Splenic Hematopoietic and Stromal Cells in Cancer Progression

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ABSTRACT

Tumor-derived secretory factors orchestrate splenic hematopoietic and stromal cells to fuel metastasis. The spleen acts as a reservoir site for hematopoietic stem and progenitor cells, which are rapidly exploited as myeloid-derived suppressor cells at the cost of tumor-reactive lymphoid cells. Splenic erythroid progenitor cells and mesenchymal stromal cells contribute directly and indirectly to both tumor immune escape and the metastatic cascade. Animal models provide valuable mechanistic insights, but their translation to a clinical setting highlights specific challenges and open issues. In this review, we envision the exploitation of the spleen as a source for novel biomarkers and therapeutic approaches.

Introduction

As the largest secondary lymphoid organ, the spleen is a crucial site of innate and adaptive immune regulation; it harbors one third of the body's immune cells, including macrophages, dendritic cells (DC), and various subsets of T and B cells (1). Among the most important functions of the adult spleen are the capacities to recycle iron from senescent erythrocytes, to remove blood-borne antigens, and to act as a storage site for monocytes, platelets, and red blood cells. In accordance with its fetal activity, splenic hematopoietic stem and progenitor cells (HSPC) produce lineage-descendant blood cells in cancer and inflammation (1, 2). In patients with solid cancers, with the exception of non–small cell lung cancer (NSCLC) and melanoma, the unique (immune) microenvironment of the spleen does not frequently harbor macroscopic metastasis (3). However, akin to disseminated tumor cells in the bone marrow (4, 5), the splenic niche may induce cellular dormancy and provide a sanctuary to escape immune surveillance. We here discuss the spatiotemporal dynamics of splenic hematopoietic and stromal cells in tumor–host interaction and cancer progression. Of note, the (re)emergence of immuno-oncology provided a catalyst to the concept of the spleen as an immune "barometer" (6).

Cellular and Structural Composition of the Spleen

The spleen is intraperitoneally located in the upper left quadrant of the abdominal cavity, posterior to the stomach and anterior to the left hemidiaphragm, where it is protected by the thoracic cage (ribs 9–11; ref. 7). Histo-anatomically, the parenchyma of the spleen is composed of the white and red pulp, separated either by the marginal zone (MZ) in rodents or a two-compartment MZ surrounded by perifollicular zone in humans. The "lymphoid" white pulp encompasses the periarteriolar lymphoid sheaths (PALS) and the lymphoid follicles, primarily consisting of T and B cells, respectively, whereas the "hematogenous" red pulp is organized into monocyte-rich splenic cords (known as Billroth's cords) situated between blood-filled sinusoids, which give this area its characteristic red appearance (7). Macrophages phagocytose infectious organisms, aging blood cells, and particulate matter when they pass through the "filter" between Billroth's cords and the sinusoids lumen (2, 7, 8). The transit area (i.e., MZ), in turn, contains a large number of unique resident immune cells, including MZ macrophages, marginal metallophilic macrophages, and MZ B cells, which effectively scavenge antigens (Ags; refs. 2, 7–10).

Various chemokines secreted by specialized stromal cells such as follicular dendritic cells (FDC) and podoplanin/gp38$^+$ fibroblastic reticular cells (FRC) are essential to the proper functioning of the white pulp (11–13). For instance, FDCs produce C-X-C motif chemokine ligand (CXCL)13, which attracts CXCR5$^+$ MZ B cells carrying Ags scavenged from the arterial blood. Once in the white pulp area, MZ B cells deliver these Ags to resident FDCs, allowing subsequent FDC-mediated Ag presentation to lymphocytes (14, 15). In addition, the network of FRCs produces an extracellular matrix- and chemokine-rich [e.g., C-C motif chemokine ligand (CCL)19 and CCL21] conduit that supports the rendezvous between rare, specific, naive CCR7$^+$ T cells and activated DCs (16). Although tumor-draining lymph nodes are considered to be the privileged sites for Ag presentation to T cells, the spleen acts as a major Ag filter and may thus activate T cells, which could consequently eliminate tumors or could even establish cellular dormancy. Indeed, a subset of T cells in the tumor microenvironment (TME) of the MMTV-PyMT mammary cancer mouse model shows similar genetic characteristics to splenic T cells (17). Importantly, given that more cells pass through the spleen than through all other secondary lymphoid organs combined (18), the spleen may additionally influence circulating lymphocytes that subsequently migrate to the tumor. Yet, performing splenectomy in tumor transplantation mouse models suggest a spleen-independent contribution of T- and B-cell infiltration into primary tumor sites (19). In contrast, a splenic reservoir is indeed suggested for monocytes as further discussed (20).

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The Spleen as Stimulator of Cancer Progression

Independent studies indicate that transplantable tumor mouse models such as 4T1 mammary cancer (21, 22), H22 hepatoma (23), B16 melanoma (24), and genetically engineered mouse models (GEMM) for tumors such as $Kras^{LSL\text{-}G12D/+}$;$p53^{fl/fl}$ (KP) lung adenocarcinoma (20), abrogate blood cell development in the bone marrow. As a response, the spleen compensates for this by HSPC infiltration and extramedullary hematopoiesis (EMH) that is skewed to myelopoiesis by tumor-derived factors such as G-CSF and GM-CSF (**Fig. 1**). The rapid switch to emergency splenic EMH supporting the bone marrow as primary hematopoietic site is a physiological mechanism used by normal tissues under stress conditions. For example, β-adrenergic stress-induced signaling abrogates anemic responses in the bone marrow through the establishment of splenic EMH (25). Moreover, immune challenges such as infection require splenic EMH aiding the bone marrow that supplies additional myeloid cells to effector sites, a process that depends on natural killer cells (26). Mass cytometric characterization of the immune landscape in eight common mouse models of cancer (three mammary cancer, two melanoma, and one pancreatic/colorectal/glioblastoma models) demonstrated that the tumor load alters the systemic immune landscape and that this tumor macroenvironmental alteration is dynamic because it can be reverted by the surgical removal of the tumor (27). All models exhibited expansions in the splenic myeloid compartment at the cost of lymphoid B and T cells. This myelopoiesis was especially dominant in the three mammary cancer models (4T1, AT3, and MMTV-PyMT), and was independent from the applied mouse strain backgrounds (BALB/c, C57BL/6, and FVB/N, respectively; ref. 27).

Tumor-induced myeloid-derived suppressor cells (MDSC) potently impede anticancer immunity (28). Both morphology and phenotype distinguish monocytic (M-MDSC) from polymorphonuclear (PMN-MDSC) subtypes (which are functionally and biochemically distinct) from their mature counterparts: macrophages, monocytes, or dendritic cells; and neutrophils, mast cells, basophils, or eosinophils, respectively. In mice, differential expression of the Ly6C and Ly6G surface markers define M-MDSC ($CD11b^{+}Ly6C^{hi}Ly6G^{-}$) and PMN-MDSC ($CD11b^{+}Ly6C^{lo}Ly6G^{+}$) populations (hi: high expression and lo: low expression; refs. 29, 30); however, further fine tuning of MDSC subtypes requires additional markers. In humans, the equivalent of PMN-MDSCs is defined as $CD11b^{+}CD14^{-}CD15^{+}$ or $CD11b^{+}CD14^{-}CD66b^{+}$ and of M-MDSC as $CD11b^{+}$ $CD14^{+}HLA\text{-}DR^{-/lo}CD15^{-}$.

Indications of myeloid cell activity in 4T1 tumor-bearing mice are splenomegaly (21) and increased splenic or systemic levels of chitinase 3-like 1 (CHI3L1) and lipocalin 2 (LCN2; refs. 31, 32). Splenic MDSCs are highly dispersed across the MZ and are in close contact with T cells in the white pulp region (33). They can engulf circulatory apoptotic bodies and other extracellular vesicles, present tumor Ags to splenic $CD8^{+}$ T cells, and release reactive oxygen species (ROS) and nutrient-deprivation enzymes such as nitric oxide synthase (NOS) and arginase (Arg), collectively resulting in tumor-specific $CD8^{+}$ T-cell tolerance (1, 33). The spleen also holds a reservoir of monocytes that are

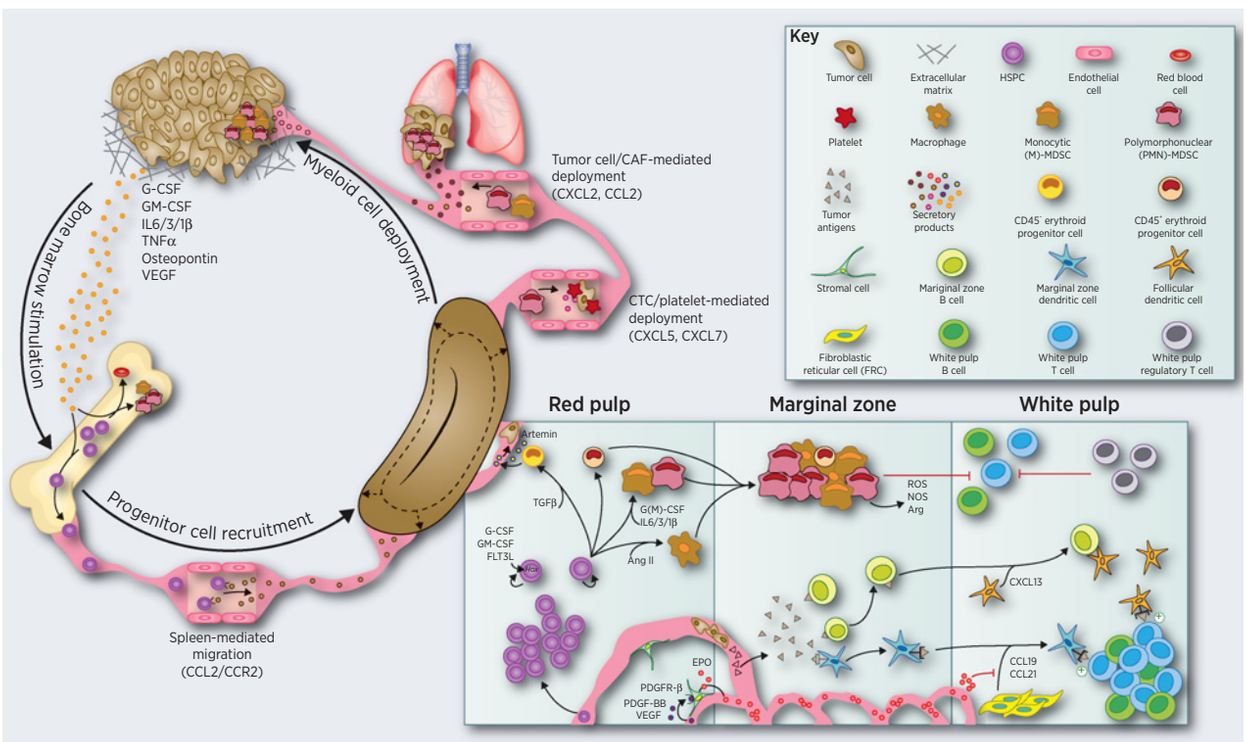

Figure 1.

Schematic representation of the tumor–bone marrow–spleen communication upon cancer progression. The tumor secretome activates myelopoiesis in the bone marrow as well as the release of HSPCs to the spleen for EMH. Myeloid cells derived from splenic EMH create an immunosuppressive environment both locally and upon recruitment to primary tumor and metastatic sites, leading to cancer progression and further release of tumor-derived secretory factors. Presented key mechanisms are based on insights from mouse models. Dynamic cyclic processes of bone marrow stimulation, progenitor cell recruitment, and myeloid cell deployment are indicated. For detailed description see main text.

en masse recruited to the primary tumor as tumor-associated macrophages (TAM), first identified in the KP GEMM (20). Local TME conditions, such as hypoxia (34) and lactic acid accumulation (35), result in functional specialization to benefit local tumor demands (28). In accordance, splenectomy in orthotopic transplant models subsequently showed a decreased number of M- and PMN-MDSCs as well as monocytes at both the primary tumor and its metastatic sites (19).

MDSC subtypes complement each other during metastasis and it is now recognized that their relative quantity varies over time at the tumor site, although model-dependent differences exist (36–39). MDSC phenotypic definitions are based upon comparison of relative expression levels of some markers. Due to this complexity it is important to use cells from control mice or healthy donors as baseline situation (40). Moreover, optimal MDSC characterization includes a functional proof of their T-cell suppressive capacity (41). In addition, PMN-MDSCs may readily be identified by the same markers as neutrophils, although recently characterized markers such as lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and fatty acid transport protein 2 (FATP2), as well as a specific PMN-MDSC gene signature have been suggested to improve their detection and enrichment (42–44). Another methodology that may assist in this differentiation is ficoll density gradient centrifugation, which separates immature PMN-MDSC (lower density) from mature neutrophils (higher density; ref. 40).

Erythrocytes are generated in the bone marrow, but stressors such as tumor-induced anemia stimulate splenic EMH. In hosts with a large tumor load, the spleen accumulates $CD45^+$ (45) and $CD45^-$ (46) erythroid progenitor cells (EPC), which both are $Ter\text{-}119^+/CD71^+$, as a result of splenic EMH. $CD45^+$ EPCs produce immunosuppressive ROS in mice bearing large tumors of Lewis lung cancer (LLC) or B16.F10 melanoma (45). The systemic suppression of $CD8^+$ T-cell activation by $CD45^+$ EPC leads to enhanced susceptibility to infection (45), but their role in metastatic progression has not yet been investigated. In contrast, $CD45^-$ EPCs produce artemin, a neurotrophic factor that stimulates hepatocellular carcinoma (HCC) growth and tumor-related death in animal models (46). Human patients with HCC show splenic $artemin^+$ Ter-cells and significantly elevated serum artemin correlates with poor prognosis (46).

Heterogeneous populations of endothelial and mesenchymal stromal cells construct the different splenic environments. In the white pulp area, there is an anatomical as well as phenotypic similarity between lymph node and spleen mesenchymal stromal cells such as $podoplanin/gp38^+$ FRCs, $MadCAM1^+$ marginal reticular cells, and B zone reticular cells either expressing CXCL12 or CXCL13 (2, 7, 8, 11). In the red pulp area, specialized endothelial cells and fibroblasts interact with leukocytes and erythrocytes (2, 7, 8, 11). Despite knowledge on the role of some of these non-hematopoietic cells in inflammation (47), infection (48), and EMH (49), further research is needed to determine whether these cells are actively reprogrammed by the tumor and can influence splenic responses.

FRCs are the most studied stromal cell type in secondary lymphoid structures. In the B16.F10 melanoma model, FRCs in tumor-draining lymph nodes are transcriptionally reprogrammed upon early tumor development (50). This reprogramming is reminiscent of the "cancer-associated fibroblast" (CAF) state observed in many solid tumors. Fibroblast activation markers such as podoplanin/gp38, S100A4, α-smooth muscle actin, endosialin/CD248 all showed increased expression, and reduced secretion of FRC-derived chemokine CCL21 and cytokine IL7 was accompanied by altered composition and aberrant localization of T and B cells (50). Whether similar changes occur in the spleen and whether these translate to a clinically relevant functional outcome (e.g., increased immunosuppression or tumor promotion) remains to be investigated.

Tumor-Derived Regulatory Signals of Splenic Responses

A tumor's secretome drives splenic responses of myelo- and erythropoiesis either directly or indirectly through the bone marrow (**Fig. 1**). Both G-CSF and GM-CSF stimulate the bone marrow production of MDSCs from HSPCs (51–53). However, these growth factors can also induce HSPC release from the bone marrow to allow compensatory EMH in the spleen. More specifically, G-CSF promotes the release of enzymes that disrupt the binding between CXCL12 on bone marrow stromal cells and CXCR4 on HSPCs (54, 55). VEGF further facilitates HSPC release by binding VEGFR2 on endothelial cells in the bone marrow, triggering vascular dilation and systemically mobilizing HSPCs (56). Splenic recruitment of circulating HSPCs involves the CCR2/CCL2 axis with the receptor CCR2 being expressed by HSPCs and with splenic $VE\text{-}cadherin^+$ endothelial cells (33) as well as $nestin^+$ splenocytes (57) acting as major sources for the monocyte chemoattractant CCL2.

Tumor-derived cytokines, such as G-CSF, GM-CSF, IL6, IL3 and IL1β, direct splenic HPSCs mainly towards M- and PMN-MDSCs (1, 57). Tumor resection or neutralization of IL1 and G-CSF restores the splenic immune landscape, demonstrating the high plasticity of the splenic immune state (27).

Tumor-derived bone-related proteins, including osteopontin and bone morphogenetic protein 4 (BMP4), impact splenic granulopoiesis. Although osteopontin has a stimulatory role (58), BMP4 inhibits PMN differentiation of HSPCs through a reduced tumor secretion of G-CSF (59). Interestingly, full maturation of HSPCs is mostly abrogated as G-CSF synergizes with GM-CSF and the FMS-like tyrosine kinase 3 ligand (FLT3L) to expand and retain the HSPCs in an immature state through Hox gene overexpression (60). This mechanism creates an HSPC niche that further supports EMH and yields MDSC. In addition, TNFα released from activated T cells at tumor sites stimulates splenic emergency myelopoiesis, thereby suppressing tumor-specific immune responses created by the adaptive immune system (61). The tumor-derived Ang II precursor, angiotensinogen, together with the HSPC-expressed angiotensin-converting enzyme (ACE) generate the vasoconstricting peptide angiotensin (Ang) II, which differentiates splenic HSPCs and deploys them as TAMs throughout cancer progression in the KP GEMM (62).

Moreover, tumor-secreted factors amplify the suppression of adaptive immunity through regulating splenic stromal cells. Platelet-derived growth factor (PDGF)-BB and VEGF stimulate splenic PDGFR-β-expressing stromal cells to produce erythropoietin (EPO). EPO stimulates the erythroid progenitor pool and robust splenic erythropoiesis (63, 64), as well as impairs splenic T cell movement by inhibiting FRC expression of CCL19 and CCL21 (65). Erythropoiesis can be further stimulated by TGFβ and Smad3 activation, important factors in splenic CD45− Ter-119+/CD71+ cell accumulation (46). Finally, VEGF directly inhibits splenic T-cell and B-cell development through VEGFR2 signaling in tumor-bearing mice (66).

Overall, it is increasingly accepted that the spleen receives and subsequently responds to systemic tumor-derived signals such as soluble factors and extracellular vesicles to generate erythroid and myeloid pro-metastatic signals. Chemokines further recruit splenic cells to tumor sites. Tumor cells, but also CAFs secrete CCL2 to recruit M-MDSCs and CXC chemokines to recruit PMN-MDSCs, respectively (28, 67, 68). Release of splenic PMN-MDSCs into the systemic

circulation can also be mediated by clusters of platelets and CTCs through the secretion of CXCL5 and CXCL7 (69).

The Road Ahead: Challenges and Opportunities

Challenges

Proposed mechanisms of splenic contribution to cancer progression originate in large part from mouse models. Although mouse and human spleens share common physiology, some structural differences exist, which should be taken into consideration when translating preclinical discoveries into patients' clinical benefit. First, in humans, the red pulp predominates the spleen, whereas the white pulp occupies more volume in mice and rats. Second, the MZ in humans is divided into an inner and an outer compartment surrounded by a perifollicular zone. (7, 8). Third, in humans the circulation system is totally open, as no connection from capillaries to sinuses can be found in the red pulp area (7, 8). Definitively, more research using advanced imaging techniques are necessary to fully understand the differences between human and rodent spleens. Nevertheless, the extramedullary myeloid response as indicated by the quantity of PMN- and M-MDSCs in mouse tumor models and cancer patients are very similar when compensating for body scale differences (20). Although there is ample evidence that MDSC subtypes accumulate in a wide range of human tumors and correlate with clinical outcome (reviewed in ref. 70), their splenic origin still warrants confirmation. Moreover, the 4T1 transplantation model is overrepresented in preclinical studies, being very attractive due to its profound splenomegaly, EMH and high secretion of the granulopoietic growth factors G-CSF and GM-CSF. Models such as the tumor transplantable LLC (45, 63, 71) and the Hepa1–6 hepatoma (46, 57), as well as the KP GEMM (20, 62), have increased splenic EMH but display splenomegaly to a far lesser extent than the 4T1 model. Splenic changes occur in animal models with a wide range of metastatic burden (e.g., AT3, weakly metastatic; MMTV-PyMT, moderately metastatic; and 4T1, highly metastatic; ref. 21) and in some animal models splenectomy impacts metastasis (19). Therefore, the splenic contribution to cancer progression should be investigated across a wide spectrum of cancer subtypes to create a more representative picture. The impact of splenectomy on tumor progression and myeloid cell content in mouse models should take into consideration potential confounders such as the spatiotemporal context, including the timing of surgery, the tumor biology, the choice of mouse model, and the operative procedure, which may each influence the study outcome (19, 72–74). Detailed and transparent reporting of all these experimental parameters could shed light on the differences observed after splenectomy and will lead to a more harmonized approach. Other animal models such as pets with spontaneously occurring neoplasms, can be considered as a valuable and still under used resource of investigating the splenic contribution to metastasis. Companion animals can help in gaining insights on tumor biology and finally may be enrolled in therapeutic trials that might act as a bridge to the clinic applications (75).

Clinically meaningful conclusions on splenic functioning in cancer requires in-depth documentation based on both liquid and tissue biopsies from cancer patients across all stages of disease, including research autopsy program samples.

Therapeutic opportunities

Cancer therapy can both influence the immunosuppressive and immuno-stimulatory circuitries within the TME. Moreover, there is a robust, growing body of preclinical data suggesting that cancer therapy does not only rely on direct cytostatic/cytotoxic effects, but also involves therapy-induced immune responses, ultimately facilitating or deterring cancer progression (76–79). As outlined below, these findings provide opportunities to exploit the splenic niche via systemic therapy (not within the scope of this review), radiotherapy, splenectomy, and nanoparticle (NP) therapeutics.

Recently, radiotherapy has come under the spotlight as an immunomodulator to enhance antitumor immunity in the clinic (80, 81, and reviewed in 82 and 83). Unfortunately, we currently lack robust evidence to establish the relationship between radiotherapy-induced immunomodulation (in either direction) and specific splenocyte populations; a notable exception is a preclinical study where radiotherapy (≥15 Gy) of the local tumor bed of mice bearing B-16-OVA melanoma raised the fraction of T_{reg} cells in the spleen (84). Radiotherapy-induced lymphopenia is well established as an independent predictor of decreased overall survival across multiple cancer types in humans (80, 85–87) and indeed, several authors have found that high-dose (∼50 Gy) splenic irradiation increases the risk for the development of more severe lymphopenia (88–92). In contrast, by lowering radiation doses to the circulating blood pool and lymphoid tissues, reduced immunosuppressive effects are elicited (80). It can thus be hypothesized that lower radiation doses to circulating lymphocytes by delineation of the heart, large vessels, lymph nodes, the thymus (in children) and the spleen, as regions at risk, has the potential to decrease the immunosuppressive effects of radiotherapy (e.g., by reducing radiotherapy-induced lymphopenia). This so-called "lymphocyte-sparing radiotherapy" strategy is particularly relevant for the spleen (80, 81), as (preclinical) studies established that splenic (and nodal) T cells are more radiosensitive compared with those located in the liver or gut (93–96). It is likely that the net effect of splenic irradiation will depend on a number of factors, including the volume of the irradiated field, the dose, and the fractionation. Nonetheless, it might be detrimental to perturb the splenic (immunological) niche with radiotherapy, especially within the context of (radio)-immunotherapeutics. Accordingly, the implementation of splenic-dose volume constraints seems indicated (97).

Although splenectomy has been used in the past as a means of treating several cancers, including Hodgkin's lymphoma, it is now rarely indicated due to availability of superior therapeutic alternatives. In addition, concerns have also been raised about the deleterious immunological and thrombotic consequences of splenectomy. For instance, early clinical data suggest that spleen preservation might be beneficial (in terms of immunological recovery) in patients with gastric cancer receiving immunochemotherapy after gastrectomy, as evidenced by higher IL2 production compared with those patients undergoing splenectomy (98). In brief, splenectomy is currently indicated for a limited number of cancer patients such as those with advanced ovarian cancer (in 20% of cases to obtain no gross residual disease; ref. 99), pancreatic cancer requiring distal (or total) pancreatectomy, and splenic flexure colon cancer or gastric cancer with direct splenic extension. Unfortunately, to our knowledge, no robust associations between immune status and outcome data have been reported for patients with cancer with splenectomy versus those without. Therefore, clinical investigators should be urged to systematically record the clinical, immunologic, and hematopoietic data of these patients, to answer the key unresolved question: whether splenectomy independently worsens oncological outcomes (especially when performed prior to the administration of a therapy with an immune component).

Upon systemic injection, NP therapeutics are specifically enriched in the liver and spleen. In the liver due to presence of Kupffer cells that line the lumen of the sinusoids and in the spleen due to its large number of phagocytic mononuclear cells and its particularly suited blood vessel and flow characteristics (100–106). Although splenic clearance of NPs has largely been viewed as a limitation of nanotherapeutic delivery, this also implies that the spleen might be a rational target of NP-based drug delivery in cancer. For instance, the delivery of anti–programmed death (PD)-1 encapsulated in biodegradable synthetic polymer NPs to the spleen resulted in a potent antitumor response in a murine melanoma model (107). In addition, nanoscale applications of tumor antigen packaging and delivery to augment splenic T-cell responses may represent opportunities for NP therapeutics. Challenges ahead are to define the NP carrier technology (e.g., liposomes, virus-like particles/virosomes, extracellular vesicles, metals, polymers, or DC vaccines), the payload (e.g., immunomodulator or tumor Ags), and the splenic target cell population (e.g., FRCs, myeloid cells, or lymphocytes) that should be utilized. Functionalization of NPs with biomimetic surfaces may further enhance splenotropism. Nevertheless, splenotropic NPs with a diverse array of immunomodulatory payloads represent a promising area of research that could provide therapeutic benefits both in case of cancers directly involving the spleen (e.g., splenic MZ lymphoma and metastases) and distant cancers susceptible to immune-mediated elimination (e.g., NSCLC) by NP-educated splenocytes.

Biomarker opportunities

The splenic niche is currently under investigation as a noninvasive imaging biomarker. For instance, the restriction level of the spleen as measured by diffusion-weighted magnetic resonance imaging, was a proxy for tumor load in multiple myeloma patients and was also associated with therapy response and prognosis (108). Further, baseline splenomegaly was predictive of survival in two independent cohorts of pancreatic ductal adenocarcinoma patients receiving FOLFIRINOX chemotherapy (109). Interestingly, colorectal cancer patients receiving neoadjuvant chemotherapy for liver metastasis surgery had a larger splenic volume increase than those who did not, which correlated with a nonalcoholic fatty liver disease and a worse survival (110). Similarly, dynamic temporal changes in spleen volume have been observed in NSCLC patients receiving chemoradiation (111), although their prognostic/predictive utility is yet unknown. Increased splenic metabolic activity, defined through the uptake of ^{18}F- fluorodeoxyglucose (FDG) measured by PET, was prognostic in patients with local breast (112), bile duct (113), gastric (114), and rectal cancer (115). Recently, our group extended this observation to locally advanced cervical cancer; we noted that those patients with a high splenic FDG uptake had a denser immune infiltrate in the primary tumor (cfr splenic "immune barometer") and were more likely to have disease progression and less likely to achieve pathologic complete response than those with a low or no splenic glucose uptake (116).

Before translating these interesting noninvasive biomarkers, including splenic volume or metabolic activity, into clinical settings, they should be validated prospectively in well-defined populations in a multicentric design.

Concluding Remarks

Animal models reveal that splenic hematopoietic and stromal cells are central players in tumor immunology and metastasis. The remarkable potential of single-cell resolution techniques (such as RNA-seq and mass cytometry) in combination with time-controlled splenectomy in animal models will further unlock mechanisms of splenic contribution to primary tumor and (pre-) metastatic niche formation, colonization, and dormancy. The potential clinical applications of tumor-induced splenic changes have been undervalued and underutilized. On the basis of the findings critically presented in this review, we suggest that acknowledging protective or deleterious (but therapeutically targetable) contributions of the spleen in cancer therapy could prove to be a sophisticated approach with applications across tumor types. Perhaps the major opportunity of characterizing the splenic niche is to develop therapeutic strategies that can synergize with conventional cancer therapeutics to shift the immunoediting balance toward elimination (of tumor-associated Ags) rather than escape.

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References

1. Bronte V, Pittet MJ. The spleen in local and systemic regulation of immunity. Immunity 2013;39:806–18.
2. Golub R, Tan J, Watanabe T, Brendolan A. Origin and immunological functions of spleen stromal cells. Trends Immunol 2018;39:503–14.
3. Budczies J, von Winterfeld M, Klauschen F, Bockmayr M, Lennerz JK, Denkert C, et al. The landscape of metastatic progression patterns across major human cancers. Oncotarget 2015;6:570–83.
4. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer 2007;7:834–46.
5. Sai B, Xiang J. Disseminated tumour cells in bone marrow are the source of cancer relapse after therapy. J Cell Mol Med 2018;22:5776–86.
6. Markel JE, Noore J, Emery EJ, Bobnar HJ, Kleinerman ES, Lindsey BA. Using the spleen as an in vivo systemic immune barometer alongside osteosarcoma disease progression and immunotherapy with alpha-PD-L1. Sarcoma 2018; 2018:8694397.
7. Mebius RE, Kraal G. Structure and function of the spleen. Nat Rev Immunol 2005;5:606–16.
8. Lewis SM, Williams A, Eisenbarth SC. Structure and function of the immune system in the spleen. Sc Immunol 2019;4:eaau6085.
9. A-Gonzalez N, Guillen JA, Gallardo G, Diaz M, de la Rosa JV, Hernandez IH, et al. The nuclear receptor LXRalpha controls the functional specialization of splenic macrophages. Nat Immunol 2013;14:831–9.

10. Pillai S, Cariappa A. The follicular versus marginal zone B lymphocyte cell fate decision. Nat Rev Immunol 2009;9:767–77.
11. den Haan JM, Mebius RE, Kraal G. Stromal cells of the mouse spleen. Front Immunol 2012;3:201.
12. Ruddle NH, Akirav EM. Secondary lymphoid organs: responding to genetic and environmental cues in ontogeny and the immune response. J Immunol 2009; 183:2205–12.
13. Mueller SN, Germain RN. Stromal cell contributions to the homeostasis and functionality of the immune system. Nat Rev Immunol 2009;9:618–29.
14. Cinamon G, Zachariah MA, Lam OM, Foss FW Jr, Cyster JG. Follicular shuttling of marginal zone B cells facilitates antigen transport. Nat Immunol 2008;9:54–62.
15. Arnon TI, Horton RM, Grigorova IL, Cyster JG. Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress. Nature 2013;493: 684–8.
16. Gunn MD, Kyuwa S, Tam C, Kakiuchi T, Matsuzawa A, Williams LT, et al. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. J Exp Med 1999;189: 451–60.
17. Spitzer MH, Carmi Y, Reticker-Flynn NE, Kwek SS, Madhireddy D, Martins MM, et al. Systemic immunity is required for effective cancer immunotherapy. Cell 2017;168:487–502.
18. Ford WL, Gowans JL. The traffic of lymphocytes. Semin Hematol 1969;6:67–83.
19. Stoth M, Freire Valls A, Chen M, Hidding S, Knipper K, Shen Y, et al. Splenectomy reduces lung metastases and tumoral and metastatic niche inflammation. Int J Cancer 2019;145:2509–20.
20. Cortez-Retamozo V, Etzrodt M, Newton A, Rauch PJ, Chudnovskiy A, Berger C, et al. Origins of tumor-associated macrophages and neutrophils. Proc Natl Acad Sci U S A 2012;109:2491–6.
21. DuPre SA, Hunter KW Jr. Murine mammary carcinoma 4T1 induces a leukemoid reaction with splenomegaly: association with tumor-derived growth factors. Exp Mol Pathol 2007;82:12–24.
22. Liu M, Jin X, He X, Pan L, Zhang X, Zhao Y. Macrophages support splenic erythropoiesis in 4T1 tumor-bearing mice. PLoS One 2015;10: e0121921.
23. Li B, Zhang S, Huang N, Chen H, Wang P, Li J, et al. Dynamics of the spleen and its significance in a murine H22 orthotopic hepatoma model. Exp Biol Med 2016;241:863–72.
24. Kamran N, Li Y, Sierra M, Alghamri MS, Kadiyala P, Appelman HD, et al. Melanoma induced immunosuppression is mediated by hematopoietic dysregulation. Oncoimmunology 2018;7:e1408750.
25. McKim DB, Yin W, Wang Y, Cole SW, Godbout JP, Sheridan JF. Social stress mobilizes hematopoietic stem cells to establish persistent splenic myelopoiesis. Cell Rep 2018;25:2552–62.
26. Jordan S, Ruzsics Z, Mitrovic M, Baranek T, Arapovic J, Krmpotic A, et al. Natural killer cells are required for extramedullary hematopoiesis following murine cytomegalovirus infection. Cell Host Microbe 2013;13:535–45.
27. Allen BM, Hiam KJ, Burnett CE, Venida A, DeBarge R, Tenvooren I, et al. Systemic dysfunction and plasticity of the immune macroenvironment in cancer models. Nat Med 2020;26:1125–34.
28. Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. Trends Immunol 2016;37: 208–20.
29. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 2008;181:5791–802.
30. Movahedi K, Guilliams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, et al. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. Blood 2008;111:4233–44.
31. Steenbrugge J, Breyne K, Demeyere K, De Wever O, Sanders NN, Van Den Broeck W, et al. Anti-inflammatory signaling by mammary tumor cells mediates prometastatic macrophage polarization in an innovative intraductal mouse model for triple-negative breast cancer. J Exp Clin Cancer Res 2018; 37:191.
32. Steenbrugge J, Vander Elst N, Demeyere K, De Wever O, Sanders NN, Van Den Broeck W, et al. Comparative profiling of metastatic 4T1- vs. non-metastatic Py230-based mammary tumors in an intraductal model for triple-negative breast cancer. Front Immunol 2019;10:2928.
33. Ugel S, Peranzoni E, Desantis G, Chioda M, Walter S, Weinschenk T, et al. Immune tolerance to tumor antigens occurs in a specialized environment of the spleen. Cell Rep 2012;2:628–39.
34. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1alpha regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med 2010;207:2439–53.
35. Li W, Tanikawa T, Kryczek I, Xia H, Li G, Wu K, et al. Aerobic glycolysis controls myeloid-derived suppressor cells and tumor immunity via a specific CEBPB Isoform in triple-negative breast cancer. Cell Metab 2018;28:87–103.
36. Mosely SI, Prime JE, Sainson RC, Koopmann JO, Wang DY, Greenawalt DM, et al. Rational selection of syngeneic preclinical tumor models for immunotherapeutic drug discovery. Cancer Immunol Res 2017;5:29–41.
37. Ouzounova M, Lee E, Piranlioglu R, El Andaloussi A, Kolhe R, Demirci MF, et al. Monocytic and granulocytic myeloid derived suppressor cells differentially regulate spatiotemporal tumour plasticity during metastatic cascade. Nat Commun 2017;8:14979.
38. Kumar S, Wilkes DW, Samuel N, Blanco MA, Nayak A, Alicea-Torres K, et al. DeltaNp63-driven recruitment of myeloid-derived suppressor cells promotes metastasis in triple-negative breast cancer. J Clin Invest 2018;128:5095–109.
39. Piranlioglu R, Lee E, Ouzounova M, Bollag RJ, Vinyard AH, Arbab AS, et al. Primary tumor-induced immunity eradicates disseminated tumor cells in a syngeneic mouse model. Nat Commun 2019;10:1430.
40. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun 2016;7:12150.
41. Bruger AM, Dorhoi A, Esendagli G, Barczyk-Kahlert K, van der Bruggen P, Lipoldova M, et al. How to measure the immunosuppressive activity of MDSC: assays, problems and potential solutions. Cancer Immunol Immunother 2019; 68:631–44.
42. Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. Sci Immunol 2016;1:aaf8943.
43. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, et al. Fatty acid transport protein 2 reprograms neutrophils in cancer. Nature 2019; 569:73–8.
44. Alshetaiwi H, Pervolarakis N, McIntyre LL, Ma D, Nguyen Q, Rath JA, et al. Defining the emergence of myeloid-derived suppressor cells in breast cancer using single-cell transcriptomics. Sci Immunol 2020;5:eaay6017.
45. Zhao L, He R, Long H, Guo B, Jia Q, Qin D, et al. Late-stage tumors induce anemia and immunosuppressive extramedullary erythroid progenitor cells. Nat Med 2018;24:1536–44.
46. Han Y, Liu Q, Hou J, Gu Y, Zhang Y, Chen Z, et al. Tumor-Induced generation of splenic Erythroblast-like Ter-cells promotes tumor progression. Cell 2018; 173:634–48.
47. Wei G, Jie Y, Haibo L, Chaoneng W, Dong H, Jianbing Z, et al. Dendritic cells derived exosomes migration to spleen and induction of inflammation are regulated by CCR7. Sci Rep 2017;7:42996.
48. Weiss L. Barrier cells in the spleen. Immunol Today 1991;12:24–9.
49. Inra CN, Zhou BO, Acar M, Murphy MM, Richardson J, Zhao Z, et al. A perisinusoidal niche for extramedullary haematopoiesis in the spleen. Nature 2015;527:466–71.
50. Riedel A, Shorthouse D, Haas L, Hall BA, Shields J. Tumor-induced stromal reprogramming drives lymph node transformation. Nat Immunol 2016;17: 1118–27.
51. Steube KG, Meyer C, Drexler HG. Secretion of functional hematopoietic growth factors by human carcinoma cell lines. Int J Cancer 1998;78:120–4.
52. Casbon AJ, Reynaud D, Park C, Khuc E, Gan DD, Schepers K, et al. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. Proc Natl Acad Sci U S A 2015;112: E566–75.
53. Hong IS. Stimulatory versus suppressive effects of GM-CSF on tumor progression in multiple cancer types. Exp Mol Med 2016;48:e242.
54. Lapidot T, Kollet O. The essential roles of the chemokine SDF-1 and its receptor CXCR4 in human stem cell homing and repopulation of transplanted immune-deficient NOD/SCID and NOD/SCID/B2m(null) mice. Leukemia 2002;16: 1992–2003.
55. Dale DC. Editorial: the mysteries of the spleen. J Leukoc Biol 2016;100:249–51.
56. Lim S, Zhang Y, Zhang D, Chen F, Hosaka K, Feng N, et al. VEGFR2-mediated vascular dilation as a mechanism of VEGF-induced anemia and bone marrow cell mobilization. Cell Rep 2014;9:569–80.
57. Wu C, Ning H, Liu M, Lin J, Luo S, Zhu W, et al. Spleen mediates a distinct hematopoietic progenitor response supporting tumor-promoting myelopoiesis. J Clin Invest 2018;128:3425–38.

58. Kim EK, Jeon I, Seo H, Park YJ, Song B, Lee KA, et al. Tumor-derived osteopontin suppresses antitumor immunity by promoting extramedullary myelopoiesis. Cancer Res 2014;74:6705–16.
59. Cao Y, Slaney CY, Bidwell BN, Parker BS, Johnstone CN, Rautela J, et al. BMP4 inhibits breast cancer metastasis by blocking myeloid-derived suppressor cell activity. Cancer Res 2014;74:5091–102.
60. Sio A, Chehal MK, Tsai K, Fan X, Roberts ME, Nelson BH, et al. Dysregulated hematopoiesis caused by mammary cancer is associated with epigenetic changes and hox gene expression in hematopoietic cells. Cancer Res 2013; 73:5892–904.
61. Al Sayed MF, Amrein MA, Buhrer ED, Huguenin AL, Radpour R, Riether C, et al. T-cell-secreted TNFalpha induces emergency myelopoiesis and myeloid-derived suppressor cell differentiation in cancer. Cancer Res 2019;79:346–59.
62. Cortez-Retamozo V, Etzrodt M, Newton A, Ryan R, Pucci F, Sio SW, et al. Angiotensin II drives the production of tumor-promoting macrophages. Immunity 2013;38:296–308.
63. Xue Y, Lim S, Yang Y, Wang Z, Jensen LD, Hedlund EM, et al. PDGF-BB modulates hematopoiesis and tumor angiogenesis by inducing erythropoietin production in stromal cells. Nat Med 2011;18:100–10.
64. Greenwald AC, Licht T, Kumar S, Oladipupo SS, Iyer S, Grunewald M, et al. VEGF expands erythropoiesis via hypoxia-independent induction of erythropoietin in noncanonical perivascular stromal cells. J Exp Med 2019;216:215–30.
65. Li Y, Yue H, Yang S, Yuan D, Li L, Zhao J, et al. Splenomegaly induced by anemia impairs T cell movement in the spleen partially via EPO. Mol Immunol 2019; 112:399–405.
66. Huang Y, Chen X, Dikov MM, Novitskiy SV, Mosse CA, Yang L, et al. Distinct roles of VEGFR-1 and VEGFR-2 in the aberrant hematopoiesis associated with elevated levels of VEGF. Blood 2007;110:624–31.
67. Yang X, Lin Y, Shi Y, Li B, Liu W, Yin W, et al. FAP Promotes immunosuppression by cancer-associated fibroblasts in the tumor microenvironment via STAT3-CCL2 signaling. Cancer Res 2016;76:4124–35.
68. Kumar V, Donthireddy L, Marvel D, Condamine T, Wang F, Lavilla-Alonso S, et al. Cancer-associated fibroblasts neutralize the anti-tumor effect of CSF1 receptor blockade by inducing PMN-MDSC infiltration of tumors. Cancer Cell 2017;32:654–68.
69. Labelle M, Begum S, Hynes RO. Platelets guide the formation of early metastatic niches. Proc Natl Acad Sci U S A 2014;111:E3053–61.
70. Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S, Bronte V. Myeloid-derived suppressor cell heterogeneity in human cancers. Ann N Y Acad Sci 2014;1319:47–65.
71. Shand FH, Ueha S, Otsuji M, Koid SS, Shichino S, Tsukui T, et al. Tracking of intertissue migration reveals the origins of tumor-infiltrating monocytes. Proc Natl Acad Sci U S A 2014;111:7771–6.
72. Prehn RT. The paradoxical effects of splenectomy on tumor growth. Theor Biol Med Model 2006;3:23.
73. Higashijima J, Shimada M, Chikakiyo M, Miyatani T, Yoshikawa K, Nishioka M, et al. Effect of splenectomy on antitumor immune system in mice. Anticancer Res 2009;29:385–93.
74. Sevmis M, Yoyen-Ermis D, Aydin C, Bilgic E, Korkusuz P, Uner A, et al. Splenectomy-induced leukocytosis promotes intratumoral accumulation of myeloid-derived suppressor cells, angiogenesis and metastasis. Immunol Invest 2017;46:663–76.
75. Porrello A, Cardelli P, Spugnini EP. Oncology of companion animals as a model for humans. an overview of tumor histotypes. J Exp Clin Cancer Res 2006;25: 97–105.
76. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015; 28:690–714.
77. Engblom C, Pfirschke C, Pittet MJ. The role of myeloid cells in cancer therapies. Nat Rev Cancer 2016;16:447–62.
78. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol 2017;17:97–111.
79. Shaked Y. The pro-tumorigenic host response to cancer therapies. Nat Rev Cancer 2019;19:667–85.
80. Lambin P, Lieverse RIY, Eckert F, Marcus D, Oberije C, van der Wiel AMA, et al. Lymphocyte-sparing radiotherapy: the rationale for protecting lymphocyte-rich organs when combining radiotherapy with immunotherapy. Semin Radiat Oncol 2020;30:187–93.
81. Will immunotherapy really change radiotherapy? Lancet Oncol 2019;20: 1642–4.
82. Deutsch E, Chargari C, Galluzzi L, Kroemer G. Optimising efficacy and reducing toxicity of anticancer radioimmunotherapy. Lancet Oncol 2019;20: e452–e63.
83. Walker J, Loo BW Jr. Radiotherapy and immunotherapy-shining further together. JAMA Oncol 2019;5:1291–2.
84. Schaue D, Ratikan JA, Iwamoto KS, McBride WH. Maximizing tumor immunity with fractionated radiation. Int J Radiat Oncol Biol Phys 2012;83:1306–10.
85. Grossman SA, Ellsworth S, Campian J, Wild AT, Herman JM, Laheru D, et al. Survival in patients with severe lymphopenia following treatment with radiation and chemotherapy for newly diagnosed solid tumors. J Natl Compr Canc Netw 2015;13:1225–31.
86. Terrones C, Specht C, Maraldo MV, Lundgren J, Helleberg M. Lymphopenia after radiotherapy and risk of infection. Open Forum Infect Dis 2017;4:S702.
87. Sun GY, Wang SL, Song YW, Jin J, Wang WH, Liu YP, et al. Radiation-induced lymphopenia predicts poorer prognosis in patients with breast cancer: a Post Hoc analysis of a randomized controlled trial of postmastectomy hypofractionated radiation therapy. Int J Radiat Oncol Biol Phys 2020;108:277–85.
88. Chadha AS, Liu G, Chen HC, Das P, Minsky BD, Mahmood U, et al. Does unintentional splenic radiation predict outcomes after pancreatic cancer radiation therapy? Int J Radiat Oncol Biol Phys 2017;97:323–32.
89. Liu J, Zhao Q, Deng W, Lu J, Xu X, Wang R, et al. Radiation-related lymphopenia is associated with spleen irradiation dose during radiotherapy in patients with hepatocellular carcinoma. Radiat Oncol 2017;12:90.
90. Saito T, Toya R, Matsuyama T, Semba A, Oya N. Dosimetric predictors of treatment-related Lymphopenia induced by palliative radiotherapy: predictive ability of dose-volume parameters based on body surface contour. Radiol Oncol 2017;51:228–34.
91. Joseph N, McWilliam A, Kennedy J, Haslett K, Mahil J, Gavarraju A, et al. Post-treatment lymphocytopaenia, integral body dose and overall survival in lung cancer patients treated with radical radiotherapy. Radiother Oncol 2019;135: 115–9.
92. Grassberger C, Hong TS, Hato T, Yeap BY, Wo JY, Tracy M, et al. Differential association between circulating lymphocyte populations with outcome after radiation therapy in subtypes of liver cancer. Int J Radiat Oncol Biol Phys 2018; 101:1222–5.
93. Qu Y, Zhang B, Liu S, Zhang A, Wu T, Zhao Y. 2-Gy whole-body irradiation significantly alters the balance of CD4+ CD25- T effector cells and CD4+ CD25+ Foxp3+ T regulatory cells in mice. Cell Mol Immunol 2010;7:419–27.
94. Cao M, Cabrera R, Xu Y, Liu C, Nelson D. Different radiosensitivity of CD4(+) CD25(+) regulatory T cells and effector T cells to low dose gamma irradiation in vitro. Int J Radiat Biol 2011;87:71–80.
95. Pugh JL, Sukhina AS, Seed TM, Manley NR, Sempowski GD, van den Brink MR, et al. Histone deacetylation critically determines T cell subset radiosensitivity. J Immunol 2014;193:1451–8.
96. Persa E, Balogh A, Safrany G, Lumniczky K. The effect of ionizing radiation on regulatory T cells in health and disease. Cancer Lett 2015;368:252–61.
97. Blais E, Bourdais R, Simon JM, Sargos P, Troussier I, Jenny C, et al. Proposition of splenic dose-volume constraints to prevent severe lymphopenia after chemoradiation for pancreatic cancer. Int J Radiat Oncol Biol Phys 2018;102:e69.
98. Okinaga K, Iinuma H, Kitamura Y, Yokohata T, Inaba T, Fukushima R. Effect of immunotherapy and spleen preservation on immunological function in patients with gastric cancer. J Exp Clin Cancer Res 2006;25:339–49.
99. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. Ann Oncol 2019;30:672–705.
100. Sou K. Advanced drug carriers targeting bone marrow. In:Sezer AD, ed. Recent advances in novel drug carrier systems. Croatia: InTech. 2012. p. 419–436.
101. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol 2015;33:941–51.
102. Jindal AB. Nanocarriers for spleen targeting: anatomo-physiological considerations, formulation strategies and therapeutic potential. Drug Deliv Transl Res 2016;6:473–85.
103. Chesson CB, Zloza A. Nanoparticles: augmenting tumor antigen presentation for vaccine and immunotherapy treatments of cancer. Nanomedicine 2017;12: 2693–706.
104. Kishimoto TK, Maldonado RA. Nanoparticles for the induction of antigen-specific immunological tolerance. Front Immunol 2018;9:230.

105. Zhuang J, Holay M, Park JH, Fang RH, Zhang J, Zhang L. Nanoparticle delivery of immunostimulatory agents for cancer immunotherapy. Theranostics 2019;9: 7826–48.
106. Nance E, McKenna M. Challenges and barriers. In: Chung EJ, Leon L, Rinaldi C, eds. Nanoparticles for biomedical applications: fundamental concepts, biological interactions and clinical applications. Cambridge: Elsevier; 2020. p. 89–107.
107. Ordikhani F, Uehara M, Kasinath V, Dai L, Eskandari SK, Bahmani B, et al. Targeting antigen-presenting cells by anti-PD-1 nanoparticles augments antitumor immunity. JCI insight 2018;3:e122700.
108. Rasche L, Kumar M, Gershner G, Samant R, Van Hemert R, Heidemeier A, et al. Lack of spleen signal on diffusion weighted MRI is associated with high tumor burden and poor prognosis in multiple myeloma: a link to extramedullary hematopoiesis? Theranostics 2019;9:4756–63.
109. Aarnink A, Richard C, Truntzer C, Vincent J, Bengrine L, Vienot A, et al. Baseline splenic volume as a surrogate marker of FOLFIRINOX efficacy in advanced pancreatic carcinoma. Oncotarget 2018;9: 25617–29.
110. Saez-Carlin P, Garcia-Botella A, Diez-Valladares LI, Ortega Medina L, Mendez R, Gonzalez JCM, et al. Splenic volume as a biomarker of hepatic damage after chemotherapy in patients with resected colorectal liver metastases (CRLM). Clin Transl Oncol 2020;22:1180–6.
111. Wen SW, Everitt SJ, Bedo J, Chabrot M, Ball DL, Solomon B, et al. Spleen volume variation in patients with locally advanced non-small cell lung cancer receiving platinum-based chemo-radiotherapy. PLoS One 2015;10:e0142608.
112. Bang JI, Yoon HJ, Kim BS. Clinical utility of FDG uptake within reticuloendothelial system on F-18 FDG PET/CT for prediction of tumor recurrence in breast cancer. PLoS One 2018;13:e0208861.
113. Pak K, Kim SJ, Kim IJ, Kim DU, Kim K, Kim H, et al. Splenic FDG uptake predicts poor prognosis in patients with unresectable cholangiocarcinoma. Nuklearmedizin 2014;53:26–31.
114. Yoon HJ, Kim BS, Moon CM, Yoo J, Lee KE, Kim Y. Prognostic value of diffuse splenic FDG uptake on PET/CT in patients with gastric cancer. PLoS One 2018; 13:e0196110.
115. Kim SY, Moon CM, Yoon HJ, Kim BS, Lim JY, Kim TO, et al. Diffuse splenic FDG uptake is predictive of clinical outcomes in patients with rectal cancer. Sci Rep 2019;9:1313.
116. De Jaeghere EA, Frederiek L, Lippens L, De Man K, Van Bockstal M, Van de Vijver K, et al. Splenic metabolic activity as biomarker in cervical cancer. Ann Oncol 2019;30:v47.