



A paradigm shift in cancer nanomedicine: from traditional tumor targeting to leveraging the immune system

Alexandros Marios Sofias¹, Francis Combes^{2,3}, Steffen Koschmieder⁴, Gert Storm^{5,6,7} and Twan Lammers^{1,5,6}

¹ Department of Nanomedicine and Theranostics, Institute for Experimental Molecular Imaging, Faculty of Medicine, RWTH Aachen University, Aachen, Germany

² Laboratory of Gene Therapy, Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

³ Cancer Research Institute Ghent (CRIG), Ghent, Belgium

⁴ Department of Medicine (Hematology, Oncology, Hemostaseology, and Stem Cell Transplantation), Faculty of Medicine, RWTH Aachen University, Aachen, Germany

⁵ Department of Pharmaceutics, Utrecht University, Utrecht, The Netherlands

⁶ Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands

⁷ Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Twenty-five years after the approval of the first anticancer nanodrug, we have to start re(de)fining tumor-targeted drug delivery alongside advances in immuno-oncology. Given that cancer is characterized by an immunological imbalance that goes beyond the primary tumor, we should focus on targeting, engaging, and modulating cancer-associated immune cells in the tumor microenvironment (TME), circulation, and immune cell-enriched tissues. When designed and applied rationally, nanomedicines will assist in restoring the immunological equilibrium at the whole-body level, which holds potential not only for cancer therapy, but also for the treatment of a range of other disorders.

Introduction

Cancer nanomedicine still focuses primarily on delivering chemotherapeutic drugs directly to cancer cells (Table 1). Here, we aim to contribute to a paradigm shift in which nanoparticles (NPs) are no longer packed with chemotherapeutics for the direct eradication of cancer cells, but instead loaded with immunomodulatory agents and targeted to cancer-associated immune cells. We describe various possibilities for *in vivo* immune cell targeting (in contrast to their *ex vivo* manipulation), and propose to target immune cell populations inside and outside the TME. Preclinical as well as clinically already translated NPs can be repurposed for such an endeavor because they can prolong the circulation half-life of therapeutic agents and shift their biodistribution profile toward target tissues and/or cells [1–4].

Traditional tumor targeting in nanomedicine

As mentioned earlier, the cancer nanomedicine field has historically invested heavily in directly killing cancer cells. The hope behind this traditional approach is that nanomedicines improve the accumulation of chemotherapeutic agents in tumor tissue and limit their off-target localization, which ultimately increases the efficacy:toxicity ratio [5]. The common consideration in this regard is that nanomedicine formulations preferentially accumulate in cancerous lesions based on enhanced vascular leakiness and defective lymphatic drainage associated with solid malignancies [6,7]. In such cases, a high degree of intratumor accumulation is the desired outcome. In many situations, however, the injected nanomedicine dose that reaches the tumor is low, averaging between 0.1 and 10% of the injected dose, both in animal models and patients [8]. This implies that 90–99.9% of the injected nanodrug dose ends up in organs and tissues other than the tumor (e.g., liver and spleen), or is rapidly cleared. In addition, significant heterogeneity in tumor accumulation is observed, both within a tumor in an

Corresponding authors: Sofias, A.M. (asofias@ukaachen.de), Lammers, T. (tlammers@ukaachen.de)

TABLE 1

Nanomedicine publication trends

Scopus search (January 8, 2021)	Number of studies per year				
	2016	2017	2018	2019	2020
'nanomedicine' AND 'chemotherapy'	276	295	370	416	470
'nanomedicine' AND 'immunotherapy'	67	74	128	177	247
'nanomedicine' AND 'immunomodulation'	27	37	27	38	42

individual patient as well as between different tumors in different patients. This complicates clinical translation [9,10].

Targeting the immune system with nanomedicines

Even with targeting capabilities increased beyond 10%, nanomedicine treatment cannot guarantee improved therapeutic responses in patients with cancer. This is particularly true when nanodrugs are used as monotherapies. Remission is often temporary and typically followed by relapse resulting from the re-establishment of pro-tumorigenic conditions, such as by progenitor immune cells [11] or reactivation of cancer stem cells [12]. In such situations, we consequently often end up in a continuous vicious circle of 'detection, treatment, response, and relapse'. This implies that truly curative anticancer therapy requires more holistic treatment concepts, which include not only direct eradication of cancer cells by chemotherapeutic agents, but also modulation of cancer growth-promoting phenomena that occur inside and outside of tumor tissue, involving, most importantly, the immune

system. In this context, it is crucial to understand that the long circulation properties that various nanomedicines have upon systemic administration will benefit their accumulation in anticipated target organs by avoiding rapid clearance by phagocytes in liver and spleen [13]. Therefore, nanomedicine formulations used for immune cell targeting must be able to evade clearance from the blood stream, be easily functionalizable with targeting moieties, and be loadable with different types of payload. These features benefit from the notion that nanomedicines are a versatile and readily available toolbox compared with other (bio)technological tools; for instance, conventional antibodies can only be directed against one therapeutic target, and microscale drug delivery systems do not have long-circulation properties.

Targeting immune cells in the tumor microenvironment

The immune system strongly affects tumorigenesis and malignant disease progression. This notion has resulted in the development

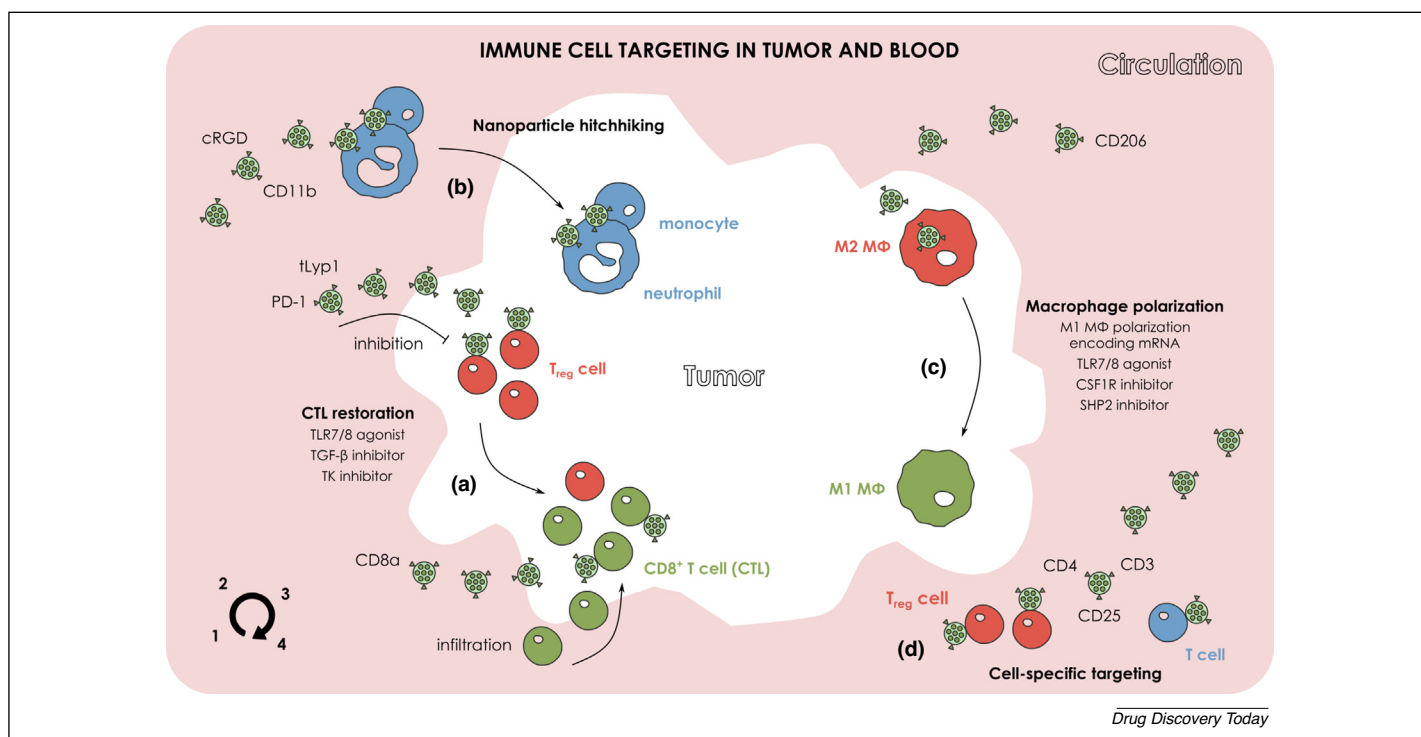


FIGURE 1

Nanoparticle (NP) targeting of the immune system in tumor and blood. Nanotherapies can aim for holistic manipulation of the immune system. (a) NPs can help to inhibit regulatory T cells, elicit a strong CD8⁺ T cell infiltration and, consequently, restore cytotoxic T lymphocyte (CTL) populations in the tumor microenvironment (TME). (b) NPs can exploit the inherent tumor-homing capabilities of myeloid immune cells and be delivered to tumors via immune cell hitchhiking. (c) NPs can be used to directly inhibit the activity of M2-like macrophages in the TME, and deliver immunomodulatory cargo that can polarize M2-like macrophages toward M1-like macrophages. (d) NPs can be specifically modified to target or inhibit specific immune cell subpopulations in the circulation. Please see main text for abbreviation definitions.

TABLE 2

Nanomedicines targeting immune cells in tumor, blood, and immune cell-enriched organs^a

Nanoparticles	Targeting decoration	Payload	Payload function	Tissue target	Cell target	Major outcome	Refs
PLGA-based polymeric micelles	CD8a, PD-1	R848 SD-208	TLR7/8 agonist TGF- β inhibitor	Tumor, blood, spleen, LN	CD8 ⁺ T cell, PD-1 ⁺ T cell	<ul style="list-style-type: none"> ↑ Survival ↑ Tumor-infiltrating CD8⁺ T cells Sensitized tumors to anti-PD-1 therapy 	[18]
PLGA-lipid hybrid NPs	tLyp1	Imatinib	Tyrosine kinase inhibitor	Tumor	FoxP3 ⁺ Treg cell	<ul style="list-style-type: none"> ↑ Survival ↑ Tumor inhibition ↓ FoxP3⁺ Treg cells ↑ CD8⁺ T cells 	[19]
β -cyclodextrin NPs	–	R848	TLR7/8 agonist	Tumor	M2 macrophage	<ul style="list-style-type: none"> Potentiated anti-CTLA-4 therapy Polarized M2→M1 macrophages Controlled tumor growth Protected against tumor rechallenge Potentiated anti-PD-1 therapy 	[20]
Liposomes, nanoemulsions	cRGD	(For imaging purposes)		Blood	Neutrophils, Ly6C [–] monocytes	NP hitchhiking with phagocytes in breast cancer	[24]
Liposomes	CD206	BLZ945 SHP099	CSF1R inhibitor SHP2 inhibitor	Tumor	M2 macrophages	<ul style="list-style-type: none"> Polarized M2→M1 macrophages ↑ Phagocytic capabilities ↑ Antitumor efficacy 	[21]
	cRGD	Edaravone	Neuroprotective	Blood	Neutrophils, monocytes	NP hitchhiking with phagocytes in cerebral ischemia	[26]
	CD3, CD4, CD25, Ly6C	CD45 siRNA TNF siRNA	(For testing CD45 silencing) To inhibit expression of proinflammatory mediator TNF α	Blood, LN	CD3 ⁺ T cells, CD4 ⁺ T cells, CD25 ⁺ Treg cells, Ly6C ⁺ monocytes	<ul style="list-style-type: none"> ↓ Infarct volume Cell-specific siRNA delivery ↓ Colitis by targeting Ly6C⁺ proinflammatory monocytes in IBD model 	[28]
	Ly6C	IL10-modified mRNA	Expressing anti-inflammatory cytokine IL10	Spleen	Ly6C ⁺ monocytes	<ul style="list-style-type: none"> ↑ IL10 in liver, spleen, and colon ↓ Colitis by targeting Ly6C⁺ monocytes in IBD model 	[29]
	–	gp70 RNA	Encoding endogenous antigen of Moloney murine leukemia virus	LN, BM, spleen, lung	DCs	<ul style="list-style-type: none"> Activation of NK, T, and B cells ↑ Survival ↓ Tumor growth 	[46]
	–	OVA RNA	Encoding ovalbumin epitope expressed in B16F10 cell line			<ul style="list-style-type: none"> Induction of systemic INFα in patients Priming and amplification of T cells against antigens in patients 	
	–	Hemagglutinin RNA	Encoding influenza virus hemagglutinin				
	–	NY-ESO-1 RNA, MAGE-A3 RNA, tyrosinase RNA, TPTE RNA	Encoding tumor antigens for clinical use				
HDL nanoformulation	–	(For imaging purposes)		Spleen, BM, blood	Neutrophils, Ly6C ⁺ monocytes, Ly6C [–] monocytes	<ul style="list-style-type: none"> ↑ Infiltration of Ly6C⁺ monocytes in intermediate atherosclerosis ↑ Infiltration of neutrophils in advanced-stage atherosclerosis Recruitment of myeloid cells in myocardial infarction 	[33]

TABLE 2 (Continued)

Nanoparticles	Targeting decoration	Payload	Payload function	Tissue target	Cell target	Major outcome	Refs
Lipid NPs	SORT molecules	hEPO mRNA or IL-10 mRNA	To verify expression of hEPO and IL-10	Spleen, liver, lung	Macrophages, B cells, T cells, others	Organ-specific targeting Effective mRNA expression in organ-specific manner Effective gene editing in organ-specific manner	[34]
		Cre mRNA	To activate tdTom expression				
		Cas9 protein/sgTom1	To improve delivery of Cas9 RNPs				
		Cas9 mRNA/sgPTEN	To edit PTEN for anticancer purposes				
		Cas9 mRNA/sgPCSK9	To edit PCSK9 for antiatherosclerotic purposes				
	–	gp100 mRNA, TRP2 mRNA	Encoding tumor-associated antigens	LN	DCs, neutrophils, macrophages, B cells	Successful anticancer vaccination ↑ Cytotoxic CD8 ⁺ T cell response ↑ Survival ↓ Tumor growth Polarized M2→M1 macrophages	[43]
Polymeric NPs	CD206	IRF5 mRNA, IKK β mRNA	Encoding M1 macrophage polarization factors	Tumor	M2 macrophages	↑ Survival ↑ T cell infiltration Control tumor metastases Potential to reprogram human macrophages	[23]
Polymeric micelles	–	Adpgk R848 CpG	Peptide neoantigen TLR7/8 agonist TLR9 agonist	LN	DCs, macrophages	Successful anticancer vaccination ↑ Cytotoxic CD8 ⁺ T cell response ↑ Survival ↓ Tumor growth Potentiating anti-PD-1 therapy	[45]
Polymeric NPs, gold nanorods	CD11b	Pyropheophorbide-a (not loaded)	Photosensitizer	Blood	Neutrophils	↑ Neutrophil tumor infiltration upon photosensitization ↑ Survival ↓ Tumor growth	[27]
Polymer/lipid TransIT reagent	–	CD3 × CLDN6 mRNA, CLDN18.2 × CD3 mRNA, EpCAM × CD3 mRNA, CD3 × (CLDN6) ₂ mRNA	<i>In vitro</i> -transcribed mRNA encoding bispecific antibodies against T cells, cancer cells, epithelial cells	Liver	T cells	↑ Plasma levels of bifunctional proteins Elimination of advanced xenograft tumors Activated T cells in cell target-specific manner ↑ Cytotoxic T cells in tumors	[47]

^a Selected representative publications demonstrate the use of NPs for immunomodulatory, NP-mediated transportation, and immune cell-tracking purposes in cancer and inflammatory diseases.

of 'nano' concepts aimed at targeting and modulating the tumor immune micro-environment (TIME [14]) to promote immune cell-mediated anticancer responses (Fig. 1 and Table 2) [15–17]. First results have been encouraging. For example, polymeric NPs loaded with a Toll-like receptor (TLR)-7/8 agonist and transforming growth factor (TGF)- β inhibitor, were functionalized with CD8a and programmed cell death protein 1 (PD-1) to target intratumoral PD-1⁺ or CD8⁺ cytotoxic T cells. Specific delivery of immunomodulatory cargo to these cells primed the cytotoxic activity of CD8⁺ T cells in the tumor, allowed for enhanced infiltration of these cells, and sensitized the TIME for better response to subsequent antibody-based anti-PD-1 immunotherapy [18]. Another interesting example is targeting forkhead box P3 (FoxP3⁺) regulatory T cells with hybrid polymeric-lipid NPs surface functionalized with the peptide tLyp1. These NPs were loaded with the kinase inhibitor imatinib to inhibit regulatory T cells in the TIME. Combining them with anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) antibodies reduced the numbers of intratumoral regulatory T cells and elevated the number of cytotoxic CD8⁺ T cells [19]. Similar conceptual approaches have been conceived for modulating myeloid cells inside the TIME. These have included the design of β -cyclodextrin NPs containing TLR7/8 agonists [20], as well as liposomes functionalized with a CD206-targeting ligand loaded with Colony stimulating factor 1 receptor (CSF1R) and Src homology 2 (SH2) domain-containing phosphatase 2 (SHP2) inhibitors [21], with the aim of exploiting the phenotypic plasticity of macrophages [22] and inducing their polarization from an immuno-suppressive M2-like toward a tumor-suppressive M1-like phenotype. This phenotypic change enhances the phagocytic capability of macrophages, helps to control tumor growth, and protects against tumor rechallenge. Macrophage polarization and TIME modulation can also be achieved through NP-mediated genetic reprogramming. Loading polymeric NPs with mRNA encoding the M1-inducing proteins interferon regulatory factor 5 (IRF5) and IKK β and targeting of these NPs to M2-like macrophages induced their repolarization toward an antitumor phenotype in three different tumor models [23]. In animal models, this approach showed an efficacy against not only primary tumors, but also metastasis, and the NPs further proved to be active in reprogramming human macrophages.

Targeting circulating immune cells

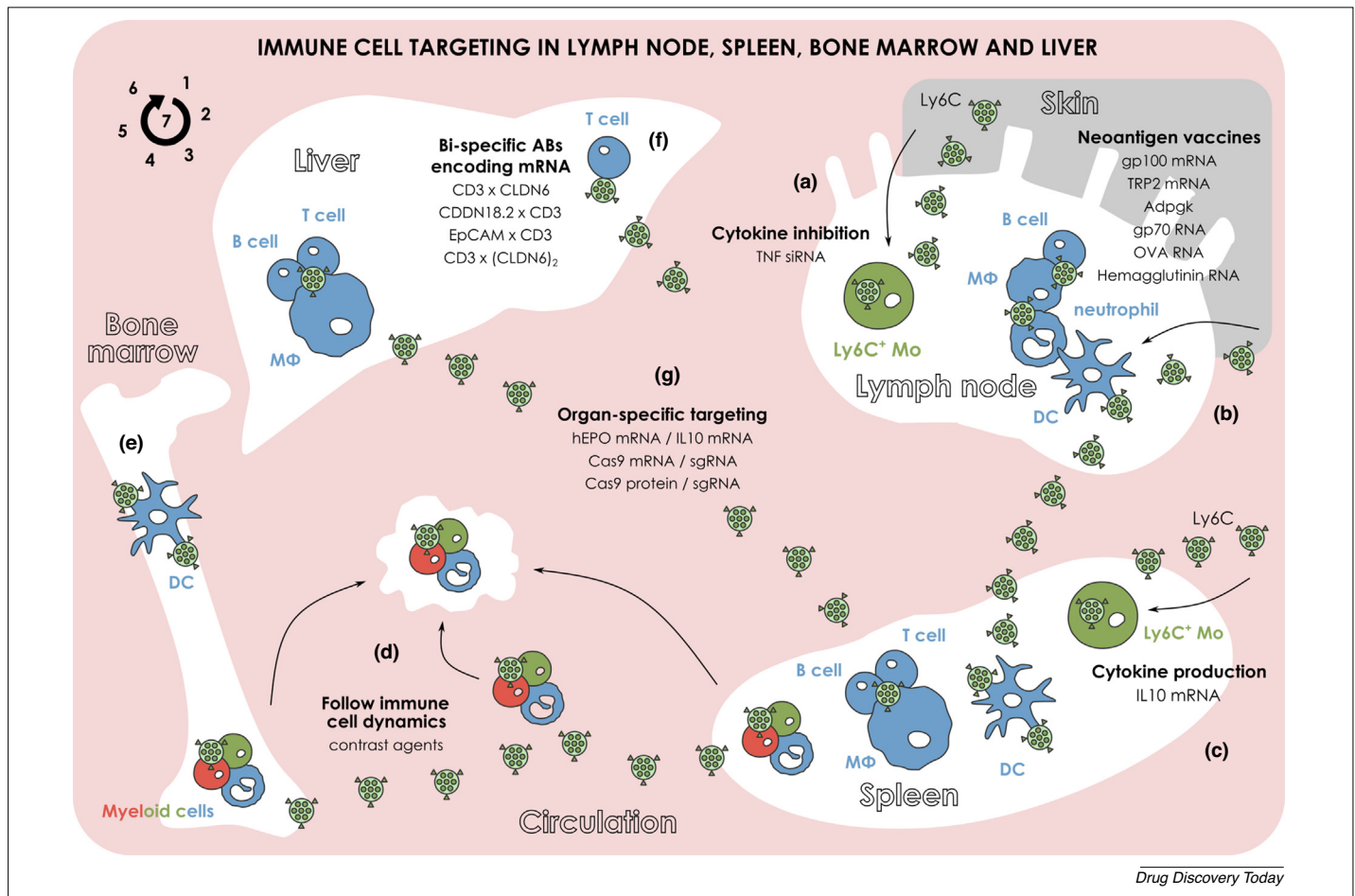
Circulating immune cells can recognize and interact with nanomedicine formulations in the bloodstream before homing to diseased areas (Fig. 1 and Table 2). For example, investigation of $\alpha v \beta 3$ -integrin-specific cRGD liposomes and nanoemulsions revealed that, in addition to targeting integrins on the tumor endothelium, circulating phagocytes (predominantly neutrophils) take up these NPs and transport them into tumor tissue [24]. This observation rationalizes the use of immune cells as drug delivery vehicles [25]. The interaction of cRGD liposomes with circulating phagocytes also resulted in co-migration into ischemic brain tissue [26], a tissue that is difficult to reach using conventional drug targeting strategies. Although the cRGD liposomes in this study were loaded with edaravone (i.e., a neuroprotective agent, aiming to lower infarct volumes), similar approaches can be envisaged for increasing anticancer and/or immunomodulatory drug deposition in the brain. Besides cRGD, circulating neutro-

phils have also been targeted by decorating polymeric NPs and gold nanorods with anti-CD11b. Subsequent TIME priming via photosensitization, with the photosensitizer pyropheophorbide-a, resulted in enhanced infiltration of the NP-loaded neutrophils in tumor tissue upon illumination with laser light [27]. Analogously, surface decoration of lipid NPs with monoclonal antibodies resulted in a modular liposome platform suitable for immune cell-specific small interfering (si)RNA delivery [28]. This platform showed impressive versatility in specifically targeting different immune cell populations when functionalized with CD3, CD4, CD25, and Ly6C antibodies. Therapeutically, anti-Ly6C-decorated liposomes loaded with siRNA successfully inhibited the expression of the proinflammatory mediator tumor necrosis factor (TNF)- α in Ly6C⁺ monocytes in a colitis model. The same modular platform was also applied for mRNA delivery in an inflammatory bowel disease (IBD) model, targeting Ly6C⁺ monocytes and inducing the expression of the anti-inflammatory interleukin (IL)-10 [29]. These examples support the exploration of cancer nanomedicine engineering toward targeting immunomodulatory cargo to circulating immune cells [30]. Furthermore, by determining the composition of tumor-infiltrating immune cells and by exploiting immune cell-NP interactions [24,31,32], immune cells can function as chariots for delivering therapeutic cargo to tumors and metastases.

Targeting myeloid and lymphoid immune cell-enriched tissues

Targeting immune cells entails delivery to tissues enriched in immune cells, such as bone marrow (BM), liver, lymph nodes (LNs), and spleen (Fig. 2 and Table 2) [33–36]. Research to achieve organ-specific targeting has enabled the development of a lipid NP platform that allowed for the targeted delivery of mRNA or gene editing in a tissue-specific manner [34,37]. By capitalizing on the biophysical properties of different lipid components, three major categories of lipid NPs achieved specific targeting to spleen, liver, or lungs and a direct association of the NPs with residual macrophages, B cells, and T cells. Loading these NPs with various therapeutic RNAs (i.e., hEPO mRNA and IL-10 mRNA) or Cas9 mRNA/single-guide (sg)RNA combinations (i.e., Cas9 mRNA plus sgPTEN, and Cas9 mRNA plus sgPCSK9) demonstrated organ-specific action for applications in inflammatory disease, cancer, and atherosclerosis. Such a redirection could benefit therapeutic approaches focusing on eliminating malignant cells responsible for blood disorders [38], or on reversing the aftermath of such conditions, such as BM fibrosis [39], splenomegaly [40], and splenic lymphomas [37].

NPs developed for delivering mRNA vaccines also target immune cells [41,42]. A subcutaneously injected lipid NP mRNA vaccine loaded with gp100 mRNA or tyrosinase-related protein 2 (TRP2) mRNA led to improved mRNA translation in antigen-presenting cells [i.e. dendritic cells (DCs), neutrophils, macrophages, and B cells]. The resulting increased transfection rate in regional LNs elicited a strong CD8⁺ T cell-mediated response against melanoma [43]. Along the same line of thinking, nanomedicine has also been strongly integrated in neoantigen vaccine approaches [44]. A subcutaneously administered micellar nanovaccine delivered a peptide neoantigen (Adpgk) together with a TLR7/8 agonist (R848) and a TLR9 receptor agonist (CpG) to immature DCs residing in LNs [45]. Combining this nanovaccine



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FIGURE 2

Nanoparticle (NP) targeting of the immune system in immune cell-enriched organs. (a) Subcutaneous administration of NPs can lead to lymph node targeting. Surface decoration of such NPs can allow for the specific targeting of lymph node-resident monocytes and stimulate cytokine inhibition. (b) NPs can deliver neoantigen RNA cargo in specific cells in lymph nodes and spleen to elicit a personalized anticancer response. (c) Surface decoration of NPs with various motifs can enable the targeting of spleen-resident monocytes and the stimulation of cytokine production. (d) NPs functionalized with contrast agents can be used for targeting myeloid cells in various tissues (e.g., bone marrow and spleen) to visualize their transportation to a cancerous or inflammatory lesion. (e) Similarly to the delivery of neoantigen vaccines in lymph node and spleen, targeting of NPs in dendritic cells in the bone marrow can also be used to elicit anticancer responses. (f) NPs can deliver specific antibody-encoding mRNAs in the liver for targeting T cells, cancer, and epithelial cells and eventually stimulating anticancer T cell responses. (g) By synthesizing NPs with desired biophysical characteristics, organ-specific targeting can be achieved and used for delivering RNA therapeutics or applying gene editing. Please see main text for abbreviation definitions.

with anti-PD-1 treatment resulted in regression of Adpgk-positive colorectal cancer. Lymphoid tissues can also be targeted for neoantigen mRNA expression after systemically injecting lipoplexes [46]. Indeed, an intravenously injected lipoplex delivered cancer-specific antigen-encoding RNA (i.e., gp70 RNA, OVA RNA, and hemagglutinin RNA) to DCs in spleen, BM, LNs, and lungs, and elicited strong antigen-specific responses against melanoma and colon carcinoma via activation of NK cells, B cells, and T cells. Of note, this lipid NP platform constitutes the first nanomedicine-based neoantigen vaccination in clinical evaluation, displaying promising targeting of lymphoid DCs in the spleen, LNs, and BM with various RNAs encoding tumor-specific antigens. Additionally, mRNA-encoding bispecific antibodies can be directed to the liver by using T cell-specific NPs [47]. These therapeutic NPs were based on a single mRNA strand that produced a combination of antibodies against T cells, cancer cells, and epithelial cells [via CD3 x CLDN6 mRNA, CLDN18.2 x CD3 mRNA, EpCAM x CD3 mRNA, and CD3 x (CLDN6)₂ mRNA], enabling stable and prolonged

production of high plasma levels of bifunctional proteins targeting T cells and activating them in a cell-specific manner [47].

Finally, NPs have been used as tools for imaging-assisted immune cell tracking. High density lipid (HDL) nanobiologics can be taken up by myeloid cells in the spleen and BM, enabling the visualization of myeloid cell dynamics in atherosclerosis and myocardial infarction [33]. The functionalization of these NPs with different contrast agents allowed for the utilization of complementary imaging modalities, which together illustrated distinct immune cell migration patterns at different disease stages. For instance, it was found that the infiltration of Ly6C⁺ monocytes was dominant in intermediate-stage atherosclerosis, whereas neutrophil infiltration was dominant in advanced-stage atherosclerosis [33].

Benefits of in vivo immune cell targeting

Targeting strategies involving immune cells typically rely on *ex vivo* methodologies. For example, *ex vivo* decoration of monocytic

myeloid cells with IFN γ immunomodulatory ‘backpacks’ polarized these cells toward an M1-like antitumor phenotype [48]. Their subsequent intratumoral injection not only preserved this phenotype, but remarkably also polarized neighboring tumor-associated macrophages toward an M1-like antitumor phenotype. Furthermore, *ex vivo* methodologies were developed for conjugating NPs to the cell surface of T cells. This led to the development of an IL-15-loaded nanogel backpack attached to the surface of T cells [49]. This technology had multiple therapeutic benefits, including increased delivery of IL-15, intratumoral increase of CD8⁺ T cells, and, consequently, an improved chimeric antigen receptor (CAR)-T cell response [50]. Given that immune cell isolation, *ex vivo* manipulation, and subsequent systemic re-infusion can lead to the rapid recognition of the injected cells by the mononuclear phagocyte system via, for example, efferocytosis [51,52], we speculate that such *ex vivo* approaches can be refined and improved by performing direct *in vivo* targeting of immune cells. *In vivo* immune cell targeting also provides the possibility of inhibiting (or even killing) immune cells in the circulation, thereby modulating the number and type of immune cells infiltrating tumors and metastases. The utilization of NPs for such concepts bypasses a key drawback of NP design and traditional tumor targeting, that is, the sometimes very rapid recognition of nanoformulations by the immune system. Last but not least, at the patient level, direct *in vivo* targeting approaches circumvent labor-intensive immune cell isolation, *ex vivo* manipulation, and re-injection into the patient.

Nanomedicine-assisted immune cell imaging

Immunomodulatory strategies require profound knowledge of immune cell count and composition in disease conditions. This knowledge can be obtained by developing NP-assisted methodologies that can accurately visualize through multiscale and multimodal imaging techniques the presence of a specific immune cell subset in the target tissue [53,54]. The target tissue can be the pathological site (e.g., a tumor), as well as immunoregulating organs (e.g., BM and spleen). NP libraries can be constructed based on well-known manufacturing procedures, which allow for tuning of NP accumulation in different tissues and cell types by controlling parameters such as composition, size, surface decoration, and ligand density [31,55]. This engineering versatility is important because each cancer case bears its own immunological signature [56,57]. Given that this immunological signature can change over time, such alternations could be assessed via multiple rounds of

NP-assisted imaging. In this regard, NPs will act as a supportive tool to biopsies that are typically not performed multiple times. A major application, in which this approach can be extended, is to decipher between hot and cold tumors, a process that typically is evaluated via biopsy and *ex vivo* histological analysis [58]. Therefore, such a strategy will allow improved monitoring patients in terms of disease progression and response to therapy, and will assist in (re)allocating patients to treatment groups. In the long run, we foresee that the systematic visualization of NP-immune cell engagement will benefit not only cancer applications, but various pathologies strongly characterized by immune cell abnormalities.

Concluding remarks

We anticipate that immune system modulation by means of targeted nanomedicines will have a prominent role in future oncological interventions. Classification of patients and disease stage based on their immunological signature [59] is already established as an important prognostic marker for achieving good treatment outcomes. In this regard, expanding our toolbox with nanomedicines that selectively target certain immune cell populations can help (re)direct the immune system against tumor progression and recurrence. Shifting our experimental attention from traditional tumor targeting toward more extensive engagement of the immune system will open a new era in nanomedical cancer therapy.

Conflict of interests

S.K. reports research funding from Novartis, Janssen, AOP Orphan Pharmaceuticals AG, and Bristol-Myers Squibb as well as consultancy honoraria from Novartis, Incyte/Ariad, Bristol-Myers Squibb, AOP Orphan Pharmaceuticals AG, Pfizer, Celgene, Bayer, Roche, CTI, and Shire. T.L. reports research funding and consultancy honoraria from Cristal Therapeutics.

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