple extra-intestinal manifestations including, among others, the skin (erythema nodosum and pyoderma gangrenosum), eyes (uveitis and episcleritis), joints (multiple forms of arthritis), and liver (primary sclerosing cholangitis).[3] Moreover, long-standing IBD is associated with a significant propensity for the development of several cancers, such as an aggressive form of colorectal cancer and the frequently fatal cholangiocarcinoma. These risks necessitate strict patient surveillance, which often further results in reduced quality of life and subjects patients to heavy psychological consequences.[4]

While IBD has been extensively studied in the last four decades, both in animal models and in humans, treatment options remain disappointing, nonspecific, and associated with multiple systemic adverse effects. Treatment options generally target the mucosal and systemic inflammatory process and include immunosuppressive and immune modulatory interventions such as corticosteroids, nonsteroidal anti-inflammatory drugs, antibiotics, azathioprine, and methotrexate in addition to agents that inhibit inflammatory cytokines and effector cells such as monoclonal antibodies to tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and novel monoclonal antibodies against interleukin 12 (IL-12) and IL-23 that are under clinical evaluation. Additional strategies under clinical investigation that aim to block effector cell recruitment into the lesions include monoclonal antibodies to α_4 and $\alpha_4\beta_7$ integrins and to mucosal addressin cell adhesion molecule 1 (MAdCAM-1) and inhibitors of endothelial intercellular adhesion molecule 1 (ICAM-1) expression. All of these modalities feature limited efficacy and are associated with severe infectious, metabolic, and neoplastic side effects that, at times, are more severe than IBD itself.[5] The resultant disease course is that of

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exacerbations and remissions, with significant morbidity, reduction in quality of life, and frequent need for surgical intervention along the course of disease.

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Intensive research in recent years, mostly in small animal models, has been aimed at deciphering major cellular subsets and molecular pathways that are involved in the pathogenesis of IBD. These studies elucidated an impressive and diverse set of new therapeutic targets, including small molecules and small interfering RNAs, which enable precise molecular manipulation of IBD-associated pathways.[6] These modalities are highly effective in reverting intracellular processes and thus can potentially be employed in the treatment of IBD, either alone or in a combination of currently used therapies. However, a lack of suitable platforms for delivery to their cellular targets greatly limits the applicability of these modalities as IBD treatments.

Potential IBD-Associated Therapeutic Targets for Nanomedicine-Based Intervention. Several currently recognized major cellular and molecular players in IBD might potentially serve as useful therapeutic targets. The adoptive and innate immune arms cooperate in the induction and perpetuation of the mucosal inflammatory process in IBD and are the major subset targeted by currently employed therapeutic interventions. Adoptive cells involved with IBD include multiple T cell subsets such as TH1 (CD), TH2 (UC), and TH17 (both) and potentially B cells. Innate subsets involved in the IBD inflammatory process include neutrophils, natural killer cells, and members of the mononuclear phagocyte lineage including macrophages and dendritic cells.[7] Indeed, mononuclear phagocytes are critically involved in the maintenance of tissue integrity, as well as the initiation and control of innate and adaptive immunity. In homeostatic conditions, subsets of *lamina propria* mononuclear phagocytes sample the gut lumen through the intestinal epithelial tight junctions. By doing so, they coordinate and regulate the delicate balance between immune tolerance to harmless food antigens and commensal microorganisms and rapid initiation of the immune response to harmful pathogens. Impaired dendritic cell function, due to genetic and environmental causes, breaches this balance and results in the development of IBD.[8,9] Moreover, conditional ablation of intestinal anti-inflammatory *lamina propria* mononuclear phagocytes results in induction of severe autoimmune colitis, a process governed by TNF-α secretion. Altogether, it is believed that altered balance between these cellular subsets and their disregulated response toward components of the microbiome leads to massive local secretion of cytokines such as TNF-α, IFN-γ, IL-17, and IL-1β. Indeed, polymorphisms in the IL-23 receptor have been associated with IBD as well as other autoinflammatory and autoimmune disorders.[10,11] Moreover, immune-mediated mechanisms of tolerance are fundamentally important in preservation of mucosal tolerance toward the "healthy" microbiome. Abrogation of such tolerance-inducing mechanisms, such as IL-10 deficiency in mice or deficiency in IL-10 responsiveness in humans, is strongly associated with intestinal autoinflammation.[12−14]

Another attractive potential target is the intestinal epithelial layer,[15−17] an extensive one-cell-thick layer that separates the dense luminal microbial ecosystem from the inner sterile milieu through a complex network of molecular seals. In IBD, this barrier function is compromised, resulting in enhanced exposure of the *lamina propria* compartment to intact microbes or microbial elements.[18] Novel, nonbarrier functions shed new light at the epithelial cell being a critical "hub" for the regulation of the innate immune response. These include innate sensing of pathogens or damage-associated molecular patterns by epithelial toll-like receptors, nod-like receptors, inflammasomes, and lectin receptors.[15] Indeed, mutations in the nod-like receptor Nod2 are strongly implicated in Crohn's disease susceptibility in humans.[19] Other epithelial functions include cellular proliferation and anchoring of cell matrix molecules, which is critical for mucosal repair during chronic injury. Mice that are deficient in the mucosal repair molecule prostaglandin E receptor 4 become highly susceptible to induced colitis, while polymorphisms in proximity to this gene in humans are linked to susceptibility to IBD.[20] Other important epithelial functions, which may bear direct relevance to IBD, are mucus production by goblet cells and antimicrobial peptide secretion by Paneth cells in the small intestine. Alterations in any of these functions may result in enhanced microbiome influx into the inflamed, injured, and permeable intestine, promoting a continuous inflammatory state.

Other emerging therapeutic targets for IBD are the extracellular matrix and the intestinal microbiome. Key molecules in the extracellular matrix, including matrix metaloproteases among others, are increasingly recognized as pivotal in switching on the immune response, enabling inflammatory cell migration, and participating in the mucosal healing response. Specific abrogation of specific matrix metaloproteases was recently shown to ameliorate colitis

substantially in mouse models.[21] The microbiome is a highly diverse microbial ecosystem whose intimate interaction with the host mucosal interphase is crucial for tolerance maintenance and for the antipathogenic immune response. Alteration in composition and function of the intestinal microbiome, termed dysbiosis, is considered a cornerstone for the development of IBD.[22] While antibiotic and probiotic treatments have been associated with variable clinical effects, specific targeting of "colitogenic" taxa, delivery of therapeutics supporting tolerogenic microbial stains (termed prebiotics), or factors involved with host–microbiome interactions may prove efficacious as novel therapeutic modalities.[23] It is, therefore, expected that the next therapeutic modality in treating IBD could use targeted nanomedicines that deliver therapeutic cargos to bacteria or to epithelial cells that secrete substances that affect the microbiome.

Cell-Specific Targeted Nanoparticles for IBD Treatment. Recent developments in nanotechnology utilize an interdisciplinary approach to generate nanoparticles (NPs) with high specificities toward subsets of cells, which thereby create new opportunities for novel targeted therapies and specific molecular imaging that will aid in disease diagnosis and management. These targeted NPs—also termed nanomedicines—are made from natural biological materials such as lipids, sugars, proteins, or nucleic acids or from synthetic polymers and, unlike conventional therapies, have the potential to offer more effective treatment with significantly reduced adverse effects through specific interactions with diseased cells.[24,25] These NPs enable enhanced specificity in the delivery of large amounts of therapeutic or imaging payloads, have the capacity to deliver multiple payloads simultaneously, and can incorporate mechanisms to overcome biological barriers in the form of targeting agents that can direct the drug NPs into diseased cells without collateral damage to healthy tissues.

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The use of NPs in IBD is still in its infancy. Several strategies to direct NPs into the gut mucosa during IBD have been documented, mainly for local (rectal) use.[26−28] Local delivery of therapeutic molecules loaded in NPs to the inflamed colon could be a promising strategy only for UC patients, where the disease is located in the proximal end of the large intestine and the anus. A recent study investigated how drug-loaded polymeric NPs target the site of inflammation and analyzed the influence of different colon-specific delivery strategies.[27] Three different polymeric NPs were formulated using ovalbumin as a model drug: pH-sensitive NPs were made with eudragit S100; mucoadhesive NPs were created with trimethylchitosan (TMC); and a mix of polymers—poly(lactic-*co*-glycolic acid) (PLGA), poly(ethylene glycol)-PLGA (PEG-PLGA), and the diblock copolymer, PEG-poly(ε-caprolactone) (PEG-PCL)—were used to obtain sustained drug delivery. Furthermore, ligands targeting leukocytes (*e.g.*, mannose) or the inflamed colon (a specific peptide) were grafted on the PEG chain of PCL. Interaction of NPs with the intestinal epithelium was explored using Caco-2 monolayers designed to mimic an inflamed epithelium and then visualized using confocal laser microscopy. The TMC NPs had the highest apparent permeability for ovalbumin in the untreated model. However, in the inflamed model, there was no difference between TMC NPs, PLGA-based NPs, and eudragit NPs. The uptake of NPs in the inflamed mouse colon was assessed in a horizontal diffusion chamber. Mannose-grafted PLGA NPs showed the highest accumulation of ovalbumin in inflamed colon. On the basis of these results, it was suggested that active targeting of macrophages and dendritic cells may be a promising approach for targeting the colon in IBD.[27]

In animal studies, luminal uptake into inflamed mucosal areas has been shown to be size-dependent.[29−31] In a recent study,[32] Schmidt *et al.* investigated the potential of NP and microparticle (MP) uptake into the rectal mucosa of human IBD patients. Fluorescently labeled placebo NPs 250 nm in diameter and MPs 3.0 μm in diameter were prepared. Two hours after rectal application to patients with CD or UC, confocal laser endomicroscopy was performed to visualize the particles in inflamed mucosal areas. In biopsies, *ex vivo* mucosal transport processes were investigated in miniaturized chambers. Overall, 33 patients with IBD (19 patients with CD, 14 patients with UC) and six healthy controls were tested.[32] A significantly enhanced accumulation of MPs in ulcerous lesions was observed (covered area = 1.28%, range 0.83−3.45%, *vs* 0% in controls; p = 0.011), while NPs were visible only in traces on mucosal surfaces of all patients. This study strengthens the importance of selective systemic delivery of therapeutic payloads directly into subsets of leukocytes and perhaps also to gut epithelial cells since IBD is a systemic, multifactorial

disease. To address this need, we previously developed a strategy that utilized a natural pathological process.[33] The b_7 intergrins are exclusively expressed on leukocytes and are dramatically upregulated during IBD. Moreover, circulating b_7-expressing lymphocytes home to the inflamed gut during IBD.[34−36] We developed a robust strategy to target these circulating lymphocytes *in vivo* and showed that we can deliver therapeutic payloads in a b_7-specific manner using lipid-based NPs covalently coated with an antibody raised against the b_7 integrin.[33,37] This first proof-of-principle study showed that selectivity *in vivo* using NPs targeted to leukocytes is feasible.[38]

RNA interference (RNAi) payloads are currently available as IBD research tools and might be applicable for clinical use. RNA interference has emerged as a powerful strategy for suppressing gene expression, offering the potential to accelerate *in vivo* drug target validation dramatically as well as the promise to create novel therapeutic approaches if it can be effectively applied *in vivo*.[39,40] The main aim of RNAi applied *in vivo* is to achieve safe, gene-specific silencing *via* tissue-specific targeting using efficient amounts of RNAi molecules. *In vivo* delivery of RNAi could be accomplished *via* local or systemic administration routes, depending on the tissues/organs that are targeted. Various approaches for local delivery of RNAi molecules have recently been applied in animal models. Techniques to deliver drugs into the gastrointestinal (GI) tract can include the provision of drugs in solution. However, such drugs will be directly affected by the pH of the stomach and are likely to degrade under acidic pH conditions. To circumvent degradation by stomach acidic pH or small intestine digestive enzymes, high drug doses or frequent administration are commonly used, and adverse effects may be challenging. Enemas are often used to target drugs to the colon, but the procedure is cumbersome and is associated with high risk of local complications, including bleeding or perforation. Thus, there is an unmet need for targeted strategies to specific areas in the GI tract, particularly the colon but also the upper GI track such as in the case of CD patients. As the terminal end of the digestive tract, the colon is challenging to target with intact and quantitative amounts of drug. From oral uptake (saliva enzymes) to colon (pH 7, higher pressure), through the stomach (pH 1−3) and the small intestine (enzymatic release and pH 3−6), drugs face deleterious environments.[41] The fact that nucleic acids in general are exposed to phagocytosis and nuclease degradation, and are membrane-impermeable, makes systemic RNAi delivery more complicated and challenging in general and particularly if the target cells are not localized to one pathological site but are spread systemically.

To address this challenge, systemic delivery strategies have been developed to target subsets of leukocytes. As discussed above, b_7-integrin-targeted and stabilized NPs have been used to packaged RNAi payloads and deliver them into b_7-expressing lymphocytes *in vivo* in an intestinal inflammation model. This strategy was also used to reveal that cyclin D1 could serve as a potential novel, anti-inflammatory target for IBD.[33] Oral delivery of TNF-α siRNAs was also developed using polymeric NPs and showed a proof-of-concept efficacy in the dextran sodium sulfate-induced colonic autoinflammation model.[42,43] Although these are highly promising examples, many challenges still need to be addressed when using RNAi to manipulate leukocytes *in vivo*. Among these challenges are immune toxicity, which needs to be carefully examined (including complement activation, induction of pro-inflammatory cytokines, interferon responses, and interference with natural coagulation cascades), and deposition of the oligos and the NPs at the cellular level.

Use of Nanoparticles for Diagnosis and Follow-Up of IBD Patients. Irritable bowel disease, and in particular that affecting the small intestine, is often difficult to diagnose mainly because of difficulties accessing many regions of the small intestine. Although innovative strategies, such as the use of capsule endoscopy, offer novel ways of diagnosing and following up lesions in the small intestine, these approaches are costly, invasive, and may be associated with complications such as aspiration and capsule retention, the later more prevalent in patients with strictures or fistulas. In addition, long-term follow-up of colonic IBD is warranted in long-term patients as a means of surveillance for the aggressive and hard-to-diagnose colorectal carcinoma that is prevalent in these patients. Even though periodic surveillance colonoscopy is recommended for these patients, the invasive and cumbersome procedure suffers from low compliance rates even among at-risk patients. Alternative approaches, such as virtual colonoscopy, necessitate similar preparation and bowel inflation and are often problematic in sensitivity and specificity.

Systemic molecular-based imaging for diagnosis and follow-up of IBD would provide more global information on disease extent and severity, while avoiding many of the current limitations of existing modalities. Furthermore, such approaches would be less subject to operator-dependent variability and would enable the kinetic assessment of lesions over time.

The infiltration and activation of dendritic cells, macrophages, and T cells play key roles in the development of IBD. These cells, and other molecular markers in the process such as chemokines, cytokines, and receptors of the immune system, can be used as markers for scintigraphic imaging. Currently, colonoscopy and/or small-bowel follow-through are considered by most to be the gold standard imaging techniques for the diagnosis of

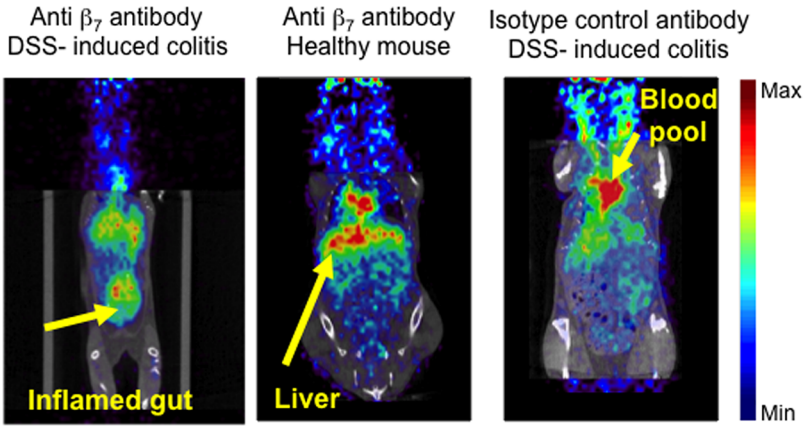

Figure 1. Representative images of microPET/CT show the distribution of ^{64}Cu-labeled anti-β_7 monoclonal antibodies (mAb) in mice, 48 h after administration as a strategy for disease management in inflammatory bowel disease.[50] The mAb raised against the integrin subunit β_7 shows higher uptake in the gut of mice with colitis (left) than in control mice (middle). Antibodies can also accumulate in inflamed tissue nonspecifically. The representative image on the right shows low uptake of nonspecific (isotype control) mAb in inflamed gut and confirms that the mAb uptake in dextran sodium sulfate-induced colitis (left) is specific (color scale = microPET image of radionuclide distribution, gray scale = microCT anatomical image).

IBD. However, since the majority of patients need long-term follow-up, it would be ideal to rely on a noninvasive technique with good compliance. With the possibility of intravascular specific contrast agents for blood enhancement, computed tomography (CT) scans, ultrasonography, and magnetic resonance imaging (MRI) are all able to detect increases in biological or endoscopic signs of disease activity (vessel dilation, wall thickening, wall stratification with thickening of the submucosa, wall and mesenteric hypervascularity, lymph node enlargement, and enhancement). Nanoparticles could prove highly useful as contrast agents. Some examples include superparamagnetic iron oxide NPs, which have notably been used with MRIs in diagnostic radiology;[44] gold NPs;[45,46] titanium dioxide NPs; and gadolinium NPs.[47−49]

Dual-modality contrast agents, such as radiolabeled NPs, are promising candidates for a number of diagnostic applications because they combine the advantages of two different imaging modalities, namely, combining single-photon emission computed tomography or positron emission tomography imaging with MRI or CT.[44] Molecular imaging is also becoming an important strategy in radiology. Cell-specific receptors are being labeled using specific antibodies that are conjugated to contrast agents or radiometals. Recently, Dearling *et al.* reported a nanotechnology strategy using ^{64}Cu-labeled anti-β_7 integrin antibody to detect intestinal inflammation by microPET/CT imaging.[50,51] This integrin represents a promising therapeutic target, as discussed above, but could also be used for the detection of CD and UC because of its specificity on a small subsets of leukocytes and its involvement in lymphocyte recruitment to the inflamed gut and not to other principal organs such as the lungs or liver. In this proof-of-concept study, the selective uptake of anti-β_7 integrin ^{64}Cu-labeled antibody in the gut of animals with experimentally induced colitis suggested that integrin β_7 may be a promising target for radioimmunodetection of colitis, which would aid in the diagnosis, assessment, and therapy guidance of IBD (see Figure 1). Indeed, this could become a new disease management modality when tested clinically in humans.

FUTURE OUTLOOK

The development of nanomedicine for disease theranostics is progressing at a fast pace and is driven by principles from diverse fields of science in the postgenomic age. Nano-based theranostics are made possible by integrating the application of molecular biomarkers across the range of tests and interventions reaching from disease susceptibility testing and monitoring of clinical outcomes resulting from interventions. However, the terms "nanotheranostics" or "theranostics using nanomedicine" usually reflect the ability of nanocarriers to report on a specific pathological site or cell and to deliver drugs simultaneously directly into these cells.[52] One such strategy that might be relevant for IBD is to use multistage, silicon-based NPs that can entrap therapeutic payloads with gadolinium-based contrast agents that enhance T_1 contrast for MRI use.[53] Other potential options include a turn-on, near-infrared, cyanine-based probe for noninvasive intravital optical imaging of hydrogen peroxide.[54] This strategy can report on inflamed areas using intravital optical imaging, and at the same time, H_2O_2 can be used as a therapeutic strategy for gut inflammation if small amounts are delivered to the appropriate site in the gut.

Although the number of nanomedicines that are reaching the clinic is dramatically increasing, most of them thus far are cancer treatments.[24,55] We propose that novel theranostic nanomedicines will be developed for IBD, combining novel therapeutic and molecular imaging modalities such as combinations of targeted NPs that incorporate

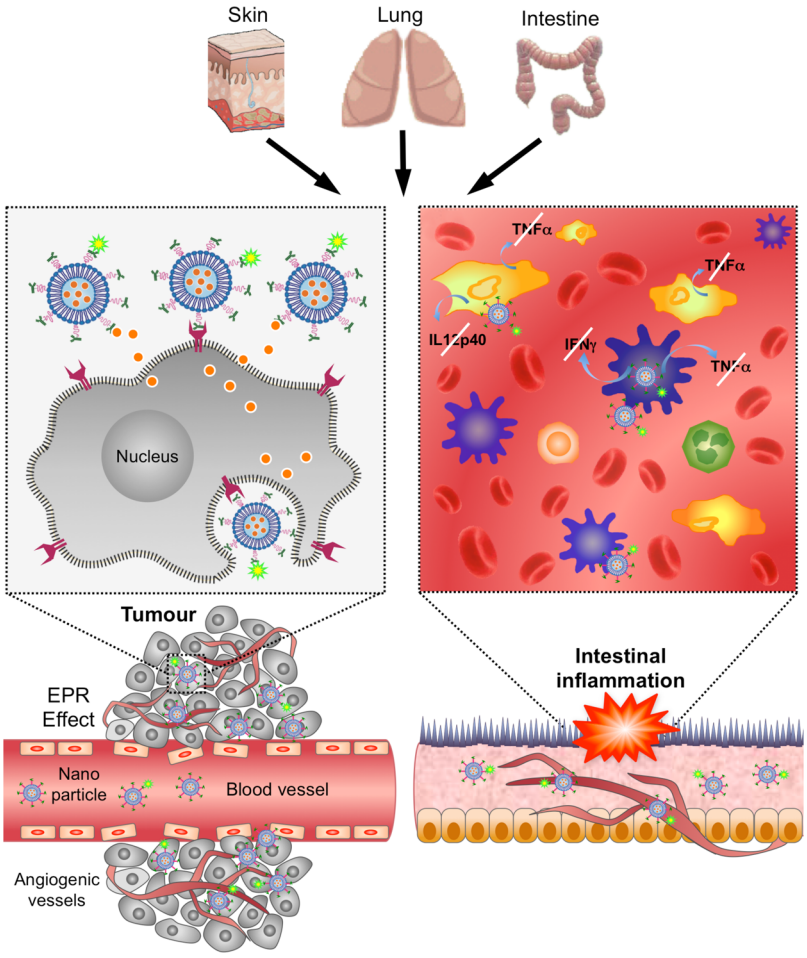

Figure 2. Schematic representation of different mechanisms by which nanoparticles (NPs) can deliver drugs and report on mucosal disease. Lipid-based NPs (as representative nanocarriers) can be employed in a variety of inflammatory and neoplastic mucosal disorders such as in the lungs, skin, and gut. Passive tissue targeting is achieved by extravasation of NPs through increased permeability of the vasculature and ineffective lymphatic drainage (EPR effect). Active cellular targeting (inset, left side) can be achieved by functionalizing the surfaces of NPs with ligands that promote cell-specific recognition and binding. The NPs can release their contents in close proximity to the target cells, attach to the membrane of the cell and act as an extracellular sustained-release drug depot, or internalize into the cell. Anti-inflammatory payloads (inset, right side) such as small molecules, RNAi, and proteins can manipulate the function of pro-inflammatory subsets of leukocytes and shift the response toward an anti-inflammatory response or *vice versa* (depending on the response). The delivery of therapeutic or imaging payloads (green star bound to the monoclonal antibodies that decorate the NPs) may be induced systemically or locally (such as luminal delivery in the gut).

> The development of nanomedicine for disease theranostics is progressing at a fast pace and is driven by principles from diverse fields of science in the postgenomic age.

therapeutic payloads (such as siRNAs targeting specific genes) with imaging agents such as ^{64}Cu that can be coupled to a cell-surface-specific targeting moiety (see Figure 2). Furthermore, individualized IBD characteristics such as cellular receptor expression, pathway activation, and cytokine profile could be assessed and used to design personalized nano-based targeting vectors and payloads. As such, the use of host and microbiome RNA sequencing in patients clustering to IBD subgroups, such as nonresponders *versus* responders, may reveal new therapeutic targets that can be downregulated by specific siRNAs or upregulated by microRNAs as a means of treatment for specific patients. Thus, we believe that these novel strategies could indeed represent a long-sought new class of personalized medicine in IBD treatment and management. This combined nanotheranostic approach—which will include new therapeutic payloads, possibly coupled with and incorporated into existing "conventional" treatment strategies—combined with novel molecular imaging approaches may revolutionize the management of IBD.

Conflict of Interest: The authors declare no competing financial interest.

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