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Review

Control of Metastasis by NK Cells

Alejandro López-Soto,[1,*] Segundo Gonzalez,[1] Mark J. Smyth,[2] and Lorenzo Galluzzi[3,4,5,*]
[1]Departamento de Biología Funcional, Área de Inmunología, Universidad de Oviedo, Instituto Universitario de Oncología del Principado de Asturias (IUOPA), 33006 Oviedo, Asturias, Spain
[2]Immunology of Cancer and Infection Laboratory, QIMR Berghofer Medical Research Institute, Herston, QLD 4006, Australia
[3]Department of Radiation Oncology, Weill Cornell Medical College, New York, NY 10065, USA
[4]Sandra and Edward Meyer Cancer Center, New York, NY 10065, USA
[5]Université Paris Descartes/Paris V, 75006 Paris, France
*Correspondence: lopezsalejandro@uniovi.es (A.L.-S.), deadoc@vodafone.it (L.G.)
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The metastatic spread of malignant cells to distant anatomical locations is a prominent cause of cancer-related death. Metastasis is governed by cancer-cell-intrinsic mechanisms that enable neoplastic cells to invade the local microenvironment, reach the circulation, and colonize distant sites, including the so-called epithelial-to-mesenchymal transition. Moreover, metastasis is regulated by microenvironmental and systemic processes, such as immunosurveillance. Here, we outline the cancer-cell-intrinsic and -extrinsic factors that regulate metastasis, discuss the key role of natural killer (NK) cells in the control of metastatic dissemination, and present potential therapeutic approaches to prevent or target metastatic disease by harnessing NK cells.

Introduction

Most patients with cancer succumb to advanced neoplasms in the context of metastatic disease, i.e., upon the dissemination of malignant cells from primary lesions to distant anatomical locations. Over the past decades, extensive efforts have been devoted to the characterization of the molecular and cellular mechanisms underlying such a detrimental aspect of cancer cell biology, resulting in several, partially reconcilable models of metastasis (Massague and Obenauf, 2016). Arguably, the best known of such models points to the existence of a sequential series of events called the ''metastatic cascade'' through which malignant cells: (1) proliferate locally to form a primary lesion as they progressively acquire additional genetic alterations, invade the local microenvironment, and stimulate angiogenesis; (2) migrate toward newly formed or pre-existent capillary vessels or sentinel lymph nodes to eventually reach the circulation; (3) survive as circulating tumor cells (CTCs) until they encounter conditions that are permissive for extravasation; (4) extravasate and colonize distant tissues until they reach a (pre)metastatic niche; and (5) persist in a relatively silent state as disseminated tumor cells (DTCs) or micrometastases for a highly variable period of time, until they resume proliferation to establish clinically detectable metastatic disease (macrometastases) (Massague and Obenauf, 2016) (Figure 1). According to the so-called seed and soil hypothesis, the preference exhibited by each type of cancer to colonize a specific target organ would be determined by the fact that malignant cells can seed metastatic lesions exclusively in particular microenvironments that are supportive for malignant outgrowth (Paget, 1989).

Although detailed discussion of the metastatic cascade goes beyond the scope of this article, it should be noted that one of the major corollaries of this model is that metastatic spread invariably occurs as a late event in tumor progression. Such an assumption has driven the implementation of nationwide early detection programs for several tumor types, including colorectal cancer (based on the detection of occult fecal blood) and breast carcinoma (based on radiographic signs of disease) (Woloshin et al., 2012). These initiatives were highly successful at increasing the proportion of tumors diagnosed at an early stage. However, whereas in the case of colorectal cancer, early detection was accompanied by a significant decrease in metastatic disease and consequent increase in overall survival, the same did not hold true for breast carcinoma (Welch et al., 2016). Corroborating these clinical findings, early metastatic dissemination (occurring before the establishment of histologically detectable primary tumors) has been robustly documented in rodent models of mammary and pancreatic carcinogenesis (Harper et al., 2016; Hosseini et al., 2016; Rhim et al., 2012). Thus, although the metastatic cascade model has been highly instrumental for our understanding of metastasis, accumulating preclinical and clinical data suggest that metastatic spread also occurs at the very early stage of disease and that CTCs or DTCs can persist for prolonged periods prior to the formation of macrometastases. Moreover, it appears that some cancers (including breast carcinoma) accumulate extensive phenotypic and functional heterogeneity as they form macrometastases, while others (including colorectal carcinoma) accrue much less diversity (Turajlic and Swanton, 2016). Finally, it has become clear that metastasis is governed not only by cancer-cell-intrinsic mechanisms that endow malignant cells with new functions but also by microenvironmental and systemic processes (Peinado et al., 2017). In particular, it appears that metastatic dissemination is under tonic control by natural killer (NK) cells, a specialized and finely regulated population of innate lymphoid cells (ILCs) that mediate cytotoxic functions independent of MHC-mediated antigen presentation (Cerwenka and Lanier, 2016; Guillerey et al., 2016) (Box 1 and Table 1).

Here, we summarize the major mechanisms that regulate metastatic spread at both the cancer-cell-intrinsic and -extrinsic level, discuss the prominent role of NK cells in the control of metastasis, and present potential therapeutic approaches to

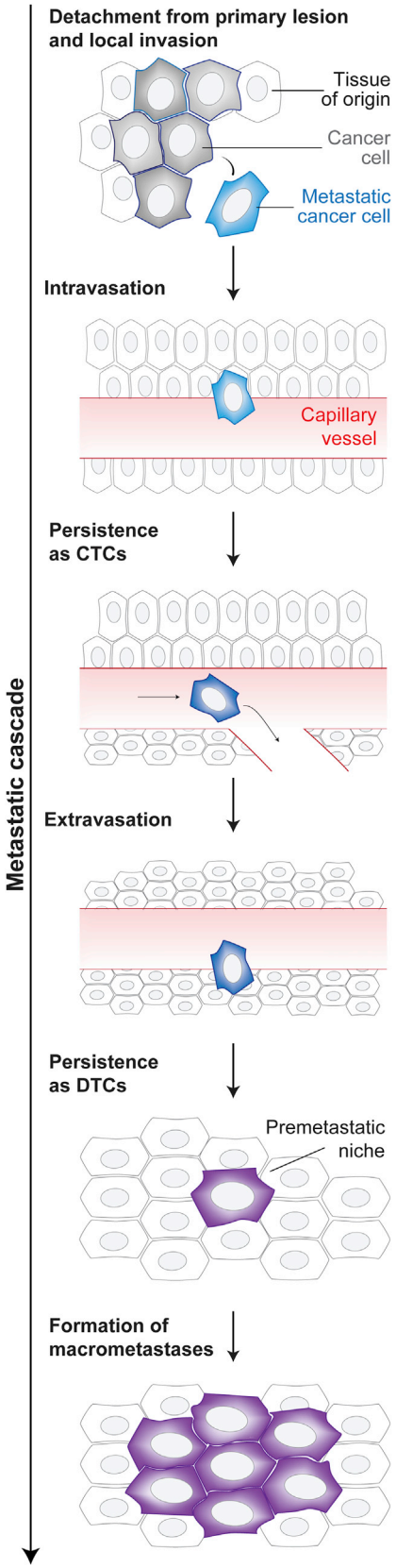

Figure 1. The Metastatic Cascade
Metastasis involves the detachment of malignant cells from the primary tumor, their intravasation and persistence in the blood as circulating tumor cells (CTCs), their extravasation and persistence at pre-metastatic niches as disseminated tumor cells (DTCs), and eventually their reactivation to generate clinically detectable macrometastatic lesions.

harness NK cells for the prevention or treatment of metastatic dissemination.

Metastatic Dissemination of Cancer Cells

Solid tumors shed a surprisingly high amount of cells into the circulation, yet only a few of them successfully form macrometastases, implying that stringent selection occurs throughout the metastatic cascade. Such a quasi-Darwinian process involves the interplay between cancer-cell-intrinsic factors, which mainly depends on the rapid (genetic and epigenetic) diversification of malignant cells proliferating locally, and microenvironmental determinants that operate within primary lesions, systemically and at colonization sites (Massague and Obenauf, 2016).

Cancer-Cell-Intrinsic Factors

Malignant cells must acquire at least three intrinsic features to form macrometastases. First, they must detach from the primary lesion without undergoing anoikis (a specific form of regulated cell death driven by loss of anchorage) and resist adverse microenvironmental conditions potentially encountered throughout the metastatic cascade. Besides relying on improved adaptive stress responses, suppressed cell death signaling (Yu et al., 2012; Zhang et al., 2009), and escape from immunosurveillance (Spranger et al., 2015), this involves the acquisition of superior metabolic flexibility (McDonald et al., 2017). Second, they must be able to interact with and potentially remodel the extracellular matrix (ECM) to allow for their local and distant invasion (Erler et al., 2006). Third, they must exhibit sufficient motility to reach a capillary vessel and expose surface molecules allowing for intra- and extravasation. Most of these features are cooperatively acquired along with the epithelial-to-mesenchymal transition (EMT) (Rhim et al., 2012). Considerable efforts have been dedicated to the elucidation of the cancer-cell-intrinsic programs that are responsible for the preferential colonization of the lung (Padua et al., 2008), brain (Bos et al., 2009), or bone (Kang et al., 2003) by specific tumors.

In summary, primary neoplasms, including early-stage lesions, contain malignant cell subpopulations intrinsically endowed with distinct metastatic potential owing to epigenetic and genetic alterations.

Cancer-Cell-Extrinsic Factors

The metabolic, stromal, and immunological microenvironments have a major impact on metastasis. Most solid tumors are hypoxic (at least in some areas), resulting in the activation of the transcription factor hypoxia-inducible factor 1 (HIF-1). Besides controlling the expression of several glycolytic enzymes, HIF-1 favors metastatic dissemination by transactivating genes involved in anoikis resistance, ECM remodeling, angiogenesis, and local immunosuppression (Rankin and Giaccia, 2016; Young et al., 2014). Hypoxia in the primary tumor also silences tumor suppressor genes by epigenetic mechanisms (Thienpont et al., 2016) and promotes the establishment of immunosuppression at the pre-metastatic niche (Sceneay et al., 2012). Finally, the tumor microenvironment is often characterized by low nutrient

Box 1. Regulation of NK Cell Activity

NK cells can exert robust antimetastatic functions independent of MHC-mediated antigen presentation via at least three pathways: (1) the release of PRF1- and GZMB-containing pre-formed granules; (2) the secretion of IFNG; and (3) the exposure of death receptor ligands, including FASLG and TRAIL. Thus, at odds with T lymphocytes (which require priming from antigen-presenting cells) NK cells are continuously poised to kill damaged, infected, or (pre)malignant cells (Morvan and Lanier, 2016). Such a potent cytotoxic activity is mainly regulated by the interplay between inhibitory and activatory signals originating at the plasma membrane of NK cells from NKIRs and NKARs, respectively (Table 1). NKIRs keep the effector functions of NK cells at bay upon interaction with ligands expressed by normal and healthy cells. Conversely, NKARs promote the effector functions of NK cells as they recognize a wide panel of ligands that are specifically upregulated in response to potentially detrimental perturbations of homeostasis, including DNA damage and viral infection. NKIRs and NKARs virtually operate as mutual antagonists as they contain intracellular domains that inhibit or activate the phosphorylation-dependent signal transduction cascade leading to NK cell activation (Morvan and Lanier, 2016).

Interestingly, some NKIRs and NKARs exhibit overlapping ligand specificity. Thus, while poliovirus receptor (PVR, best known as CD155) and nectin cell adhesion molecule 2 (NECTIN2, best known as CD112) stimulate NK cell effector functions upon binding to DNAM-1, they exert robust inhibitory activity upon binding to CD96, TIGIT, or poliovirus receptor related immunoglobulin domain containing (PVRIG; best known as CD112R) (Chan et al., 2014; Stanietsky et al., 2009; Zhu et al., 2016). This scenario is highly reminiscent of CTL regulation. Indeed, CD80 expressed on antigen-presenting cells can promote CTL activation upon binding to CD27 or inhibition upon binding to CTLA4 (Topalian et al., 2015). Additional receptors are important for the acquisition of full effector functions by developing NK cells. As an example, CD160 is critical for NK cells to mount efficient IFNG responses but does not affect the exocytosis of cytotoxic granules (Tu et al., 2015). Moreover, $CD56^{dim}$ NK cells rely on Fc fragment of IgG receptor IIIa (FCGR3A, best known as CD16) to recognize the Fc portion of antibodies bound to a target cell and mediate ADCC (Muntasell et al., 2017). Of note, a fraction of cancer patients spontaneously develop antibodies specific for tumor-associated antigens expressed on the surface of malignant cells (Sahin et al., 1995). Whether these antibodies efficiently drive ADCC, however, remains to be elucidated. In summary, NK cell effector functions are finely regulated by an intricate network of signals that prevents autoimmune reactions against healthy tissues, while allowing for rapid activation against damaged, infected or (pre)malignant cells.

availability, resulting in the activation of autophagy (Galluzzi et al., 2017a). This adaptive stress response mediates robust cytoprotective effects and has been involved in virtually all steps of the metastatic cascade (Galluzzi et al., 2015b).

The normal stroma (in its cellular and structural components) generally prevents local invasion as well as metastatic seeding at distant sites. However, malignant cells acquire the ability to condition the stroma in multiple ways to establish feedforward circuitries that *de facto* support metastasis. These include the lysyl oxidase (LOX)-dependent crosslinking of collagen (Levental et al., 2009), the exosome-dependent conditioning of pre-metastatic niches (Hoshino et al., 2015), and the transforming growth factor β 1 (TGFB1)-dependent conversion of normal fibroblasts into tumor-supporting cells (Avgustinova et al., 2016; Viel et al., 2016). Along similar lines, the normal lung stroma generally enforces DTC dormancy, an antimetastatic effect that is antagonized by cancer-cell-derived factors (Gao et al., 2016; Sun et al., 2010).

Multiple immune cell populations promote metastasis, either because they establish an immunosuppressive microenvironment within primary lesions or because they contribute to conditioning the pre-metastatic niche. These include $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells (Vences-Catalan et al., 2015), myeloid-derived suppressor cells (MDSCs) (Vences-Catalan et al., 2015), platelets (Best et al., 2015), conventional $CD11b^+Ly6G^+$ neutrophils (Liu et al., 2016; Wculek and Malanchi, 2015), macrophages (Georgoudaki et al., 2016), as well as stromal cell populations with immunological activities such as glial cells (Zhang et al., 2015b). Conversely, T_H1 $CD4^+$ T cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) indirectly suppress metastatic dissemination by contributing to the adaptive immunological control of primary tumors and by preserving its normal vascularization (Senovilla et al., 2012; Tian et al., 2017), while non-conventional $Ly6G^-$ neutrophils (Hanna et al., 2015) and NK cells selectively exert innate antimetastatic effects (as detailed below). Finally, several cytokines besides TGFB1 (e.g., interleukin 5 [IL-5]), as well as the complement system, reportedly contribute to the metastatic spread of malignant cells (Boire et al., 2017; Vadrevu et al., 2014; Zaynagetdinov et al., 2015).

In summary, several cancer-cell-extrinsic factors of nutritional, stromal, and immunological nature regulate the metastatic cascade.

Role of NK Cells in the Immunosurveillance of Metastasis

Although the role of NK cells in the control of primary tumors remains a matter of debate (Box 2), an inverse correlation between high amounts of circulating or tumor-infiltrating NK cells and the presence of metastases at clinical presentation has been demonstrated in patients with gastrointestinal sarcoma (GIST) (Delahaye et al., 2011), as well as gastric (Ishigami et al., 2000), colorectal (Coca et al., 1997), renal (Donskov and von der Maase, 2006), and prostate carcinomas (Gannon et al., 2009). Along similar lines, high expression levels of NK cell activatory receptors (NKAR) (Box 1) or improved NK cell cytotoxicity have been linked with good prognosis in multiple cohorts of cancer patients with or at risk of metastatic disease. For example, patients bearing primary prostate carcinomas infiltrated with NK cells expressing high levels of killer cell lectin-like receptor K1 (KLRK1, best known as NKG2D) and natural cytotoxicity triggering

Table 1. Nomenclature of Main Receptor/Ligand Pairs Involved in NK Cell Regulation

Receptor	Aliases	Ligands	Aliases
NKARs[a,b]			
CD160	BY55, NK1, NK28	MHC[c] class I	Not applicable
		TNFRSF14	ATAR, CD270, HVEA, HVEM, LIGHTR, TR2
CD226	DNAM-1, DNAM1, PTA1, TLiSA1	NECTIN2	AI325026, AI987993, CD112, HVEB, MPH, PRR2, Pvr, PVRL2, PVRR2, Pvs
		PVR	3830421F03Rik, CD155, D7Ertd458e, HVED, mE4, Necl-5, NECL5, PVS, Taa1, TAGE4
CD244	2B4, NAIL, NKR2B4, Nmrk, SLAMF4	CD48	AI449234, AW610730, Bcm-1, BCM1, BLAST, BLAST-1, BLAST1, hCD48, mCD48, MEM-102, SLAMF2, Sgp-60
CRTAM	CD355	CADM1	2900073G06Rik, 3100001I08Rik, AI987920, BL2, IGSF4, IGSF4A, NECL2, Necl-2, RA175, RA175A, RA175B, RA175C, RA175N, SgIGSF, ST17, sTSLC-1, SYNCAM, synCAM1, TSLC1
FCGR3A	CD16, CD16A, FCG3, FCGR3, FCGRIII, FCR-10, FCRIII, FCRIIIA, IGFR3, IMD20	Fc fragment of IgG	Not applicable
KLRC2	CD159c, NKG2-C, NKG2C	H2-T23 (*Mus musculus*)	37b, 37c, H-2T23, H2-Qa1, Qa-1,Qa-1(b), Qa1, Qed-1, T18c, T18c(37), T23b, T23d
		HLA-E (*Homo sapiens*)	HLA-6.2, QA1
KLRK1	CD314, D12S2489E, KLR, NKG2-D, NKG2D	H60A (*M. musculus*)	H60
		MICA (*H. sapiens*)	MIC-A, PERB11.1
		MICB (*H. sapiens*)	PERB11.2
		RAET1A (*M. musculus*)	RAE-1alpha, Rae1alpha, Raet1
		RAET1B (*M. musculus*)	RAE-1beta
		RAET1C (*M. musculus*)	RAE-1gamma
		RAET1D (*M. musculus*)	RAE-1delta
		RAET1E	bA350J20.7, LETAL, N2DL-4, NKG2DL42, RL-4, ULBP4
		RAET1G (*H. sapiens*)	Not applicable
		RAET1L (*H. sapiens*)	ULBP6
		ULBP1	A430108B07Rik, MULT1, N2DL-1, NKG2DL1, RAET1I
		ULBP2 (*H. sapiens*)	ALCAN-alpha, N2DL2, NKG2DL2, RAET1H
		ULBP3 (*H. sapiens*)	N2DL-3, NKG2DL3, RAET1N
NCR1	CD335, LY94, NK-p46, NKp46	Heparan sulfates	Not applicable
		VIM	CTRCT30, HEL113
		Viral HA[d]	Not applicable
NCR2 (*H. sapiens*)	CD336, LY95, NK-p44, NKp44, dJ149M18.1	Heparan sulfates (*H. sapiens*)	Not applicable
		KMT2E (*H. sapiens*)	HDCMC04P, MLL5, NKp44L
		PCNA (*H. sapiens*)	ATLD2
		Viral HA	Not applicable
NCR3 (*H. sapiens*)	1C7, CD337, LY117, MALS, NKp30	BAG6 (*H. sapiens*)	BAG-6, BAT3, D6S52E, G3
		Heparan sulfates	Not applicable
		NCR3LG1 (*H. sapiens*)	B7-H6, B7H6, DKFZp686O24166
TNFRSF9	4-1BB, CD137, CDw137, ILA	TNFSF9	4-1BB-L, CD137L, TNLG5A
NKIRs[b,e]			
CD96	1700109I12Rik, TACTILE	NECTIN2	AI325026, AI987993, CD112, HVEB, MPH, PRR2, Pvr, PVRL2, PVRR2, Pvs
		PVR	3830421F03Rik, CD155, D7Ertd458e, HVED, mE4, Necl-5, NECL5, PVS, Taa1, TAGE4

(*Continued on next page*)

Table 1. *Continued*

Receptor	Aliases	Ligands	Aliases
KIR2DL1 (*H. sapiens*)	CD158A, KIR-K64, KIR221, NKAT, NKAT-1, NKAT1, p58.1	HLA-B (*H. sapiens*)	AS, B-4901, HLAB
		HLA-C (*H. sapiens*)	D6S204, HLA-JY3, HLAC, HLC-C, MHC, PSORS1
KIR2DL2 (*H. sapiens*)	CD158B1, CD158b, NKAT-6, NKAT6, p58.2	HLA-A (*H. sapiens*)	HLAA
		HLA-C (*H. sapiens*)	D6S204, HLA-JY3, HLAC, HLC-C, MHC, PSORS1
KIR2DL3 (*H. sapiens*)	CD158B2, CD158b, GL183, KIR-023GB, KIR-K7b, KIR-K7c, KIR2DS5, KIRCL23, NKAT, NKAT2, NKAT2A, NKAT2B, p58	HLA-C (*H. sapiens*)	D6S204, HLA-JY3, HLAC, HLC-C, MHC, PSORS1
KIR2DL4 (*H. sapiens*)	CD158D, G9P, KIR-103AS, KIR-2DL4, KIR103, KIR103AS	HLA-G (*H. sapiens*)	MHC-G
KLRA (*M. musculus*)	Ly-49, Ly49	MHC Class I (*M. musculus*)	Not applicable
KLRC1	CD159A, NKG2, NKG2A	H2-T23 (*M. musculus*)	37b, 37c, H-2T23, H2-Qa1, Qa-1,Qa-1(b), Qa1, Qed-1, T18c, T18c(37), T23b, T23d
		HLA-E (*H. sapiens*)	HLA-6.2, QA1
LAG3	CD223, LAG-3, Ly66	MHC Class II	Not applicable
LILRB1 (*H. sapiens*)	CD85J, ILT-2, ILT2, LIR-1, LIR1, MIR-7, MIR7, PIR-B, PIRB	MHC Class I	Not applicable
		Viral UL18	Not applicable
PDCD1	CD279, hPD-1, hPD-I, hSLE1, Ly101, PD-1, PD1, SLEB2	CD274	B7-H, B7H1, PD-L1, PDCD1L1, PDCD1LG1, PDL1
		PDCD1LG2	bA574F11.2, B7DC, Btdc, CD273, PD-L2, PDCD1L2, PDL2
PVRIG	CD112R, C7orf15, Gm36869	NECTIN2	AI325026, AI987993, CD112, HVEB, MPH, PRR2, Pvr, PVRL2, PVRR2, Pvs
		PVR	3830421F03Rik, CD155, D7Ertd458e, HVED, mE4, Necl-5, NECL5, PVS, Taa1, TAGE4
TIGIT	VSIG9, VSTM3, WUCAM	NECTIN2	AI325026, AI987993, CD112, HVEB, MPH, PRR2, Pvr, PVRL2, PVRR2, Pvs
		PVR	3830421F03Rik, CD155, D7Ertd458e, HVED, mE4, Necl-5, NECL5, PVS, Taa1, TAGE4

[a]NKAR, NK cell activatory receptor.
[b]In *Mus musculus* and *Homo sapiens*, unless otherwise specified (source https://www.ncbi.nlm.nih.gov/gene).
[c]MHC, major histocompatibility complex.
[d]HA, hemagglutinin.
[e]NKIR, NK cell inhibitory receptor.

receptor 1 (NCR1, best known as NKp46) did not develop metastases 1 year after surgery (Pasero et al., 2016). Likewise, high interferon gamma (IFNG) production by circulating NK cells exposed to autologous dendritic cells (DCs), as well as expression of specific natural cytotoxicity triggering receptor 3 (NCR3, best known as NKp30) splicing variants (i.e., NKp30A or NKp30B rather than NKp30C), were found to have positive and independent prognostic value for long-term survival in patients with GIST under imatinib mesylate treatment (Delahaye et al., 2011; Menard et al., 2009) and patients with neuroblastoma upon induction chemotherapy (Semeraro et al., 2015). Finally, cytotoxic NK cells (defined as $CD56^{bright}CD16^{+}$ or $CD56^{dim}CD57^{+}KIR^{+}$ cells) have been documented in tumor-infiltrated lymph nodes from patients with melanoma, although no prognostic or predictive value has been attributed to this finding (Ali et al., 2014; Messaoudene et al., 2014). These observations suggest that NK cells mediate clinically relevant antimetastatic effects.

An extensive preclinical literature lends further support to this notion and elucidates (part of) the molecular mechanisms through which NK cells control metastasis. NK cells efficiently kill human and mouse metastatic melanoma cells *in vitro* and *in vivo*, reflecting the overexpression of ligands for natural cytotoxicity triggering receptor 2 (NCR2, best known as NKp44), NKp46, and CD226 (best known as DNAM-1) by the latter (Lakshmikanth et al., 2009). In line with this notion, the EMT has been linked to the upregulation of multiple NKG2D ligands, including MHC class I polypeptide-related sequence A (MICA), MICB, and various UL16 binding proteins (ULBPs), coupled to the loss of NK cell inhibitory receptor (NKIR) (Table 1) ligands such as cadherin 1 (CDH1, best known as E-cadherin), which renders potentially metastatic cells susceptible to recognition and elimination by NK cells (Li et al., 2009b; Lopez-Soto et al., 2013). Mice in which *Mcl1* (which codes for an inhibitor of regulated cell death from the Bcl-2 family) is selectively deleted from $NKp46^{+}$ cells are characterized by the complete absence of

Box 2. Control of Primary Solid Tumors by NK Cells

In 2001, pioneering work from the Schreiber lab demonstrated that *Rag2*$^{-/-}$ mice (which lack B and T lymphocytes but contain normal numbers of functional NK cells) are more susceptible to chemical carcinogenesis than their WT counterparts, and that carcinogen-driven tumors originating in *Rag2*$^{-/-}$ mice are rejected with high efficiency upon inoculation in syngeneic immunocompetent mice (Shankaran et al., 2001). This study provided a critical contribution to the establishment of modern tumor immunology as it demonstrated that the adaptive immune system counteracts early oncogenesis and shapes primary tumors that escape such a control (Shankaran et al., 2001). It is now clear that adaptive immunity plays a fundamental role in the control of oncogenesis and primary tumor progression, as well as in the response of established neoplasms to treatment (Galluzzi et al., 2015a). NK cells also appear to shape developing tumors in the absence of the adaptive immunity (O'Sullivan et al., 2012). Moreover, NK cells reportedly play a prominent role in the control of hematological malignancies, including multiple forms of leukemia and lymphoma (Ilander et al., 2017; Street et al., 2004). However, to what extent NK cells limit the growth of primary solid tumors in physiological settings remains a matter of debate.

In vitro, NK cells have been shown to kill cancer cell lines of different histological origin, virtually irrespective of derivation (primary tumors versus metastatic lesions), including malignant cells with stem-like features (Ames et al., 2015; Castriconi et al., 2009; Tallerico et al., 2013). Accordingly, *Klrk1*$^{-/-}$ mice develop transgene-driven lymphomas and prostate carcinomas at increased incidence compared with WT mice (Guerra et al., 2008). Moreover, transgene-driven overexpression of NKG2D ligands renders multiple murine cancer cells that normally form tumors upon inoculation into immunocompetent syngeneic hosts sensitive to rejection (Diefenbach et al., 2001). Moreover, selective depletion experiments demonstrated a role for NK cells in the control of methylcholanthrene-driven fibrosarcoma (Smyth et al., 2000, 2001). However, *Klrk1*$^{-/-}$ mice are equally sensitive to methylcholanthrene-driven carcinogenesis as their WT counterparts (Guerra et al., 2008) and develop diethylnitrosamine-induced hepatocellular carcinomas at a comparatively increased incidence (Sheppard et al., 2017). Furthermore, *Tlr3*$^{-/-}$ mice, which are characterized by NK cell hyporesponsiveness, are more sensitive to metastatic spread than WT mice, yet do not differ from WT mice in terms of spontaneous carcinogenesis (nor in terms of primary growth of subcutaneously inoculated murine melanoma, breast carcinoma, or colorectal carcinoma cells) (Guillerey et al., 2015). Finally, NK cells generally represent a minor fraction of the immunological infiltrate of most established solid tumors in humans and have limited prognostic value compared with other tumor-infiltrating lymphocytes such as CD8$^+$ CTLs or CD4$^+$CD25$^+$FOXP3$^+$ T_{REG} cells (Senovilla et al., 2012). Thus, the current literature appears to suggest that NK cells play a prominent role in the control of metastasis but the precise role of NK cells in the immunosurveillance of primary solid tumors remains to be investigated.

circulating and tissue-infiltrating NK cells (Sathe et al., 2014). Upon intravenous challenge with low numbers of B16F10 melanoma cells, these mice rapidly develop pulmonary, hepatic, and bone metastases that require euthanasia, while their wild-type (WT) counterparts virtually accumulate no metastases at all (Sathe et al., 2014). Such an increased sensitivity can be robustly rescued by the adoptive transfer of *Cish*$^{-/-}$ NK cells, which lack an endogenous inhibitor of interleukin 15 (IL-15)-driven proliferation and hence display a hyperactive phenotype (Delconte et al., 2016; Putz et al., 2017a). *In vivo* NK cell depletion with antibodies specific for killer cell lectin-like receptor subfamily B member 1C (KLRB1C, best known as NK1.1) or asialo ganglio-N-tetraosylceramide (asialo-GM1) markedly increases the metastatic burden in immunocompetent mice inoculated with syngeneic cancer cell lines via the subcutaneous, intravenous, intracardiac, intrasplenic, or orthotopic route (Diefenbach et al., 2001; Malladi et al., 2016; Spiegel et al., 2016). Likewise, mice in which cytotoxic NK cell effectors, including IFNG, perforin 1 (PRF1), and TNF superfamily member 10 (TNFSF10, best known as TRAIL) are inactivated upon gene knockout (at the whole-body level or in an NK-cell-restricted manner) or antibody-mediated antagonism are more sensitive to metastatic disease following challenge with cancer cells (administered subcutaneously, intrasplenically, via the tail vein, or via the portal vein) or with carcinogen-driven tumors than their control counterparts (Smyth et al., 1999; Street et al., 2001; Takeda et al., 2001). Similar findings have been obtained in *Ncr1*$^{-/-}$ mice (Glasner et al., 2012), *Cd226*$^{-/-}$ mice (Chan et al., 2010; Iguchi-Manaka et al., 2008), *Tlr3*$^{-/-}$ mice (which lack a Toll-like receptor involved in the acquisition of optimal NK cell competence) (Guillerey et al., 2015), and mice in which NKp46$^+$ NK cells are specifically ablated upon the conditional knockout of interleukin-2 receptor, gamma chain (*Il2rg*) (Merzoug et al., 2014). Moreover, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (which exhibit a profound defect in both adaptive and innate lymphoid immunity) are extremely sensitive to lung or hepatic colonization from cancer cells inoculated via the tail vein, which can be prevented by the adoptive transfer of purified DNAM-1$^+$ NK cells (Martinet et al., 2015) or NK cells lacking CD96 (a potent NKIR) (Chan et al., 2014). *Tbx21*$^{-/-}$ mice (which lack a transcription factor involved in the differentiation of NK cells and T lymphocytes best known as T-bet) challenged intravenously with syngeneic melanoma or colorectal carcinoma cells are also more susceptible to metastatic dissemination than their WT littermates, a detrimental phenotype that can be largely prevented by adoptive transfer of bulk NK cells as well as purified CD27^{low}KLRG1$^+$ NK cells (Malaise et al., 2014; Werneck et al., 2008). A similar protection can also be conferred by treating *Tbx21*$^{-/-}$ mice with IL-15 (which has potent mitogenic and cytoprotective effects on NK cells) pre-complexed with interleukin 15 receptor, alpha chain (IL-15RA), resulting in the development of KLRG1$^+$ NK cells expressing the T-bet family member eomesodermin (Malaise et al., 2014). Deletion of *Cblb* (coding for an E3 ubiquitin ligase that negatively regulates effector functions in multiple lymphocyte populations) also mediates robust NK-cell-dependent antimetastatic effects in mice (Paolino et al., 2014), as does the *Cd96*$^{-/-}$ genotype

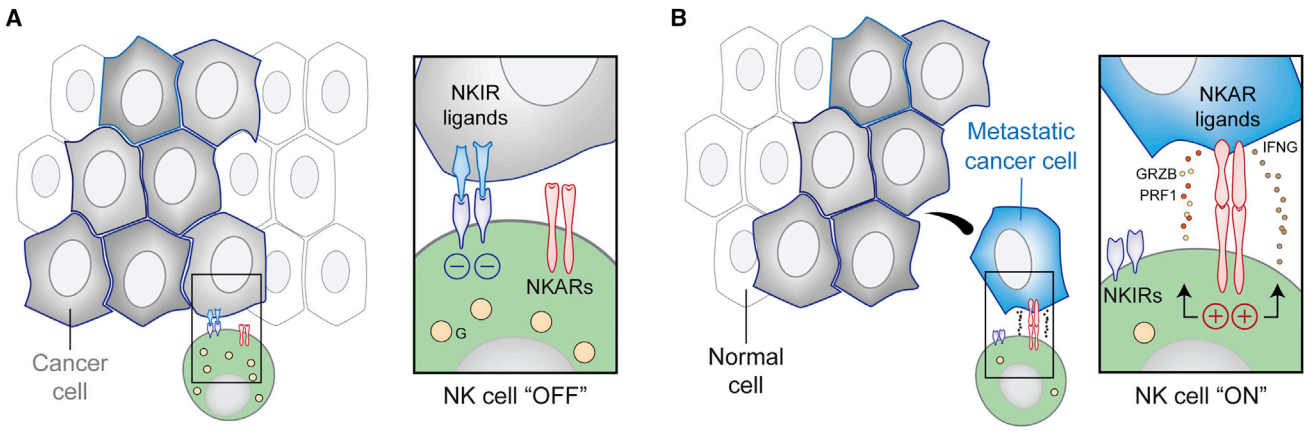

Figure 2. Immunosurveillance of Metastasis by NK Cells

(A) Normal cells and some malignant cells express multiple ligands for natural killer (NK) cell inhibitory receptors (NKIRs), hence robustly suppressing NK cell activation.

(B) However, potentially metastatic cancer cells, including cells that have undergone the epithelial-to-mesenchymal transition, lose the expression of NKIR ligands as they upregulate ligands for NK cell activatory receptors (NKARs). In this setting, NK cells become potently activated to release cytotoxic granules (G) that contain perforin 1 (PRF1) and granzyme B (GRZB), and to secrete interferon gamma (IFNG), hence mediating robust antimetastatic effects.

(Chan et al., 2014). Finally, mice in which NKp46$^+$ NK cells are engineered to express a constitutively active variant of transforming growth factor β receptor 1 (TGFBR1), which inhibits NK cell effector functions irrespective of TGFB1, have markedly enhanced susceptibility to metastasis (Viel et al., 2016). Thus, molecular defects or pharmacological agents that decrease NK cell number or activity have been extensively linked to increased metastatic colonization, whereas interventions that boost NK cell effector functions have been shown to provide robust protection against metastasis (at least in preclinical settings).

Trafficking is one of the major determinants of the antimetastatic functions of NK cells. Thus, *Spns2*$^{-/-}$ mice (which accumulate increased amounts of NK cells and CD8$^+$ CTLs within the lungs upon the absence of a sphingosine-1-phosphate transporter) exhibit decreased metastatic burden upon syngeneic cancer cell inoculation via the tail vein or directly to the spleen compared with WT mice (van der Weyden et al., 2017). Despite the fact that phosphatase and tensin homolog (PTEN) overexpression limits the cytotoxic activity of NK cells by disrupting immunological synapses (Briercheck et al., 2015), an increased metastatic burden has been documented in the lungs of mice carrying an NK-cell-specific *Pten* deletion upon inoculation of B16F10 melanoma cells via the tail vein, which was attributed to a profound alteration in NK cell trafficking to peripheral organs (Leong et al., 2015). Along similar lines, deletion of zinc finger E-box binding homeobox 2 (*Zeb2*, which codes for a transcription factor involved in NK cell development and homeostasis downstream of T-bet) from NKp46$^+$ NK cells reportedly reduces the pool of NK cells in multiple organs, rendering mice susceptible to metastatic lung colonization by syngeneic melanoma cells (van Helden et al., 2015). A similar sensitization has been observed in mice in which NKp46$^+$ NK cells were deleted of heparanase (*HPSE*) (Putz et al., 2017b). Intriguingly, physical exercise also appears to mediate antimetastatic effects in mice, owing to epinephrine- and interleukin 6 (IL-6)-dependent mobilization and redistribution of NK cells to the tumor bed (Pedersen et al., 2016).

Several signaling pathways are involved in the regulation of NK cell functions downstream of surface NK cell receptors, some of which are also harnessed by malignant cells for survival and proliferation. Thus, while pharmacological inhibition of the Janus kinase/Signal transducer and activator of transcription (JAK/STAT) axis has been proposed as a means to limit chemo- and radioresistance in multiple tumor types, including breast carcinoma (Britschgi et al., 2012), ruxolitinib (a licensed JAK1/JAK2 inhibitor) has been associated with impaired NK cell maturation and activity in patients with myeloproliferative neoplasms (who developed infectious complications to treatment) (Schonberg et al., 2015). Likewise, mice inoculated with mouse EO771 or 4T1 mammary carcinoma cells (via the tail vein, intracardiacally, or into the mammary fat pad) developed increased metastatic burden upon ruxolitinib administration (Bottos et al., 2016). However, specific members of the STAT protein family have differential effects on the NK cell compartment. For example, whereas STAT3 negatively regulates the expression of DNAM-1, IFNG, PRF1, and granzyme B (GRZB, another NK cell cytotoxic effector), hence limiting the ability of NK cells to control the dissemination of malignant cells delivered subcutaneously or intravenously (Gotthardt et al., 2014), STAT1, which is under negative control by cyclin dependent kinase 8 (CDK8), supports NK cell development and antimetastatic activity (Putz et al., 2013). Along similar lines, NK cell differentiation is severely compromised in mice upon conditional deletion of *Stat5* from the NKp46$^+$ compartment, resulting in exacerbated susceptibility to lung colonization after injection of B16F10 melanoma cells via the tail vein in spite of normal CTL responses (Eckelhart et al., 2011). Of note, the overexpression of the cytoprotective protein BCL2 rescues the developmental defect of *Stat5*$^{-/-}$ NK cells, but converts them into a pro-angiogenic cell population that supports tumor progression upon vascular endothelial growth factor A (VEGFA) synthesis (Gotthardt et al., 2016).

Taken together, these observations indicate that NK cells mediate robust antimetastatic effects that are subjected to several layers of regulation (Figure 2), some of which are actively

Box 3. Type I IFN Signaling in Anticancer Immune Responses

Besides exerting major antiviral effects (McNab et al., 2015), type I IFNs, which in humans comprise interferon beta 1 (IFNB1), interferon omega 1 (IFNW1), and multiple variants of interferon alpha (IFNA), play a fundamental role in the activation of innate and adaptive immune responses specific for malignant cells (Zitvogel et al., 2015). Type I IFNs generally operate upon binding to heterodimeric plasma membrane receptors composed of interferon alpha and beta receptor subunit 1 (IFNAR1) and IFNAR2, resulting in the activation of a JAK1- and tyrosine kinase 2 (TYK2)-driven transcriptional response, depending on various members of the STAT protein family (Ivashkiv and Donlin, 2014). A large panel of type I IFN-stimulated genes has been characterized, largely underlying the prominent antiviral and immunostimulatory functions of type I IFN signaling (Schneider et al., 2014). Of note, virtually all cells can produce type I IFNs in response to viral infection as well as to many other conditions that activate cytosolic pattern recognition receptors (PRRs), including some forms of chemotherapy and radiation therapy (Sistigu et al., 2014; Vanpouille-Box et al., 2017). Indeed, cancer cells undergoing immunogenic cell death—a variant of cell death that is sufficient to activate an adaptive immune response in immunocompetent syngeneic hosts (Galluzzi et al., 2017b)—accumulate double-stranded RNA and/or DNA in the cytoplasm, resulting in type I IFN secretion upon PRR signaling (Sistigu et al., 2014; Vanpouille-Box et al., 2017). Importantly, type I IFNs not only support anticancer immunity as they stimulate multiple immune cell effectors, including NK cells (Zitvogel et al., 2015), but also as they initiate cancer cell-intrinsic responses that ultimately favor T cell infiltration into the tumor bed (Sistigu et al., 2014). Further corroborating the importance of type I IFN signaling for tumor-targeting immune responses, progressing neoplasms often exhibit epigenetic or genetic alterations that result in limited type I IFN secretion or IFNAR1 downregulation (Bidwell et al., 2012; Katlinski et al., 2017). Multiple PRR agonists that potently stimulate type I IFN production are currently being evaluated in the clinic for their ability to boost the efficacy of chemo-, radio-, and immunotherapy (Iribarren et al., 2016).

harnessed by malignant cells to escape immunosurveillance, as discussed below.

Metastatic Subversion of NK Cell Surveillance

Malignant cells can harness a wide array of strategies to subvert recognition and elimination by NK cells, colonize distant sites and form macrometastases. On the one hand, cancer cells progressing along the metastatic cascade tend to acquire intrinsic features that limit their likelihood to be recognized or killed by NK cells. Such features encompass (but are not limited to) (1) downregulation of NKAR ligands, mostly owing to reversible epigenetic changes that involve histone deacetylation (Armeanu et al., 2005; Lopez-Soto et al., 2009); (2) upregulation of NKIR ligands, such as CD274 (best known as PD-L1) and major histocompatibility complex, class I, G (HLA-G) (Benson et al., 2010; Carosella et al., 2015); and (3) downregulation of FAS, a death receptor through which NK cells can kill their targets (Maecker et al., 2002). On the other hand, progressing malignant cells secrete numerous mediators that directly or indirectly suppress NK cell immunosurveillance. In particular, cancer cells acquire the ability to generate soluble variants of NKG2D and NKp30 ligands, which operate as molecular decoys and trigger NKAR downregulation (Schlecker et al., 2014; Zhang et al., 2015a). This process generally relies on the secretion and hyperactivation of metalloproteinases, including ADAM metallopeptidase domain 10 (ADAM10), ADAM17, and matrix metallopeptidase 14 (MMP14) (Chitadze et al., 2013; Liu et al., 2010a). Accordingly, high circulating levels of soluble NKAR ligands have been found to correlate with advanced disease stage and dismal prognosis in multiple cohorts of patients with cancer (Paschen et al., 2009; Yamaguchi et al., 2012). However, recent data indicate that soluble ULBP1 (also known as MULT1) may *de facto* boost the activity of NK cells, perhaps owing to its capacity to limit functional exhaustion linked to chronic NKG2D signaling (at least in mice) (Deng et al., 2015). The clinical relevance of this process remains to be elucidated. Finally, the secretome of progressing tumors contains little amounts of immunostimulatory and NK cell-activating molecules such as type I interferons (IFNs) (Bidwell et al., 2012) and IL-15 (Mlecnik et al., 2014) (Box 3), as it becomes ever more enriched in immunosuppressive and NK cell-inhibiting factors, including interleukin 10 (IL-10) and TGFB1 (Bruno et al., 2013; Tang et al., 2017). These agents can act directly on NK cells to reduce their cytotoxic functions (Kopp et al., 2009) or to convert them into cells that promote metastatic spread by virtue of a pro-angiogenic effect (Bruno et al., 2013). In addition, IL-10, TGFB1, and other immunosuppressive factors produced by progressing tumors can recruit immune cells that inhibit NK cell effector functions, including T_{REG} cells (Pedroza-Pacheco et al., 2013), MDSCs (Li et al., 2009a), $CD11b^{+}Ly6G^{+}$ neutrophils (Sceneay et al., 2012; Spiegel et al., 2016), and indoleamine 2,3-dioxygenase 1 (IDO1)-expressing DCs (Della Chiesa et al., 2006). Interestingly, platelets also favor the escape of malignant cells from NK cell immunosurveillance, via at least three distinct mechanisms as they (1) physically shield cancer cells from recognition (Palumbo et al., 2005), (2) produce TGFB1 (Kopp et al., 2009), and (3) transfer MHC class I molecules (which inhibit NK cells via NKIRs) to cancer cells to establish a state of pseudo-self (Placke et al., 2012). In concert with this notion, tumor-infiltrating and circulating NK cells from patients with cancer display reduced levels of NKARs and/or increased levels of NKIRs compared with NK cells from healthy donors, and such an effect is often more pronounced in patients with metastatic disease (Pasero et al., 2016; Platonova et al., 2011). Of note, some of these processes are relevant not only for malignant cells actively progressing along the metastatic cascade but also for dormant DTCs. As an example, dormancy often impinges on WNT signaling suppression by dickkopf WNT signaling pathway inhibitor 1 (DKK1), which results in downregulation of ULBPs and hence escape from NK cell immunosurveillance (Malladi et al., 2016).

Additional features of the tumor microenvironment inhibit the antimetastatic functions of NK cells, including hypoxia and stromal inflammation. The hydrolysis of extracellular ATP into AMP, which is catalyzed by ectonucleoside triphosphate

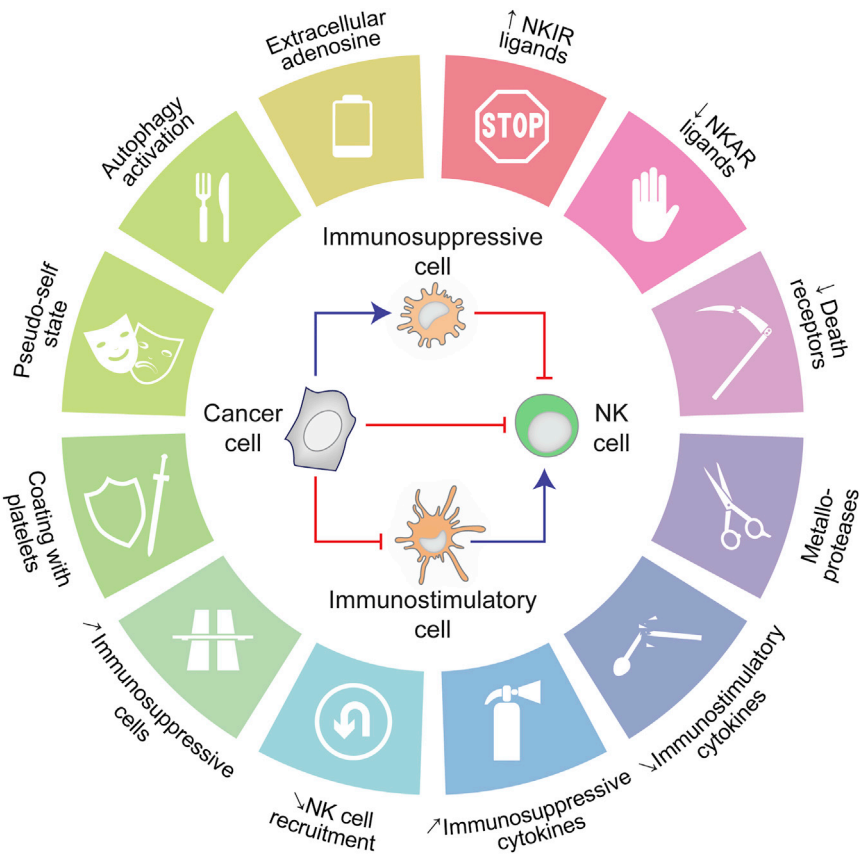

Figure 3. Mechanisms of NK Cell Evasion by Cancer Cells

Malignant cells avoid recognition and elimination by natural killer (NK) cells via multiple mechanisms that operate directly on NK cells, upon the activation of immunosuppressive cells, or upon the inhibition of immunostimulatory cells. These mechanisms include (1) upregulation (↑) of NK cell inhibitory receptor (NKIR) ligands; (2) downregulation (↓) of NK cell activatory receptor (NKAR) ligands; (3) downregulation of death receptors; (4) secretion of metalloproteinases that generate soluble NKAR ligands; (5) decreased (↘) secretion of immunostimulatory cytokines; (6) increased (↗) secretion of immunosuppressive cytokines; (7) decreased recruitment of NK cells; (8) increased recruitment of immune cells with immunosuppressive functions; (9) physical coating with platelets; (10) acquisition of a pseudo-self state; (11) hypoxia-dependent extracellular adenosine synthesis; and (12) hypoxia-dependent autophagy activation.

diphosphohydrolase 1 (ENTPD1, best known as CD39), and the subsequent conversion of AMP into adenosine, which is mediated by 5′-nucleotidase ecto (NT5E, best known as CD73), is favored by hypoxia via HIF-1 (Young et al., 2014). Adenosine exerts robust immunosuppressive effects on NK cells and other immune effector cells via autologous or heterologous adenosine A2a receptor (ADORA2A) signaling (Beavis et al., 2013; Cekic et al., 2014). Accordingly, respiratory hyperoxia (60% oxygen) facilitates the rejection of pulmonary metastases and prolongs the survival of mice bearing orthotopic breast carcinomas, an effect that fully depends on $CD8^+$ CTLs and NK cells (Hatfield et al., 2015). Inhibition of CD39 (which is highly expressed by T_{REG} cells) with polyoxometalate-1, as well as *Entpd1* deletion from the bone marrow, impairs the hepatic colonization of B16F10 melanoma and MC38 colon carcinoma cells administered to mice via the portal circulation (Sun et al., 2010). Likewise, mouse cancer cells overexpressing CD73 spontaneously (such as 4T1.2 mammary carcinoma cells) or upon genetic engineering (such as B16F10 melanoma cells transduced with a CD73-expressing retrovirus) exhibit elevated metastatic potential, which can be limited by restoration of NK cell functions by an ADORA2A antagonist (Beavis et al., 2013). The antimetastatic activity of ADORA2A antagonists can be further increased upon co-administration of monoclonal antibodies (mAbs) specific for programmed cell death 1 (PDCD1, a co-inhibitory receptor best known as PD-1) (Mittal et al., 2014) or CD73 (Young et al., 2016). This latter observation implies that CD73 and ADORA2A do not mediate NK cell inhibitory effects precisely via the same pathway. The relative contribution of ADORA2A signaling in myeloid cells to NK cell suppression remains to be elucidated.

Hypoxia also favors the escape of potentially metastatic cells from NK cell immunosurveillance by favoring the release of exosomes that contain TGFB1 and miRNAs targeting NKG2D (Berchem et al., 2016), and by activating autophagy, which limits the sensitivity of cancer cells to GZMB-dependent lysis (Baginska et al., 2013). Finally, stromal inflammation appears to have a context-dependent impact on the control of metastasis by NK cells. Thus, *Nlrp3*$^{-/-}$ mice (which lack a critical component of the inflammasome) exhibit reduced amounts of pulmonary metastases upon intravenous or orthotopic challenge with syngeneic melanoma, prostate, and breast carcinoma cells (Chow et al., 2012), but increased hepatic metastases upon intrasplenic inoculation with syngeneic colorectal carcinoma cells (Dupaul-Chicoine et al., 2015). These apparently discrepant observations have been explained by the accrued recruitment of IFNG-producing NK cells in *Nlrp3*$^{-/-}$ lungs (Chow et al., 2012), and by the reduced expression of FAS ligand (FASLG) on the surface of NK cells infiltrating *Nlrp3*$^{-/-}$ livers (Dupaul-Chicoine et al., 2015). Thus, stromal inflammation can also subvert NK cell immunosurveillance, at least in some organs.

Taken together, these observations exemplify the mechanisms through which potentially metastatic cancer cells avoid recognition and elimination by NK cells (Figure 3).

NK Cell Immunotherapy in the Management of Metastasis

Owing to the profound phenotypic, biochemical, and metabolic alterations that accompany metastasis, therapeutic interventions that are relatively efficient on primary neoplasms, including agents specifically directed against oncogenic drivers, are often unable to mediate robust antimetastatic effects (Klein, 2013). Thus, metastatic disease has long been conceived as a setting

Table 2. Examples of Therapeutic Strategies Harnessing NK Cells for Metastasis Control

Strategy	Rationale	Status	Notes	References
Anti-TNFRSF9 mAb[a]	immunostimulation	clinical	various CD137 agonistic mAbs currently tested in the clinic potentially boost NK cell effector functions	(Muntasell et al., 2017)
Anti-CD96 mAb	checkpoint inhibition	preclinical	Mediates antimetastatic effects *in vivo* that depend on NK cells	(Blake et al., 2016)
Catumaxomab	anti-CD3/EPCAM bispecific mAb	approved	stimulates T cell responses and NK cell-mediated ADCC[b] against EPCAM$^+$ cancer cells	(Seimetz et al., 2010)
Cetuximab	Anti-EGFR mAb	approved	inhibits EGFR-dependent cancer cell proliferation and drives NK cell-mediated ADCC	(Weiner et al., 2010)
Chemotherapy	upregulation of NKAR[c] ligands	approved	intracellular damage caused by chemotherapy promotes expression or exposure of NKAR ligands	(Galluzzi et al., 2015a)
CPI-444	ADORA2A antagonist	clinical	ADORA2A blockage mediates robust antimetastatic effects	(Beavis et al., 2013; Cekic et al., 2014)
Epacadostat	IDO1 inhibitor	clinical	Improves NK cell effector functions	(Liu et al., 2010b)
Immune checkpoint blockers	checkpoint inhibition	approved	multiple mAbs specific for CTLA4, PD-1, and PD-L1 may stimulate NK cell effector functions	(Buque et al., 2015)
Indoximod	IDO1 inhibitor	clinical	improves NK cell effector functions	(Liu et al., 2010b)
IL2	immunostimulation	approved	stimulates CTL[d] and NK cell expansion, but is associated with toxicity and regulatory T cell expansion	(Vacchelli et al., 2016)
IL12	immunostimulation	clinical	relatively disappointing clinical profile	(Lasek et al., 2014)
IL15	immunostimulation	clinical	safe and associated with improved CTL and NK cell effector functions in patients with cancer	(Conlon et al., 2015)
IPH2101	anti-KIR2D mAb	clinical	safe, but associated with limited efficacy, perhaps linked to unexpected NK cell inhibitory activity	(Benson et al., 2012; Benson et al., 2015; Korde et al., 2014)
IPH4301	anti-MICA/MICB mAb	preclinical	boosts NK cell effector functions *in vitro* and *in vivo*, in models of transplantable and endogenous tumors	(Morel et al., 2016)
LDC1267	TAM receptor inhibitor	preclinical	mediates antimetastatic effects *in vivo* that depend on NK cells	(Paolino et al., 2014)
Lenalidomide, pomalidomide	immunomodulatory drugs	approved	target multiple myeloma cells as they mediate immunostimulatory effects that involves NK cells	(Reddy et al., 2008; Wu et al., 2008)
Lirilumab	anti-KIR2D mAb	clinical	enhances rituximab-driven ADCC in transgenic and syngeneic murine lymphoma models	(Kohrt et al., 2014)
MEDI9447	anti-NT5E mAb	clinical	currently tested in combination with a PD-L1-targeting mAb in patients with non-small-cell lung carcinoma	(Hay et al., 2016)
Monalizumab	anti-KLRC1 mAb	clinical	boosts NK cell effector functions against cells from patients with chronic lymphocytic leukemia	(McWilliams et al., 2016)
NK cell adoptive cell transfer	NK cell expansion	clinical	feasible and safe, but associated with limited efficacy	(Aranda et al., 2015)
PFB-709	ADORA2A antagonist	clinical	ADORA2A blockage mediates robust antimetastatic effects	(Beavis et al., 2013; Cekic et al., 2014)
Polyoxometalate-1	ENTPD1 inhibitor	preclinical	mediates antimetastatic effects *in vivo*	(Sun et al., 2010)

(*Continued on next page*)

Table 2. *Continued*

Strategy	Rationale	Status	Notes	References
Radiation therapy	upregulation of NKAR ligands	approved	intracellular damage driven by radiotherapy promotes expression or exposure of NKAR ligands	(Galluzzi et al., 2015a)
Rituximab	anti-CD20 mAb	approved	Triggers apoptosis in $CD20^+$ cancer cells and NK cell-mediated ADCC	(Weiner et al., 2010)
Trastuzumab	anti-ERBB2 mAb	approved	inhibits ERBB2-dependent cancer cell proliferation and drives NK cell-mediated ADCC	(Weiner et al., 2010)
Vemurafenib	$BRAF^{V600E}$ inhibitor	approved	inhibits $BRAF^{V600E}$ signaling in melanoma cells and promotes tumor infiltration by NK cells	(Ferrari de Andrade et al., 2014; Knight et al., 2013)
Warfarin	TAM receptor inhibitor	approved[e]	Mediates antimetastatic effects *in vivo* that depend on NK cells	(Paolino et al., 2014)

[a]mAb, monoclonal antibody.
[b]ADCC, antibody-dependent cellular cytotoxicity.
[c]NKAR, NK cell activatory receptor.
[d]CTL, cytotoxic T lymphocyte.
[e]For indications other than cancer.

amenable to palliative, as opposed to therapeutic, interventions. However, recent data suggest that the tumor microenvironment—in its endothelial, stromal, and immunological components—is a promising target for the development of therapies directed against metastatic disease (Ghajar, 2015). In particular, multiple strategies aimed at the (re)activation of NK cell immunosurveillance have been shown to mediate robust therapeutic effects in preclinical models of metastatic dissemination. In addition, at least part of the efficacy of some therapeutic agents currently employed in the clinic may stem from the restoration of NK-cell-dependent immune responses against metastatic or potentially metastatic malignant cells (Table 2).

Pharmacological inhibition of TAM tyrosine kinase receptors (which are substrates for ubiquitination by CLBL) with LDC1267, which specifically inhibits TYRO3, AXL, and MERTK, mediates robust antimetastatic effects in mice challenged with B16F10 melanoma cells, an effect that is completely abrogated upon NK cell depletion with an antibody specific for NK1.1 (Paolino et al., 2014). Intriguingly, warfarin (a commonly employed anticoagulant that is well known for its antimetastatic effects) (McCulloch and George, 1989; Ryan et al., 1968) also appears to inhibit TAM receptors, which may account (at least in part) for its ability to limit metastatic dissemination (Paolino et al., 2014). At least theoretically, several chemotherapeutics and radiation therapy may favor NK cell immunosurveillance by promoting the exposure of NKAR ligands on the surface of malignant cells (Galluzzi et al., 2015a), a process that is often subverted by cancer-derived matrix metalloproteinases. Accordingly, the broad-spectrum metalloproteinase inhibitor marimastat has been successfully employed *in vitro* to enhance the recognition and elimination of malignant cells exposed to chemotherapy by NK cells (Zingoni et al., 2015). However, it remains to be elucidated whether marimastat mediates antimetastatic effects *in vivo*.

Over the past two decades, multiple mAbs have been approved for use as targeted anticancer agents in the clinic, including the CD20-targeting molecule rituximab (currently employed for the treatment of multiple hematological neoplasms), the epidermal growth factor receptor (EGFR)-targeting molecule cetuximab (currently used against colorectal carcinoma and head and neck cancer) and the erb-b2 receptor tyrosine kinase 2 (ERBB2)-targeting molecule trastuzumab (currently employed in patients with $ERBB2^+$ breast carcinoma) (Gotwals et al., 2017). Interestingly, the efficacy of these agents is not restricted to their ability to inhibit trophic signaling pathways that are required for the survival and proliferation of cancer cells but also involves antibody-dependent cellular cytotoxicity (ADCC), one of the main NK cell effector mechanisms (Box 1) (Weiner et al., 2010). Although the efficacy of ADCC may considerably suffer from antibody-intrinsic features (e.g., isotype, pattern of glycosylation) as well as from the existence of *FCGR3A* polymorphisms that reduce the ability of NK cells to bind Fc fragments (Weiner et al., 2010), considerable progress has been made in the development of second-generation antibodies with improved ADCC potential (Junttila et al., 2010). In addition, so-called bispecific trifunctional antibodies (which have dual specificity while preserving ADCC potential) may be harnessed to physically bridge malignant cells and immune effectors, including $CD8^+$ CTLs and NK cells. The most successful example of this class of drugs is the CD3- and epithelial cell adhesion molecule (EPCAM)-targeting agent catumaxomab, which is now approved in the European Union for the treatment of patients with malignant ascites from EPCAM-positive tumors (Seimetz et al., 2010).

A more direct approach to unleash the antimetastatic potential of NK cells consists of the use of mAbs that block NKIRs or activate NKARs (Box 1). Several antibodies specific for various killer cell immunoglobulin like receptor, two Ig domain (KIR2D) family members reportedly exert robust therapeutic effects in preclinical models of lymphoma and myeloma (Benson et al., 2011; Kohrt et al., 2014). Among these agents, IPH2101 has been tested in multiple cohorts of patients with myeloma (Benson

et al., 2012, 2015; Korde et al., 2014), with no safety concerns but relatively paradoxical results, possibly due to an unexpected NK cell inhibitory effect (Carlsten et al., 2016). Lirilumab, which resembles IPH2101 in specificity, has been shown to enhance rituximab-driven ADCC in transgenic and syngeneic murine lymphoma models (Kohrt et al., 2014) and is currently being evaluated in clinical trials enrolling patients with hematological malignancies (source https://clinicaltrials.gov). Monalizumab, a mAb specific for the NKIR killer cell lectin-like receptor C1 (KLRC1), has been shown to boost NK cell effector functions against cells from patients with chronic lymphocytic leukemia (McWilliams et al., 2016) and is now being tested for the treatment of this and other hematological tumors (source https://clinicaltrials.gov). The MICA- and MICB-targeting mAb IPH4301 reportedly mediates potent NK cell-stimulatory effects *in vitro* and *in vivo*, in the context of transplantable and endogenous tumors, as it prevents NKG2D downregulation on NK cells (Morel et al., 2016). Blocking CD96 with a mAb has been shown to inhibit experimental metastases in three different tumor models, an effect that depended on NK cells, DNAM-1, and IFNG (Blake et al., 2016). However, neither IPH4301 nor any CD96-blocking mAbs are currently being tested in the clinics. Yet another candidate for the (re)activation of NK cell immunosurveillance is the NKIR T cell immunoreceptor with Ig and ITIM domains (TIGIT) (Muntasell et al., 2017). *Tigit*$^{-/-}$ mice are indeed cooperatively sensitive to CD96 blockade in terms of metastasis suppression (Blake et al., 2016). The clinical potential of TIGIT-targeting agents for metastasis control remains unexplored. Finally, (at least some subsets of) NK cells express multiple receptors that have been extensively characterized for their ability to regulate CTL-mediated anticancer responses (Muntasell et al., 2017). These include (but are not limited to) tumor necrosis factor receptor superfamily, member 9 (TNFRSF9, best known as CD137), which operates as a co-stimulatory molecule, as well as PD-1 and cytotoxic T lymphocyte-associated protein 4 (CTLA4), which mediate co-inhibitory effects (Muntasell et al., 2017). Several mAbs have been developed to modulate the signaling pathways that emanate from these receptors, including the US FDA-approved checkpoint blockers targeting PD-1 (e.g., nivolumab, pembrolizumab) or PD-L1 (e.g., atezolizumab, avelumab) (Buque et al., 2015). Accumulating preclinical data suggest that part of the therapeutic effects of these agents may indeed stem from the (re)activation of NK cell immunosurveillance (Muntasell et al., 2017). In this setting, a particularly promising strategy may consist of the dual blockade of one NKIR (such as CD96) and one co-inhibitory receptor (such as CTLA4 or PD-1) (Blake et al., 2016). Additional studies, however, are required to elucidate the actual antimetastatic potential of checkpoint blockers in humans. Indeed, although some patients with metastatic cancer receiving CTLA4- or PD-1-targeting mAbs experience complete disease eradication (Topalian et al., 2015), the contribution of NK cells to this clinical outcome is unclear.

NK cell effector functions can be boosted with relatively nonspecific immunostimulatory agents, including (1) cytokines such as interleukin 2 (IL-2), interleukin 12 (IL-12), or IL-15; (2) immunomodulatory drugs, such as lenalidomide and pomalidomide; (3) IDO1 inhibitors; and (4) inhibitors of adenosinergic signaling (Guillerey et al., 2016). IL-2 has been used with some success for the treatment of renal cell carcinoma or melanoma, but its use is declining owing to considerable side effects as well as the ability of IL-2 to drive the expansion of immunosuppressive T_{REG} cells (Vacchelli et al., 2016). Novel forms of IL-2 specifically targeted to CTLs and NK cells are currently being developed (Levin et al., 2012). Promising preclinical data stimulated an intense clinical investigation on the possibility of using recombinant IL12 as an immunostimulatory agent for cancer therapy, with rather disappointing results (Lasek et al., 2014). Lately, renewed clinical interest has been generated by the use of IL-12-coding plasmids or IL-12 variants specifically targeted to the tumor microenvironment (Lasek et al., 2014). Recent data from the first-in-human clinical trial testing recombinant human IL-15 suggest that this cytokine can be safely administered to patients with renal cell carcinoma or melanoma, promoting the activation of multiple lymphocytic populations including NK cells, and may therefore have therapeutic activity (Conlon et al., 2015). Besides directly targeting multiple myeloma cells (Semeraro and Galluzzi, 2014), both lenalidomide and pomalidomide (which are currently licensed for this indication) mediate broad-spectrum immunostimulatory activities that involve improved NK cell effector functions, notably ADCC (Reddy et al., 2008; Wu et al., 2008), and are currently being investigated in the clinic for the treatment of other hematological malignancies (Semeraro et al., 2013). Indoximod and epacadostat, two relatively non-selective inhibitors of IDO1 that boost NK cell activity (Liu et al., 2010b), are being tested in combination with chemo-, radio- or immunotherapy for use in patients with various neoplasms (Buque et al., 2016). Finally, small chemicals or mAbs that inhibit the catalytic activity of CD39 or CD73, or antagonize ADORA2A receptors are at various stages of development (Buque et al., 2016). In particular, the CD73-specific antibody MEDI9447 is currently being evaluated (alone or in combination with a PD-L1-targeting agent) in patients with advanced solid tumors (Hay et al., 2016), whereas the ADORA2A antagonists CPI-444 and PBF-509 (Buque et al., 2016) are being tested together with anti-PD-L1 or anti-PD-1 antibodies in patients with non-small-cell lung carcinoma (source https://clinicaltrials.gov).

Agents that promote tumor infiltration by NK cells, such as the BRAF inhibitor vemurafenib (which is currently approved for use in melanoma patients with *BRAF* mutations), may also exert antimetastatic effects (Knight et al., 2013). Additional studies are required to elucidate whether part of the therapeutic activity of vemurafenib actually originates from the (re)activation of NK cell immunosurveillance in humans. Finally, the possibility of using NK cells for adoptive cell transfer procedures has been investigated in several patient cohorts (Aranda et al., 2015). Most of these studies involved either autologous or haploidentical unmodified NK cells expanded *ex vivo* from peripheral blood mononuclear cells, an approach that is feasible and generally safe, although associated with limited clinical efficacy (Aranda et al., 2015). More recently, efforts have been dedicated to the creation of NK cells expressing so-called chimeric antigen receptors (CARs), which endow them with antigen-specific MHC-unrestricted cytotoxic potential (Han et al., 2015). The clinical safety and efficacy of CAR-expressing NK cells remains to be elucidated.

In summary, although it is difficult to predict which of the aforementioned approaches (if any) will be successful in the clinic, NK

Table 3. Common Models for Assessing the Antimetastatic Effect of NK Cells *In Vivo*

Model	Type	Advantages	Disadvantages
Intracardiac injection	experimental	allows for metastatic dissemination at multiple sites suitable for cell lines with limited metastatic potential	requires anesthesia not suitable for the study of early invasion and intravasation heterogeneous delivery of cancer cells to different organs
Intrasplenic injection	experimental	preferential metastatic dissemination to the liver allows for visual assessment of the metastatic burden	requires anesthesia and surgery not suitable for the study of early invasion and intravasation generally requires splenectomy (to avoid death from splenic complications)
Portal vein inoculation	experimental	preferential metastatic dissemination to the liver allows for visual assessment of the metastatic burden	requires anesthesia and surgery not suitable for the study of early invasion and intravasation
Tail vein inoculation	experimental	simple procedure suitable for cell lines with limited metastatic potential may allow for visual assessment of the metastatic burden	generally associated with lethal lung colonization (poorly suitable to assess metastases in other organs) not suitable for the study of early invasion and intravasation
Orthotopic tumor establishment	spontaneous	potentially recapitulates all steps of the metastatic cascade potentially reproduces the pattern of dissemination observed in humans (especially for breast tumors) allows for the evaluation of primary tumor growth	requires anesthesia and surgery variable efficacy of metastatic dissemination (highly variable with cell type) primary tumor removal may be needed for macrometastases to develop (ethical endpoint on primary lesion)
Subcutaneous tumor establishment	spontaneous	simple procedure potentially recapitulates all steps of the metastatic cascade allows for the evaluation of primary tumor growth	setting of limited pathophysiological relevance low efficacy of metastatic dissemination (highly variable with cell type) primary tumor removal may be needed for macrometastases to develop (ethical endpoint on primary lesion)

cells represent a promising target for the development of therapeutic strategies specific for metastatic disease.

Concluding Remarks

Data accumulating over the past three decades have elucidated several processes that (at least theoretically) can be targeted to prevent or treat metastatic cancer lesions. For a long time, however, efforts have been focused on inhibiting cancer-cell-intrinsic or -dependent processes, such as the EMT and vascular alterations (Steeg, 2016). Along with the realization of the key role that the innate and adaptive arms of the immune system play in oncogenesis, tumor progression, and response to treatment (Galluzzi et al., 2015a), renewed interest has been generated by the possibility of harnessing the effector functions of NK cells against metastatic disease (Muntasell et al., 2017). Further corroborating the potential of this approach, it has been suggested that cancer-cell-intrinsic processes like the EMT might play a less relevant role in metastatic spread than initially thought (Fischer et al., 2015; Zheng et al., 2015). The precise reasons why NK cells appear to exert a preferential control on metastatic dissemination (rather than, or in addition to, primary tumor growth) remain obscure. It is tempting to speculate, yet remains to be formally elucidated, that poorly antigenic cancer cells escaping T cell immunosurveillance may become particularly vulnerable to innate immune mechanisms as they abandon the primary tumor microenvironment (which is generally immunosuppressive) and progress through the metastatic cascade. Moreover, the actual impact of NK cells on early oncogenesis may have been (at least partially) underestimated (Box 1), perhaps linked to the methodological problems associated with monitoring biological processes involving a few cells.

Each of the models commonly employed to study the ability of NK cells to control metastatic disease is associated with specific assets and limitations (Table 3). On the one hand, the direct delivery of cancer cells into the tail vein, the portal circulation, the heart, or the spleen (generally referred to as "experimental models") is advantageous as it allows for the efficient and rapid formation of metastatic lesions in one or multiple organs, but it is not suitable as a model of early metastatic dissemination and can be methodologically cumbersome. On the other hand, the establishment of subcutaneous or orthotopic tumors that subsequently generate metastases (generally referred to as "spontaneous models") would be ideal to reproduce all the steps of the metastatic cascade. However, most cancer cell lines have a relatively low metastatic potential, and primary lesions often reach ethically unacceptable dimensions well before the development of macrometastases. In some cases, the removal of primary tumors may circumvent such an issue and allows for metastatic disease to manifest (Table 3). Likewise, each of the methodological approaches most commonly employed to

deplete or inhibit NK cells has specific limitations. For instance, NK1.1-targeting antibodies deplete NK cells as well as NKT cells (a subset of lymphoid cells expressing NK cell markers and the αβ T cell receptor) (Smyth et al., 2001), anti-asialo-GM1 antibodies efficiently deplete NK cells as well as monocytes, macrophages, and basophils (Dunn et al., 2005; Nishikado et al., 2011), and pharmacological as well as genetic interventions targeting IFNG or PRF1 have a major impact on the cytotoxicity of both NK cells and CTLs (Dunn et al., 2005). The deletion of *Ncr1* and all other genetic approaches targeting NKp46^{+} cells are not fully specific for NK cells as they affect type 1 ILCs as well as a subset of type 3 ILCs (Walker et al., 2013). Finally, *Rag2*$^{-/-}$*; Il2rg*$^{-/-}$ mice lack multiple lymphoid cell populations other than NK cells, notably T lymphocytes and B lymphocytes (Shultz et al., 2007). It is paramount to take these and other limitations of the models currently employed to study the control of metastases by NK cells into careful account when data are interpreted.

Several aspects of NK cell immunosurveillance require further in-depth investigation. First, to what extent (if any) does the net antimetastatic activity of NK cells result from an interaction with the myeloid cell compartment and/or CTLs? Several reports suggest that multiple myeloid cell populations, including DCs, have a prominent impact on NK cell effector functions (Fernandez et al., 1999; O'Sullivan et al., 2012; Polansky et al., 2016). Moreover, NK cells are known for their ability to kill immature DCs, which express low amounts of MHC class I molecules, while sparing their mature counterparts (Ferlazzo and Moretta, 2014). Such an editing of the DC pool generally supports CTL priming and the establishment of tumor-targeting immunity (as opposed to tolerance) (Morandi et al., 2012). That said, NK cells have also been suggested to inhibit the ability of DCs to perform priming in a PD-1-/PD-L1-dependent manner (Iraolagoitia et al., 2016). Thus, the potential cooperation between NK cells and CTLs in the control of metastatic dissemination remains poorly investigated. In addition, it remains to be firmly established whether NK cells can kill tumor-specific CD4^{+} and/or CTLs as they do in the context of viral infection (Andrews et al., 2010; Waggoner et al., 2010, 2011). In a mouse lymphoma model that fails to directly activate NK cells (owing to robust expression of MHC class I molecules), NK cell depletion with an antibody specific for NK1.1 boosted CTL-dependent anti-tumor immunity, but this effect was mostly related to increased priming (Barber et al., 2007). More recently, an intratumoral CD56^{+}CD3^{-} cell population has been shown to potently suppress the expansion of tumor-infiltrating CTLs in an NKp46-dependent manner (Crome et al., 2017). However, neither the precise identity of these CD56^{+}CD3^{-} ILCs nor their ability to kill CTLs has been established.

Second, at which step(s) of the metastatic cascade do NK cells operate to control the growth of clinically relevant macrometastases? The vast majority of the studies published so far relied on macrometastatic burden as a single functional endpoint and hence were intrinsically unable to discriminate between antimetastatic effects related to the control of primary tumor growth, local invasion, intravasation, CTC persistence, extravasation, distant invasion, DTC persistence, reactivation, or macrometastatic growth. Refined animal models that allow for the precise monitoring of metastatic disease in each of its steps are urgently awaited to adequately address this question.

Third, to what extent (if any) do NK cells support rather than inhibit metastatic dissemination? Tumor-infiltrating CD56^{bright} CD16^{dim} NK cells display a striking phenotypic and functional resemblance to decidual NK cells (Levi et al., 2015). Decidual NK cells not only drive angiogenesis in the context of early pregnancy (Hanna et al., 2006; Lima et al., 2014) but also promote IDO1 expression by local CD14^{+} myelomonocytic cells, hence stimulating the establishment of an immunosuppressive microenvironment enriched in T_{REG} cells that underlies maternal tolerance (Vacca et al., 2010). It will be important to elucidate the impact of CD56^{bright}CD16^{dim} NK cells in metastatic disease.

Additional work is also required to elucidate whether interventions that specifically (re)activate NK cell immunosurveillance, alone or in combination with other treatments, can be successfully used in patients with cancer to prevent or treat metastatic disease. Until recently, the prospect of rigorously investigating the efficacy of immunotherapies directed against metastatic disease by monitoring survival has been a deterrent to commercial investments. Now, with the recent clinical success of multiple checkpoint blockers and the initiation of clinical trials testing these agents as neoadjuvant interventions (Buque et al., 2015), the prospect of testing immunotherapies for preventing death from distant metastasis is becoming a reality. Irrespective of these and other unknowns, NK cells represent a promising target for the development of novel strategies for the management of this deadly aspect of cancer.

AUTHOR CONTRIBUTIONS

A.L.S. and L.G. conceived and wrote the manuscript, centralized and integrated comments from co-authors, and revised the paper upon editorial feedback. A.L.S. prepared the tables under supervision by M.J.S. and L.G. M.J.S. corrected the article and provided valuable input to preparation. All authors read and approved the final content of the manuscript.

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