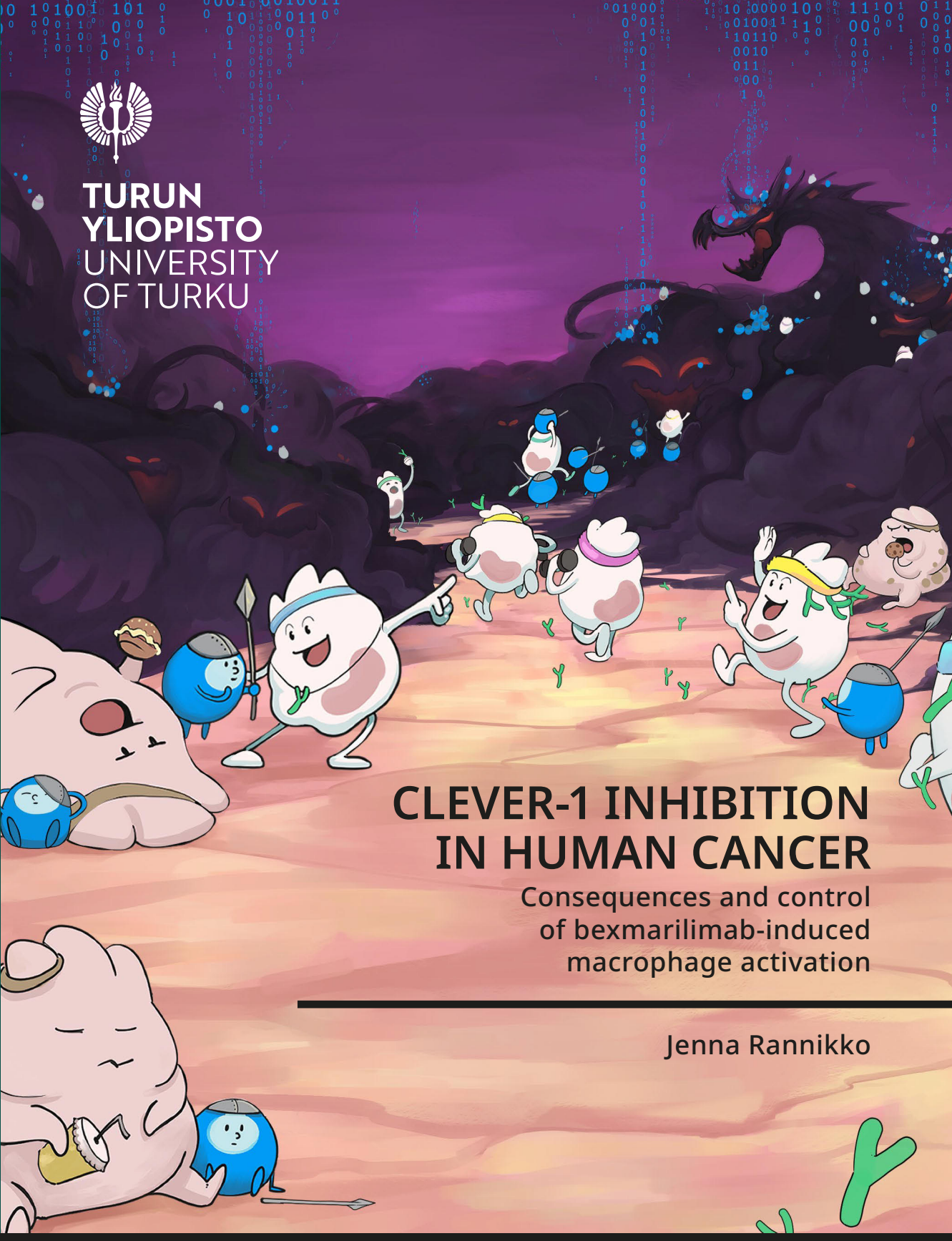

TURUN YLIOPISTO
UNIVERSITY OF TURKU

CLEVER-1 INHIBITION IN HUMAN CANCER

Consequences and control of bexmarilimab-induced macrophage activation

Jenna Rannikko

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Cover image: Activated macrophages lead the fight against cancer, Joonas Rannikko

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To all the people who helped me along the way

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ABSTRACT

Cancer immunotherapy enhances the immune system's ability to eliminate cancer cells for remarkable efficacy, but new therapies are needed to combat treatment resistance. Tumor-associated macrophages (TAMs) drive cancer progression largely through immunosuppression, and inhibiting their immunoregulatory scavenger receptor Clever-1 restores anti-tumor immunity in mice. A Clever-1-blocking humanized antibody bexmarilimab, developed to exploit this mechanism, entered first-in-human clinical testing for advanced solid tumors (MATINS trial) in 2018.

This thesis aimed to elucidate the immunological consequences of human macrophage Clever-1 blockade and to identify regulators of bexmarilimab treatment sensitivity. The presented transcriptomic, single-cell and spatially resolved analyses of clinical patient samples, patient-derived cells and tumor explants provide the first functional characterization of Clever-1 inhibition in human cancer.

We discovered bexmarilimab to disrupt tolerogenic lipid metabolism pathways and lysosomal acidification in monocytes and macrophages, resulting in their pro-inflammatory activation. In patients, monocyte and TAM reprogramming was accompanied by interferon and T-cell responses, both in the circulation and within tumors demonstrating disease stabilization. Similar immune responses occurred in one third of bexmarilimab-treated patient-derived cancer models, enabling recognition of bexmarilimab-sensitive tumor explant cultures with a gene signature identified in this thesis. Patient-derived cancer models additionally revealed that bexmarilimab-treated TAMs secrete CXCL10 for T-cell recruitment and that the tumor secretome regulates bexmarilimab responses more strongly than macrophage origin or cellular neighborhoods. Across the studies, bexmarilimab-induced immune responses were principally observed in interferon-poor tumor microenvironments, while chronic interferon priming impaired bexmarilimab-mediated macrophage activation. Additionally, responsive tumors had abundant intratumoral Clever-1^{+} TAMs and low PD-L1 expression, while lacking IL4I1^{+} TAMs and regulatory T cells. In conclusion, bexmarilimab activates macrophage- and T-cell-mediated immunity in non-inflamed tumors, which are mostly resistant to the current T-cell-directed cancer immunotherapies effective in T-cell- and interferon-rich tumors.

KEYWORDS: tumor-associated macrophages, Clever-1, immunotherapy, patient-derived cancer models, tumor immune microenvironment

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TIIVISTELMÄ

Syövän immunoterapiat vahvistavat puolustusjärjestelmän kykyä tuhota syöpäsoluja hämmästyttävin hoitotuloksin, mutta uusia hoitoja tarvitaan hoitoresistenssin voittamiseksi. Kasvaimen makrofagit edistävät syövän etenemistä ja vaimentavat kasvaimenvastaisen immuunipuolustuksen, joka voidaan syövän eläinmalleissa käynnistää uudelleen estämällä makrofagien Clever-1-haaskareseptorin immunosuppressiivinen toiminta. Tähän kehitetty humanisoitu vasta-aine, beksmarilimabi, eteni vuonna 2018 ensimmäiseen kliiniseen tutkimukseensa (MATINS-tutkimus).

Tämän väitöskirjan tavoitteena oli selvittää makrofagien Clever-1:n eston immunologiset vaikutukset ihmisessä ja tunnistaa beksmarilimabin hoitovastetta säätteleviä tekijöitä. Esitetyt sekvensointi-pohjaiset analyysit kliinisistä tutkimusnäytteistä ja potilasperäisistä syöpämalleista muodostavat ensimmäisen kuvauksen Clever-1:n estosta ihmisen syövässä.

Havaitsimme, että beksmarilimabi voimistaa monosyyttien ja makrofagien immuunivasteita estämällä niiden lipidimetabolian signalointireittejä ja lysosomien happamoitumista. Edennyttä syöpää sairastavien potilaiden verenkierrossa ja taudinkuvaltaan vakaina säilyvissä kasvaimissa beksmarilimabi sai lisäksi aikaan interferoni- ja T-soluvasteita. Vastaava immuuniaktivaatio tapahtui kolmasosassa beksmarilimabi-hoidetuista potilasperäisistä syöpämalleista, mikä mahdollisti beksmarilimabi-herkkien syöpäkudosviljelmien tunnistamisen tässä väitöskirjassa löydetyn geenienkspressioprofiilin perusteella. Lisäksi potilasperäiset syöpämallit osoittivat, että beksmarilimabi saa makrofagit houkuttelemaan T-soluja CXCL10:n välityksellä ja että kasvaimen erittämät tekijät säätelevät beksmarilimabi-vasteita makrofagien alkuperää ja naapurisoluja voimakkaammin. Beksmarilimabin aikaansaamat immuunivasteet havaittiin läpi tutkimusten lähinnä interferoni-köyhissä ympäristöissä, ja krooninen interferoni-altistus estikin beksmarilimabi-välitteisen makrofagiaktivaation. Beksmarilimabi-herkissä kasvaimissa oli myös runsaasti kasvaimensisäisiä Clever-1^{+} makrofageja, matala PD-L1-taso sekä vähän IL4I1^{+} makrofageja ja regulatorisia T-soluja. Siten beksmarilimabi aktivoi immuunipuolustuksen immunologisesti hiljaisissa kasvaimissa, jotka harvemmin hyötyvät nykyisistä T-soluihin kohdistetuista syövän immunoterapioista.

AVAINSANAT: kasvaimen makrofagit, Clever-1, immunoterapia, potilasperäiset syöpämallit, kasvaimen immunologinen mikroympäristö

Table of Contents

Abbreviations

CCL	C-C motif chemokine ligand
CCR	C-C motif chemokine receptor
CD	Cluster of differentiation (molecule)
C/EBP	CCAAT/enhancer binding protein
Clever-1	Common lymphatic endothelial and vascular endothelial receptor 1
CSF1R	Colony-stimulating factor 1 receptor
CTLA-4	Cytotoxic T lymphocyte antigen 4
CXCL	C-X-C motif chemokine ligand
CXCR	C-X-C motif chemokine receptor
CyTOF	Cytometry by time-of-flight
DAMP	Damage-associated molecular pattern
DC	Disease control
DEG	Differentially expressed gene
ECM	Extracellular matrix
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
EMP	Erythro-myeloid progenitor
EMT	Epithelial-mesenchymal transition
FBS	Fetal bovine serum
Fc(γ)R	Fragment crystallizable (γ) receptor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
H3K(4me)	histone H3 lysine (4 methylation)
HSC	Hematopoietic stem cell
ICI	Immune checkpoint inhibitor
IDO1	Indoleamine 2,3-dioxygenase 1
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
IL4I1	Interleukin-4 induced 1
IMDM	Iscove’s modified Dulbecco’s medium
LAMP-1	Lysosomal-associated membrane protein 1

LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
LXR	Liver X receptor
M1/M2	Classical/alternative macrophage activation
M-CSF	Macrophage colony-stimulating factor
MATINS	Macrophage antibody to inhibit immune suppression (clinical trial)
MHC	Major histocompatibility complex
NK	Natural killer (cell)
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death 1 ligand 1
PDEC	Patient-derived explant culture
PI3K	Phosphoinositide 3-kinase
PMA	Phorbol 12-myristate 13-acetate
PPAR	Peroxisome proliferator-activated receptor
PRR	Pattern recognition receptor
qPCR	Quantitative polymerase chain reaction
ROI	Region of interest
(sc)RNA-seq	(Single-cell) RNA-sequencing
SIRPα	Signal-regulatory protein alpha
SPARC	Secreted protein acidic and rich in cysteine
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
TAM	Tumor-associated macrophage
TCR	T-cell receptor
TGF	Transforming growth factor
T_H	Helper T (cell)
TLR	Toll-like receptor
TME	Tumor microenvironment
TNF	Tumor necrosis factor
T_{REG}	Regulatory T (cell)
TREM	Triggering receptor expressed on myeloid cells
VEGF	Vascular endothelial growth factor

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I **Rannikko, J. H.** & Hollmén, M. Clinical landscape of macrophage-reprogramming cancer immunotherapies. *Br J Cancer*, 2024; 131(4):627-40.

II Virtakoivu, R.*, **Rannikko, J. H.***, Viitala, M.*, Vaura, F., Takeda, A., Lönnberg, T., Koivunen, J., Jaakkola, P., Pasanen, A., Shetty, S., de Jonge, M. J. A., Robbrecht, D., Ma, Y. T., Skyttä, T., Minchom, A., Jalkanen, S., Karvonen, M. K., Mandelin, J., Bono, P. & Hollmén, M. Systemic blockade of Clever-1 elicits lymphocyte activation alongside checkpoint molecule downregulation in patients with solid tumors: results from a phase I/II clinical trial. *Clin Cancer Res*, 2021; 27(15):4205-20.

III **Rannikko, J. H.**, Verlingue, L., de Miguel, M., Pasanen, A., Robbrecht, D., Skyttä, T., Iivanainen, S., Shetty, S., Ma, Y. T., Graham, D. M., Arora, S. P., Jaakkola, P., Yap, C., Xiang, Y., Mandelin, J., Karvonen, M. K., Jalkanen, J., Karaman, S., Koivunen, J. P., Minchom, A., Hollmén, M. & Bono, P. Bexmarilimab-induced macrophage activation leads to treatment benefit in solid tumors: the phase I/II first-in-human MATINS trial. *Cell Rep Med*, 2023; 4(12):101307.

IV **Rannikko, J. H.**, Bono, P., Hynninen, J. & Hollmén, M. Bexmarilimab activates human tumor-associated macrophages to support adaptive immune responses in interferon-poor immune microenvironments. *Cancer Immunol Res*, 2024; 12(1):48-59.

V **Rannikko, J. H.**, Turpin, R., Boström, P., Virtakoivu, R., Harth, C., Takeda, A., Tamminen, A., Koskivuo, I. & Hollmén, M. Macrophage sensitivity to bexmarilimab-induced reprogramming is shaped by the tumor microenvironment. *J Immunother Cancer*, 2025; 13(5):e011292.

*, these authors contributed equally

1 Introduction

A specialized protective network of cells and molecules, the immune system, pervades our bodies to surveil and defend homeostasis. At its heart, immune cells eliminate threatening targets with their various functions and cross-regulate their actions to mount appropriate and coordinated responses.

This thesis book revolves around a specific type of immune cells, macrophages, which were discovered almost 150 years ago as cells clustering around foreign substances and attempting to eat them, regardless of target size. (Metchnikoff, 1893) The described process, phagocytosis, gave them their name as great eaters, macrophages. Even today, macrophage propensity for phagocytosis remains at the center of their behavior, regulating their various functions in immune defense, tissue repair and organ homeostasis. Phagocytosis is performed with the help of scavenger receptors that each recognize various substances for engulfment. Ligand binding and phagocytosis, in turn, tremendously alter macrophage function, including their gene expression and secretome, thereby pivotally governing macrophage inflammatory responses. (Canton et al., 2013) The literature review of this thesis delves deeply into macrophage biology, describing macrophage behavior under homeostasis and during immunological defense, regulation of their functions and dysregulation in cancer.

The immune system is well-equipped to fight cancer and capable of recognizing, killing and disposing malignant cells. Thanks to its continuous work, malignant cells can be eliminated before clinical detection of cancer (Dunn et al., 2002; Swann & Smyth, 2007). If cancer cells manage to escape from the immune system, cancer immunotherapy is needed to re-activate anti-tumor immune defense. Cancer immunotherapy can lead to complete remission, yet inherent and treatment-emergent therapeutic resistance are common and cause disappointing therapeutic efficacy. (Restifo et al., 2016; Sharma et al., 2017). Therefore, new therapies and methods for patient selection are continuously developed.

This thesis describes how interrupting the scavenger receptor Clever-1 (Common lymphatic endothelial and vascular endothelial receptor 1) transforms macrophage function to restore anti-tumor immunity. The presented results characterize immunological changes after human Clever-1 blockade with a novel therapeutic

antibody, bexmarilimab, and describe patients likely-to-benefit from bexmarilimab therapy. Clinical patient samples and patient-derived cancer models were extensively used in this thesis work to produce translational knowledge for the treatment of human cancer.

2 Review of the Literature

2.1 Macrophages as key players in immunity

The classification of macrophages in relation to other cell types was revised several times after their discovery, until the conception of the mononuclear phagocyte system. This system describes bone marrow precursors that generate circulating monocytes, which enter tissues to differentiate into macrophages. (van Furth et al., 1972) While we now know that this differentiation pathway describes macrophage development only after birth, it facilitates relatively easy differentiation of macrophages from human blood monocytes and mouse bone marrow cells. These and other methods have revealed the remarkably diverse functions of macrophages in organ development, disease pathologies, regulation of homeostasis and immune defense, as described in this section.

2.1.1 The origin of macrophages

First macrophages appear very early on. Primitive hematopoiesis that generates the first blood cells occurs outside the developing embryo, in the blood islands of the membranous yolk sac, which supplies the developing embryo with nutrients and oxygen. There, yolk sac progenitors and erythro-myeloid progenitors (EMPs) generate primitive macrophages that enter embryonic tissues once the blood circulation has developed (Hoeffel et al., 2015; Mass et al., 2016; McGrath et al., 2015; Moore & Metcalf, 1970; Palis et al., 1999). Unlike during later macrophage development, the primitive yolk sac-derived macrophages seem to arise without a monocyte intermediate (Hoeffel et al., 2015; Takahashi & Naito, 1993; Takahashi et al., 1989). Later on, fetal liver develops and yolk sac EMPs migrate there to generate fetal monocytes and macrophages (Gomez Perdiguero et al., 2015; Hoeffel et al., 2015; McGrath et al., 2015; Palis et al., 1999). These first two waves of macrophage generation are virtually independent of adult hematopoiesis, which relies on hematopoietic stem cells (HSCs). (McGrath et al., 2015; Schulz et al., 2012)

Remarkably, HSCs are generated by specialized endothelial cells in the embryo's aorta, shortly after the emergence of EMPs within the yolk sac (Bertrand et al., 2010; Boisset et al., 2010). They mature in the fetal liver and migrate into the bone marrow

to take over hematopoiesis at birth (Ara et al., 2003; Kieusseian et al., 2012). HSCs divide and generate progenitor cells that commit to producing specific blood cell types upon subsequent cell divisions. According to an established model, monocyte differentiation from HSCs proceeds through a common myeloid progenitor, a granulocyte/macrophage progenitor, a bipotential macrophage/dendritic cell progenitor and a common monocyte progenitor, the last of which generates monocytes to be released into circulation (Akashi et al., 2000; Fogg et al., 2006; Hettinger et al., 2013; Iwasaki et al., 2005; Kawamura et al., 2017; van Furth et al., 1972; Varol et al., 2007). More recent studies, however, have suggested two diverging routes for monocyte production, either via the granulocyte/macrophage progenitor or the macrophage/dendritic cell progenitor. The resulting monocytes would both differentiate into macrophages, but only the latter route would generate monocyte-derived dendritic cells (Liu et al., 2019; Yáñez et al., 2017). Under homeostasis, monocyte differentiation and survival are promoted by specific transcription factors (PU.1, IRF8, KLF4) and cytokines (macrophage colony-stimulating factor [M-CSF] and interleukin [IL]-34), (Dai et al., 2002; Feinberg et al., 2007; Iwasaki et al., 2005; Mossadegh-Keller et al., 2013; Olson et al., 1995; Tamura et al., 2000; Wei et al., 2010), while inflammation can upregulate additional cytokines to drive monocyte production (de Bruin et al., 2012).

New monocytes exiting the bone marrow are so-called classical monocytes, distinguished in humans by the cluster of differentiation (CD)$14^{++}CD16^{-}$ surface marker combination (Patel et al., 2017; Ziegler-Heitbrock et al., 2010). Their exit and recruitment to the sites of inflammation is driven by C-C motif chemokine receptor 2 (CCR) that binds C-C motif chemokine ligands 2 and 7 (CCL2 and CCL7) (Boring et al., 1997; Lu et al., 1998; Serbina & Pamer, 2006; Tsou et al., 2007). Outside the bone marrow, spleen acts as an additional reservoir for monocytes, allowing their storage and quick mobilization upon inflammation (Swirski et al., 2009). If classical monocytes remain in the circulation, they can sequentially differentiate into two other monocyte subtypes found in the blood, first intermediate $CD14^{++}CD16^{+}$ monocytes and then patrolling $CD14^{+}CD16^{++}$ monocytes (Passlick et al., 1989; Patel et al., 2017; Yona et al., 2013; Ziegler-Heitbrock et al., 2010). The patrolling monocytes perform important within-vessel functions, including immunosurveillance and maintenance of the vascular endothelium (Auffray et al., 2007; Carlin et al., 2013; Cros et al., 2010). Monocytes leaving the circulation and entering tissues have several possible fates, including differentiation into macrophages or dendritic cells, remaining as a monocyte-like cell or exiting the tissue undifferentiated through lymphatics (Jakubzick et al., 2013; Tamoutounour et al., 2013; Yáñez et al., 2017). These processes result in a short half-life of classical monocytes in the circulation, approximately one day (Patel et al., 2017).

Both the embryonic and HSC-dependent macrophage development contribute to macrophage populations found in adult individuals. While HSCs generate new monocytes and macrophages, embryonic macrophages can persist in several tissues, relying on local proliferation for sustaining their population size with minimal replacement by HCS-derived cells (Ginhoux et al., 2010; Gomez Perdiguero et al., 2015; Hashimoto et al., 2013; Schulz et al., 2012). The contribution of different macrophage origins, yolk sac, fetal liver or bone marrow, varies between tissues, as illustrated in **Figure 1**. At one extreme, brain microglia derive from primitive yolk sac macrophages and maintain their population size behind the blood-brain barrier with minimal contribution by fetal or HSC-derived monocytes (Ginhoux et al., 2010; Hoeffel et al., 2015). Similarly, liver Kupffer cells and skin Langerhans cells are principally derived from the fetal liver alone (Hoeffel et al., 2012; Liu et al., 2019). At the other extreme, gut macrophages undergo constant turnover and are mainly bone marrow-derived (Bain et al., 2014). In between these extremities, a mixture of both embryonic and bone marrow ontogenies can be observed in tissues such as pancreas, breast and skeletal muscle (Calderon et al., 2015; Jäppinen et al., 2019; Wang et al., 2020). Some macrophage populations, such as alveolar macrophages in the lungs and cardiac macrophages, will be gradually replaced by HCS-derived macrophages in aging individuals (Gomez Perdiguero et al., 2015; Liu et al., 2019; Molawi et al., 2014). Furthermore, specialized subpopulations with a different ontogeny than the main population can be found in some tissues performing distinct functions, such as embryonic gut macrophages that regulate enteric neuron function in intestinal motility (De Schepper et al., 2018).

Tissue-specific conditions regulate the composition of macrophage populations, determining whether there is free space for new macrophages and which macrophage ontogeny is favored. Macrophage population size in each tissue has been proposed to be regulated by niche competition, which means that new monocytes differentiate into macrophages only when niches become available through macrophage death or tissue growth (Guilliams & Scott, 2017). Additionally, disruption of homeostasis is often followed by local macrophage proliferation, monocyte influx or both (Ajami et al., 2011; Culemann et al., 2019; Davies et al., 2013; Liu et al., 2019), when a new cytokine environment overrides homeostatic mechanisms controlling macrophage density (Guilliams et al., 2020; Jenkins et al., 2013; Zhou et al., 2022). Upon resolution of inflammation, recruited macrophages may disappear, but empty niches left behind by the previous tissue-resident macrophages are refilled by newly-recruited monocyte-derived macrophages or local macrophage proliferation. (Ait Ahmed et al., 2021; Ajami et al., 2011; Scott et al., 2016) Therefore, recurrent tissue stress and damage accelerate macrophage turnover rate. Some tissue niches additionally regulate their macrophage pool composition by favoring colonization of macrophages with a specific ontogeny (van de Laar et al., 2016). When necessary,

however, monocyte-derived macrophages can assume remarkably similar transcriptomic profiles and capacity for self-maintenance as the original embryonic inhabitants (Scott et al., 2016; van de Laar et al., 2016).

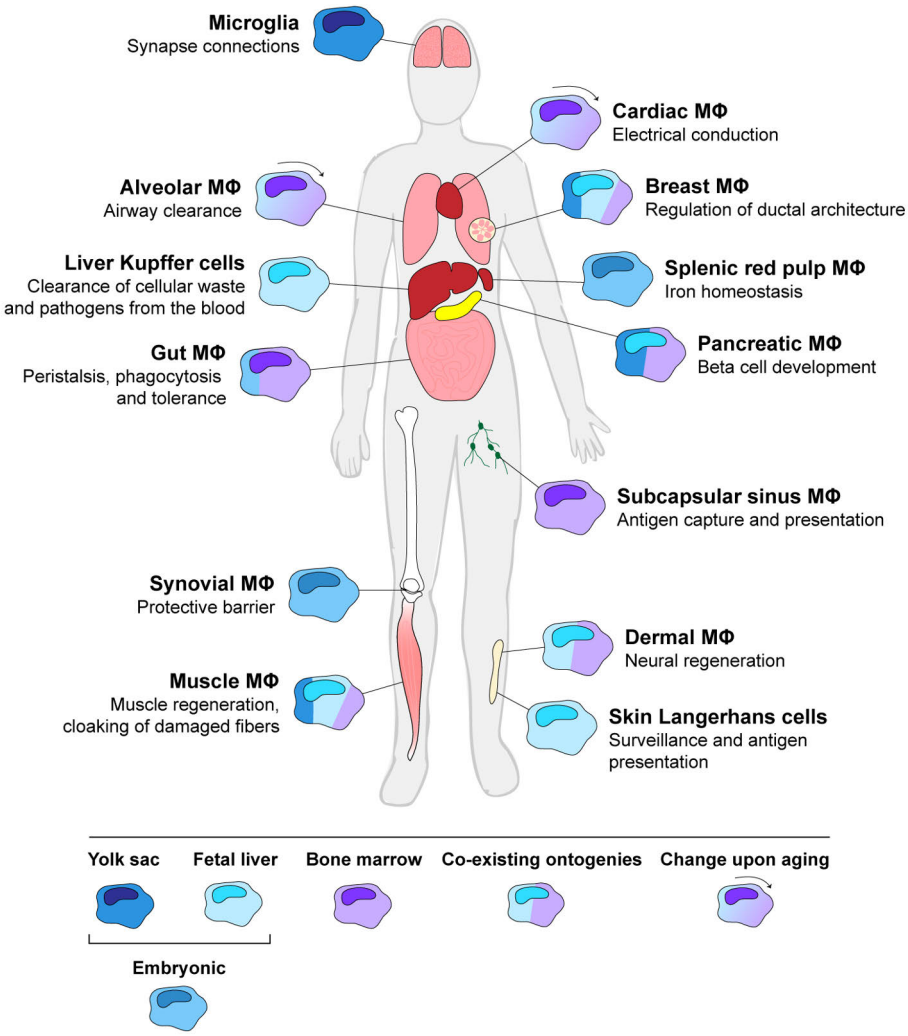


Figure 1. Origin and specialized functions of tissue-resident macrophages. The figure illustrates selected macrophage populations, connected to their organ of residence and colored by their ontogeny. Co-existing ontogenies often associate with functionally distinct subpopulations residing in the tissue, while only one of the organ's multiple macrophage subsets was depicted in the lungs, brain, spleen, joints, liver and lymph nodes. Additional references were used to review macrophage ontogeny and functions in the gut (Muller et al., 2015; Shaw et al., 2018; Smythies et al., 2005), liver (Deppermann et al., 2020; Terpstra et al., 2000; Zeng et al., 2016), lymph nodes (Mondor et al., 2019), pancreas (Banaei-Bouchared et al., 2004) and skeletal muscle (Uderhardt et al., 2019). MΦ, macrophage.

Finally, while monocyte and macrophage development has been primarily investigated using murine fate-mapping models, single-cell analyses on human embryos suggest similar macrophage development pathways in humans (Bian et al., 2020; Popescu et al., 2019).

2.1.2 Macrophages under homeostasis

Astonishingly, macrophages simultaneously perform immunological surveillance, waste disposal and remarkably sophisticated tissue-specific functions to constantly maintain body's homeostasis. The latter two roles are the focus of this section, while macrophage surveillance work is described below. **Figure 2** summarizes macrophage functions under homeostasis and after detection and elimination of threats.

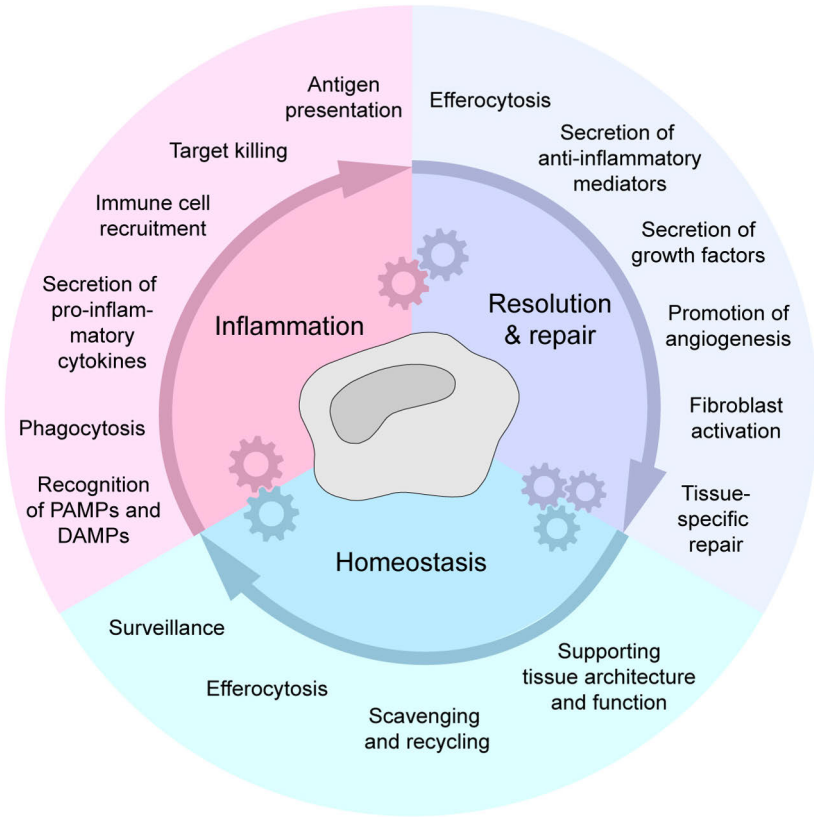


Figure 2. Key macrophage functions under homeostasis, during acute inflammation and its resolution. A single macrophage is depicted to highlight the potential of macrophages to assume different functional profiles based on the prevailing conditions.

Macrophages throughout the body engulf and dispose dying cells by phagocytosis, which is called efferocytosis (A-Gonzalez et al., 2017; deCathelineau & Henson, 2003). Dying cells release chemotactic find-me molecules to attract phagocytes (Elliott et al., 2009; Gude et al., 2008; Lauber et al., 2003) and display surface ligands for recognition by macrophage receptors, such as MERTK, Tim4 and scavenger receptors (A-Gonzalez et al., 2009; Borisenko et al., 2003; Fadok et al., 1992; Greenberg et al., 2006; Miyanishi et al., 2007; Park et al., 2009). Recognized targets will be enclosed by macrophage plasma membrane, taken inside and usually sorted for degradation within the phago-lysosomal system, which is equipped with acidic pH, catabolic enzymes and free radicals for efficient target degradation (Babior, 1999; Claus et al., 1998; Hackam et al., 1998; Parnaik et al., 2000).

As cell turnover is a constant process (Nagata, 2018), homeostatic cell death should not signal any danger. While apoptotic cell death can be inconspicuous or immunogenic, depending on the surrounding conditions and molecules exposed on the dying cells, failure to dispose apoptotic cells causes secondary necrosis and loss of plasma membrane integrity on the dying cells (Green et al., 2009; Obeid et al., 2007; Rogers et al., 2017; Vanden Berghe et al., 2010). Ensuing exposure to intracellular molecules will predispose to autoimmune diseases, where the body's immune system attacks its own tissues, such as systemic lupus erythematosus (Asano et al., 2004; Hanayama et al., 2004; Lövgren et al., 2004).

Silent, non-immunogenic or anti-inflammatory cell waste disposal by tissue-resident macrophages is therefore of paramount importance. Upon recognizing apoptotic cells, macrophages secrete anti-inflammatory molecules and suppress their pro-inflammatory mediator secretion, resulting in tolerance towards the target (A-Gonzalez et al., 2009; Fadok et al., 1998; Freire-de-Lima et al., 2006; Huynh et al., 2002; McDonald et al., 1999; Roberts et al., 2017). Mechanistically, tolerance is promoted by phagocytosis-induced changes in macrophage lipid metabolism and gene transcription, associated with specific immunosuppressive transcription factors, such as liver X receptor (LXR) (A-Gonzalez et al., 2009; Cummings et al., 2016). Resident macrophages are also programmed by local tissue environments to have higher threshold for activation after ingestion of apoptotic cells (Roberts et al., 2017). Additionally, resident macrophages can cover up necrotic damage to some extent by cloaking small damaged areas with their cellular extensions, preventing other cells from detecting the disruption (Uderhardt et al., 2019).

Besides efferocytosis, macrophages ingest and recycle various unwanted materials for re-use. For instance, macrophages in the red pulp of the spleen phagocytose aged blood cells and release their iron to be used again (Kohyama et al., 2009). In the brain, vessels, liver and adipose tissue, several macrophage subsets take up excess lipids or myelin debris, which can either prevent chronic inflammatory diseases caused by excess lipid accumulation or exacerbate the disease

pathology (Aouadi et al., 2014; Bieghs et al., 2012; Childs et al., 2016; Grajchen et al., 2020). In the lungs, alveolar macrophages clear away excess surfactant and inhaled particles (Nakamura et al., 2013; Warheit & Hartsky, 1993). Macrophages bind these and various other self- and non-self-molecules with their scavenger receptors, which have a central role in macrophage-mediated clearance (Dunne et al., 1994; Elomaa et al., 1995; Goldstein et al., 1979; Greenberg et al., 2006; PrabhuDas et al., 2017). Internalized cargo is usually recycled, and even engulfed pathogens can be broken down to fuel macrophages' own metabolism (Lesbats et al., 2025). If macrophages encounter particles that they are unable to degrade, such as tattoo pigment in the skin, they nevertheless ingest them for permanent storage (Baranska et al., 2018). Apart from this nutrient recycling, macrophages can control the body's metabolic processes more widely, regulating thermogenesis, energy consumption and lipid storage (Nguyen et al., 2011; Pirzgalska et al., 2017).

Building on to their general nature to scavenge, sort and recycle, macrophages have extremely sophisticated tissue-specific roles that ensure normal organ development and function (**Figure 1**). To name a few, macrophages in mammary tissues regulate the initial development of the ductal network as well as the cyclic changes in the mammary tissue architecture at each estrous cycle and during pregnancy (Chua et al., 2010; Gouon-Evans et al., 2000; Ingman et al., 2006). Microglia in the brain remodel synapse connections and promote synapse formation for memory and learning throughout life (Parkhurst et al., 2013; Schafer et al., 2012), while cardiac macrophages form gap junctions with cardiomyocytes and participate in electrical conduction in the heart (Hulsmans et al., 2017). Remarkably, synovial macrophages lining the joint synovial cavities express tight-junction proteins and create an epithelial-like barrier on the synovial membrane to protect the joints from chronic inflammation and arthritis by insulating damage-associated signals generated by mechanical stress (Culemann et al., 2019). Furthermore, macrophages in the bone marrow regulate HSC retention and remove expelled red blood cell nuclei to maintain functional erythropoiesis (Chow et al., 2011; Kawane et al., 2001).

Unsurprisingly, defects in these normal macrophage functions can cause severe conditions. Since the receptor for M-CSF, colony stimulating factor 1 receptor (CSF1R), is vital for monocyte and macrophage generation, its inactivation drastically decreases monocyte and macrophage numbers throughout the body (Dai et al., 2002). In humans, homozygous *CSF1R* mutations cause severe brain malformations and osteopetrosis due to disrupted microglia and osteoclast functions (Guo et al., 2019; Oosterhof et al., 2019). Similarly, the ability of alveolar macrophages to phagocytose surfactants depends on granulocyte-macrophage colony stimulating factor (GM-CSF), and mutations in its receptor genes *CSF2RA* or *CSF2RB* lead to surfactant accumulation, progressive dyspnea and pulmonary fibrosis (Suzuki et al., 2011; Suzuki et al., 2008). Undeniably, macrophages are vital

already under homeostasis, even though they are better known for their defensive work against invading pathogens.

2.1.3 Macrophage-mediated immune surveillance

Macrophages remain constantly vigilant for recognizing pathogens, foreign substances, tissue injury or other types of threats. Invading pathogens can by-pass the body's first line of defense, tight epithelial barriers of the skin, mucosa and glandular tissues, which protect the body from the outside world with anti-microbial molecules and acidic pH (Punt et al., 2019c). In the vicinity of these entry sites, macrophages form surveillance networks and search for assaults by either crawling or probing with their cellular extensions (Kubo et al., 2009; Neupane et al., 2020; Nimmerjahn et al., 2005). With their moving cellular extensions, immobile macrophage populations can cover large areas over time, such as the whole mammary duct network (Dawson et al., 2020; Nimmerjahn et al., 2005). Similarly, Langerhans cells capture pathogens in the upper skin layers by passing their cellular extensions through keratinocyte tight junctions (Kubo et al., 2009). Additionally, strategically located macrophage populations – Kupffer cells in the liver, marginal zone and metallophilic macrophages in the spleen and subcapsular sinus macrophages in the lymph nodes – recognize and capture pathogens from the blood and lymph (Aichele et al., 2003; Junt et al., 2007; McDonald et al., 2020).

When homeostasis is disrupted, macrophages react quickly. In case of tissue damage, macrophages start to move their membrane extensions towards necrotic cells within minutes (Uderhardt et al., 2019). Such sterile damage is detected via so-called danger-associated molecular patterns (DAMPs) that are molecules normally located within intact nuclei, mitochondria or plasma membrane, but get released from cells undergoing stress or non-apoptotic cell death (Davalos et al., 2005; Di Micco et al., 2016; Kayagaki et al., 2021; Punt et al., 2019c; Scaffidi et al., 2002; Zhong et al., 2022). Macrophages are equipped with several receptor families to bind and recognize these molecules, such as pattern recognition receptors (PRRs), receptor for advanced glycation end-products (RAGE) and P2Y receptors (Davalos et al., 2005; Di Micco et al., 2016; Hori et al., 1995; Kraakman et al., 2017; Zhong et al., 2022). If the damaged area surpasses macrophages' capability to cover and contain it, other immune cells, especially neutrophils, will contact the damaged cells and mount an acute inflammatory response (Uderhardt et al., 2019).

In case of pathogen invasion, macrophages recognize common pathogen-associated molecular patterns (PAMPs), such as bacterial cell wall components, viral RNA or DNA with their PRR family receptors, such as Toll-like receptors (TLRs), C-type lectin receptors, AIM2-like receptors, NOD-like receptors and cGAS-STING (stimulator of interferon genes) (Bürckstümmer et al., 2009; Franchi et al., 2006;

Punt et al., 2019c; Rock et al., 1998; Wu et al., 2013). PRRs are positioned at different subcellular locations: cell surface, cytosol and endo-lysosomal compartments, which provides information about the location of the pathogen (extracellular, intracellular or phagocytosed) and regulates downstream signaling (Kagan et al., 2008; Latz et al., 2004; Sun et al., 2013). Additionally, different receptor specificities carry information about the type of danger. For instance, specific TLR-family receptors recognize bacterial flagella, pathogen cell wall components, viral DNA or RNA (Alexopoulou et al., 2001; Hayashi et al., 2001; Hemmi et al., 2000; Takeuchi et al., 1999). If these detection mechanisms are by-passed, macrophages can still mount inflammatory responses against pathogen-induced tissue damage, as described above for sterile inflammation. Alongside macrophages, also dendritic cells, more mobile and efficient in presenting captured antigens, and mast cells, central in parasitic defense and allergic reactions, act as sentinels capable of alarming the immune system (Punt et al., 2019a; Punt et al., 2019c).

2.1.4 Macrophages in immune defense

Initial PRR engagement activates several macrophage effector functions to kick-start immune defense. Macrophages phagocytose pathogens and degrade them in acidic pH with lysosomal enzymes and reactive oxygen species generated by phagocyte oxidase and phagosome-contacting mitochondria (Babior, 1999; Claus et al., 1998; Geng et al., 2015; Hackam et al., 1998; West et al., 2011). To speed up this process, TLR signaling can activate LC3-associated phagocytosis, which enhances the delivery speed of the phagocytosed cargo to lysosomes (Sanjuan et al., 2007). Furthermore, pathogens can be coated by complement molecules that enhance pathogen uptake via macrophage complement receptors, such as VSIG4 (Helmy et al., 2006). Efficient phagocytosis alone may be sufficient to eliminate pathogens, and alveolar macrophages have been shown to dispose the majority of inhaled bacteria before their detection by the rest of the immune system, thereby limiting inflammatory damage within the lungs (Neupane et al., 2020). If macrophages themselves get invaded, cytosolic bacteria activate inflammasome protein complexes via NOD-like receptors. This results in a caspase-1-mediated lytic cell death of the infected macrophage, called pyroptosis. Invading bacteria get trapped within the dying macrophages for subsequent clearance. Additionally, pyroptosis releases caspase-1-cleaved active pro-inflammatory cytokines from the dying macrophages. (Franchi et al., 2006; Jorgensen et al., 2016; Martinon et al., 2002)

PRR-mediated recognition also triggers acute inflammation, when macrophages alongside other resident immune cells signal about danger with cytokine and chemokine secretion. Through NF-κB transcription factor, TLRs activate the

expression of pro-inflammatory cytokines, including IL-1β, IL-6 and tumor necrosis factor α (TNFα) (Alexopoulou et al., 2001; Medzhitov et al., 1997). These cytokines increase vascular permeability for immune cell arrival and anti-microbial molecule leakage from the blood. They also have systemic effects, generating the liver's acute phase response and fever. (Punt et al., 2019c) Activation of intracellular PRRs, in particular, kick-starts secretion of interferons (IFNs) that are central in combatting viruses and other intracellular targets (Alexopoulou et al., 2001; Punt et al., 2019d; Sun et al., 2013).

Secreted cytokines together with additional chemokines also recruit immune cells from the circulation. For instance, TNFα together with IL-17 induces endothelial cells to express neutrophil-recruiting C-X-C motif chemokine ligands, CXCL1, CXCL2 and CXCL5. Likewise, TNFα together with IFNγ upregulates lymphocyte-recruiting chemokines CCL5, CXCL9 and CXCL10 on the endothelium. (Griffin et al., 2012) Macrophages themselves secrete several neutrophil-recruiting chemokines in response to TLR activation, including CXCL1, CXCL2 and IL-8 (De Filippo et al., 2008; Strieter et al., 1990).

Neutrophils are the first immune cells to arrive and combat pathogens by releasing their proteolytic and anti-microbial granules or by undergoing an immunogenic cell death called NETosis, which traps pathogens and amplifies inflammation (Punt et al., 2019a, 2019c). They are followed by newly-recruited monocytes that differentiate into additional macrophages (Punt et al., 2019c) and natural killer (NK) cells that combat malignant or virus-infected cells (Punt et al., 2019b).

This initial phase of acute immune response is orchestrated by the innate immune system, capable of faster and less specific immune reactions. To activate B- and T-cell-mediated adaptive immunity, innate immune cells usually need to present pathogen-derived antigens for the lymphocytes. Antigen presentation on major histocompatibility complex (MHC)-I molecules activates antigen-specific $CD8^+$ T cells against cells displaying that particular antigen, which allows cytolytic killing of infected or malignant cells. Antigen presentation on MHC-II molecules, on the other hand, allows $CD4^+$ T cells to support the activation of other immune cells, including $CD8^+$ T cells and antigen-specific B cells that produce high-affinity antibodies against extracellular pathogens. (Punt et al., 2019a, 2019c) While dendritic cells are traditionally more efficient in supporting these processes, also macrophages can load phagocytosed peptides onto their MHC-II and MHC-I molecules for presentation on the cell surface (Harding & Geuze, 1992; Pfeifer et al., 1993; Pozzi et al., 2005; Tang-Huau et al., 2018). Antigen presenting cells additionally need to provide naïve T cells with necessary co-stimulatory signals and cytokines. They also guide differentiating $CD4^+$ T cells towards specific helper T (T_H) cell phenotypes with different functional properties. For instance, IFNγ-

secreting T_H1 cells are important in cell-mediated immunity against intracellular targets, IL-4-secreting T_H2 cells regulate antibody responses and fight extracellular pathogens, IL-17-secreting T_H17 cells combat extracellular pathogens at mucosal barriers, and IL-10-secreting regulatory T (T_{REG}) cells suppress immune responses. (Punt et al., 2019e)

During fully-fledged innate and adaptive immune responses, macrophages reside in a cytokine-rich microenvironment, which enhances their activation and effector functions. In particular, IFNγ priming potentiates macrophage responses to PRR ligands via selective chromatin remodeling and translational suppression that shift macrophage gene expression from anti- to pro-inflammatory and inhibit tolerance (Chen & Ivashkiv, 2010; Kang et al., 2019; Qiao et al., 2013; Su et al., 2015). Macrophage metabolism is also rewired during acute inflammation. PRR stimulus switches macrophage energy consumption from oxidative phosphorylation to less efficient but faster glycolysis (Oren et al., 1963; Palsson-McDermott et al., 2015; Rodríguez-Prados et al., 2010), and fatty acid oxidation to fatty acid synthesis, which facilitate macrophage phagocytosis and inflammatory cytokine production (Ecker et al., 2010; Huang et al., 2014; Vats et al., 2006). Altered metabolism also promotes histone acetylation to modify gene expression, regulation of iron and amino acid availability to restrict pathogen growth, and anti-bacterial metabolite production (Ganesan & Roy, 2019; Lauterbach et al., 2019; Nairz et al., 2007; Niño-Castro et al., 2014).

At the height of the immune response, activated B cells incapacitate extracellular pathogens and flag them for efficient destruction by macrophage phagocytosis, complement-mediated lysis or neutrophil degranulation. Activated $CD8^+$ T cells and NK cells kill infected or malignant host cells by inducing their apoptosis via cytotoxic granules or Fas-ligand interactions. (Punt et al., 2019b) The choice of effector mechanisms likely culminates in the ability to eliminate the threat with minimal collateral damage, as both excessive immune responses and failure to resolve the inflammation may have pathological consequences (Iwasaki & Medzhitov, 2015).

2.1.5 Macrophages in the resolution of inflammation and tissue repair

After the target of the inflammatory response has been eliminated, it is time to restore homeostasis. Resolution of inflammation entails shutting down pro-inflammatory mediator secretion and associated signaling pathways. Built-in, self-limiting mechanisms that protect from excessive immune activation assist in this, as pro-inflammatory mediators are rapidly catabolized and also activate counteracting pathways (Kumar et al., 2021) For instance, IFNγ-induced negative feedback

mechanisms include upregulation of a negative regulator SOCS1 and immunosuppressive enzymes indoleamine 2,3-dioxygenase 1 (IDO1) and interleukin-4 induced 1 (IL4I1) (Alexander et al., 1999; Li et al., 2016; Marquet et al., 2010; Starr et al., 1997). Repeated exposure to a pro-inflammatory mediator also dampens its pro-inflammatory effects. For instance, chronic TNFα has been shown to inhibit T-cell receptor (TCR) signaling, T-cell proliferation and cytokine production (Cope et al., 1997), while chronic IFNγ impairs $CD8^+$ T-cell cytotoxicity (Tau et al., 2001). Pro-inflammatory macrophages themselves are susceptible for self-limiting epigenetic regulation, as their enhanced glycolysis produces lactate for histone lactylation that switches on their wound healing gene expression (D. Zhang et al., 2019).

Once the support of immune cell survival fades away, many immune cell subsets, especially neutrophils, undergo apoptotic cell death (Lee et al., 1993). Macrophages clear away the apoptotic cells by efferocytosis, which further transforms their phenotype (Frasch et al., 2011; Mounier et al., 2013; S. Zhang et al., 2019). Efferocytosis alters macrophage lipid metabolism (S. Zhang et al., 2019), dampens macrophage responses to further PRR activation (Fadok et al., 1998) and suppresses their fragment crystallizable receptor (FcR)-mediated phagocytosis as well as bactericidal activity (Medeiros et al., 2009). Consequently, macrophage secretome changes from pro-inflammatory (IL-1β, IL-8, TNFα) to anti-inflammatory mediators (transforming growth factor β [TGFβ], IL-10) (Arnold et al., 2007; Byrne & Reen, 2002; Fadok et al., 1998; Mounier et al., 2013; S. Zhang et al., 2019).

Macrophages also play important roles in repairing tissue damage caused by the original assault and the ensuing immune response. The repair results in tissue regeneration, fibrosis or both, depending on the regenerative capacity of the tissue. (Kumar et al., 2021) If tissue-resident macrophages have died in the process of immune defense, new monocyte-derived macrophages assume their places (Blériot et al., 2015). Otherwise, the healing becomes aberrant, which leads to loss of the original tissue architecture (Arnold et al., 2007; Goren et al., 2009).

Restorative macrophages continue phagocytosis and anti-inflammatory cytokine secretion, and promote tissue remodeling via growth factor secretion (e.g. TGFα, TGFβ, platelet-derived growth factor and insulin-like growth factor) (Ramachandran et al., 2012; Rappolee et al., 1988). Additionally, macrophages support re-building of tissue architecture in a tissue-specific manner. For instance, dermal macrophages assist in axon sprouting after injury to restore nerve activity (Kolter et al., 2019), while monocyte-derived macrophages support myogenesis, myocyte differentiation and muscle fiber growth in injured muscles (Arnold et al., 2007; Varga et al., 2016). After chronic liver injury, efferocytic macrophages can regulate the generation of new hepatocytes from progenitor cells by upregulating Wnt-signaling (Boulter et al., 2012).

Repair by fibrosis entails formation of new vascularized connective tissue, deposition of collagen and connective tissue remodeling (Kumar et al., 2021). Macrophages secrete platelet-derived growth factor and TGFβ to support fibroblast proliferation and collagen deposition (Ploeger et al., 2013; Roberts et al., 1986; Shimokado et al., 1985), and promote angiogenesis via vascular endothelial growth factor (VEGF)-A (Thakral et al., 1979; Willenborg et al., 2012; Zordan et al., 2014). During subsequent remodeling phase, macrophages crucially degrade excess collagen to minimize damage-associated fibrosis by direct collagen uptake and secretion of collagen-degrading matrix metalloproteinases (Atabai et al., 2009; Fallowfield et al., 2007; Ramachandran et al., 2012; Zordan et al., 2014).

During inflammation and its resolution, the macrophage population itself has experienced tremendous changes and its return to homeostasis may involve monocyte recruitment or local proliferation to fill empty niches (Davies et al., 2013; Davies et al., 2011; Epelman et al., 2014; Zigmond et al., 2014). The experience of past assaults, however, changes how the macrophage population will function in the future, as we will see in the following sections.

2.2 Regulation of macrophage function

Macrophage functional repertory is impressively versatile, and yet they manage to opt for suitable actions in correct locations at the right time. This section will try to uncover how this feat is achieved.

The function of a tissue-resident macrophage is tightly coupled with its phenotypic identity that depends on ontogeny (embryonic vs. bone marrow), local tissue niche, possible inflammatory cues and time spent in the tissue (Blériot et al., 2020). In addition to these, macrophages can retain some memory of the past inflammatory conditions, which affects their responsiveness in the short term (synergistic priming) and long term (trained immunity and tolerance) (Divangahi et al., 2021). Following subsections will describe how macrophages sense and combine different inputs from their environment to regulate their functional behavior, what is the contribution of macrophage-intrinsic (ontogeny) and -extrinsic (environment) factors and how macrophage memory works.

2.2.1 Mechanisms facilitating stimulus-specific responses

With a generic core program garnished with tissue-specific functions, macrophages serve the needs of each specific tissue and help other cell types to thrive (Okabe & Medzhitov, 2016). To tailor their function to changing tissue conditions, macrophages sense various types of signals from their environment and within themselves, including the PAMPs and DAMPs described above, metabolites, such

as heme (Haldar et al., 2014) and lactic acid (Colegio et al., 2014), messenger molecules (Doyle et al., 1994) and physical or chemical factors, such as tissue stiffness (Atcha et al., 2021), pH (Lee-Rueckert et al., 2020) and osmolarity (Machnik et al., 2009). Some signals directly alter macrophage metabolism (Lee-Rueckert et al., 2020), while others are received by specific receptors and ligand-dependent transcription factors that activate signaling cascades and gene expression downstream to alter macrophage phenotype and function (Colegio et al., 2014; Haldar et al., 2014; Luecke et al., 2021).

Early studies on the regulation of macrophage function were performed *ex vivo* on murine macrophages that were activated by singular stimuli. As a result, macrophage functional phenotypes were categorized in two: IFNγ-dependent classical (M1) activation and IL-4/IL-13-driven alternative (M2) activation. These two programs were presented as opposites, with M1 macrophages acting in pathogen defense, synthetizing nitric oxide and activating T_H1-type responses, while M2 macrophages metabolized arginine to ornithine and supported T_H2-type immune responses (Dalton et al., 1993; Doyle et al., 1994; Mills et al., 2000; Stein et al., 1992). Upon studying more macrophage-regulating compounds, different subtypes under these categories were termed and thought to form a spectrum between ultimate M1 and M2 macrophages (Edwards et al., 2006; Mantovani et al., 2004). This theory has been challenged by observations of both M1 and M2 macrophage hallmarks within the same cell, calling for function-based characterization of macrophage subsets, especially in complex environments, such as cancer (Cassetta et al., 2019; S. Cheng et al., 2021; Martinez & Gordon, 2014; Szulzewsky et al., 2015). Nevertheless, terms “M1-like” and “M2-like” have remained in use, when referring to more pro-inflammatory (M1) or anti-inflammatory (M2) macrophage phenotypes and functions.

More recently, macrophage responses to specific stimuli have been decoded with the help of systems biology, as sensed signals activate complex signaling circuits (Luecke et al., 2021). For instance, PRR signaling goes through multiple levels of regulated signaling events before activating specific transcription factors. First, activated receptors associate with adaptor proteins that regulate several downstream enzymes, including ubiquitin ligases, phosphatases and kinases. Here, the signaling branches into a network of different signaling complexes (An et al., 2008; Chuang & Ulevitch, 2004; Jiang et al., 2004; Kawai et al., 1999; Shih et al., 2009; Wang et al., 2009). The signaling network often includes negative feedback loops that self-regulate and finetune pathway activity (Kearns et al., 2006; Shih et al., 2009). Furthermore, the activated receptor may be translocated from cell surface inside endosomes for the activation of additional pathways, or inside lysosomes for receptor degradation (Kagan et al., 2008; Wang et al., 2007; Zanoni et al., 2011). Eventually, combinations of different activated pathways culminate in

transcriptional responses. Central transcription factors regulating macrophage function include NF-κB and AP-1 (pro-inflammatory effects downstream of TLRs and TNF family receptors) (Shih et al., 2009; Yao et al., 1997), signal transducer and activator of transcription (STAT)-family members (mediating effects of IFNs and IL-4) (Ohmori & Hamilton, 1998) and nuclear receptors LXRα and peroxisome proliferator-activated receptor γ (PPARγ) (mediating immune suppression) (Ito et al., 2015; Ricote et al., 1998). After transcription, processes controlling mRNA stability and translation can further uncouple the end-product levels from initial transcription, as has been shown for TNFα secretion (Caldwell et al., 2014).

Importantly, the effects of transcription factor activation depend on which target genes have their transcription factor binding sites open and available for binding. Therefore, the same stimulus may produce vastly different responses on two different macrophages. Already macrophage differentiation opens and closes various chromatin areas, regulated by core macrophage lineage-determining transcription factors PU.1, c-MAF, MAFB and CCAAT/enhancer binding protein alpha/beta (CEBPα/β) that keep macrophage-specific enhancers primed with histone methylations (Feng et al., 2008; Garber et al., 2012; Ghisletti et al., 2010; Hegde et al., 1999; Kelly et al., 2000). Tissue environment also changes macrophages' chromatin state, when tissue-specific transcription factors get activated and collaborate with the afore-mentioned lineage-determining transcription factors to either activate or prime enhancers in a tissue-specific manner (Gosselin et al., 2014; Lavin et al., 2014). This will affect what programs macrophages constitutively express and what tools they have available upon encountering new stimuli. Finally, stimulus-induced transcription factors mainly activate these already primed enhancers and co-operate with the lineage-determining transcription factors to support nearby gene expression (Garber et al., 2012; Ghisletti et al., 2010; Ostuni et al., 2013).

In reality, signals activating macrophages do not act in solitude. Instead, the tissue environment provides a continuous stream of signals for macrophages to interpret. In some cases, signal combinations lead to synergistic activation and stronger responses, as observed after simultaneous activation of multiple PRRs, lipopolysaccharide (LPS)-mediated priming of anti-microbial genes or IFNγ-mediated priming of inflammatory cytokines (Foster et al., 2007; Qiao et al., 2013; Sato et al., 2000). In contrast, an otherwise pro-inflammatory TLR response can be dampened by previous PRR or LXR/RXR transcription factor activation (Foster et al., 2007; Ito et al., 2015; Sato et al., 2000). Strikingly, in the presence of apoptotic cells, a bacterial PAMP, LPS, actually induces anti-inflammatory IL-10 and TGFβ secretion instead of the usual pro-inflammatory mediators (Byrne & Reen, 2002). These combinatory responses are regulated at various levels, including but not limited to adapter protein recruitment, chromatin modifications, and enhanceosome

assembly (Foster et al., 2007; Ito et al., 2015; Merika et al., 1998; Qiao et al., 2013). In terms of systems biology, multiple signaling pathways can converge at single signaling molecules or transcription factors that act as logical gates allowing the signal to go forward in the presence of multiple ("and" gate) or any ("or" gate) of its activators (Cheng et al., 2017; Luecke et al., 2021). Furthermore, activation of specific genes or generation of distinct epigenetic marks may require signaling of sufficient strength, duration or non-oscillatory pattern (Q. J. Cheng et al., 2021; Gottschalk et al., 2016; Son et al., 2022).

In summary, macrophages interpret the signals from their environment through a lens constructed from their immediate and previous surroundings. They combine different signals into their phenotype by letting intertwining signaling cascades boost and cancel out each other in order to present functions most suited to their current niches.

2.2.2 Tissue imprinting

To carry out tissue-specific functions, macrophages in different organs have highly different transcriptomes. Only a few macrophage-specific genes are equally expressed across most macrophage populations and even genes central to pathogen defense, such as several TLRs and chemokine receptors, exhibit varying expression patterns across macrophage populations. (Gautier et al., 2012; Lavin et al., 2014) Tissue-specific macrophage identities are generated by a combination of niche-specific signals that activate tissue-specific transcription factors to finetune the core macrophage program (Gosselin et al., 2014; Lavin et al., 2014; Mass et al., 2016). For instance, retinoic acid activates GATA binding protein 6 (GATA6) in large macrophages residing within the peritoneum and other bodily cavities, while GM-CSF-induced PPARγ drives the unique alveolar macrophage phenotype (Buechler et al., 2019; Okabe & Medzhitov, 2014; Schneider et al., 2014). Even within the same organ, different macrophage subsets can arise in this manner, as is demonstrated by the spleen's LXRα-dependent marginal zone and Spi-C-controlled red pulp macrophages (A-Gonzalez et al., 2013; Kohyama et al., 2009). Yet, similar macrophage populations can develop in different tissues, if the niches are similar (Chakarov et al., 2019). Mechanistically, activated niche-specific transcription factors prime enhancers in a tissue-specific manner to facilitate tissue-specific gene expression, whereas promoters often remain similarly used across tissues (Lavin et al., 2014). These environment-imposed transcriptional programs have been shown to get activated already during embryonic development (Mass et al., 2016).

Various types of surrounding cells can constitute the niche, including stromal, endothelial, epithelial and immune cells, depending on the tissue in question (Bonnardel et al., 2019; Buechler et al., 2019; Cohen et al., 2018; Mondor et al.,

2019). They provide specific ligands, metabolites, cytokines and matrix architecture to modulate macrophage phenotypes and function (Atcha et al., 2021; Buechler et al., 2019; McWhorter et al., 2013; Mondor et al., 2019; Snelgrove et al., 2008). For instance, CD200 on lung epithelium keeps alveolar macrophages hyporesponsive via macrophage CD200 receptor (Snelgrove et al., 2008).

Macrophage adaptation to the niche is not immediate, as newly-recruited monocyte-derived macrophages acquire some of the tissue-resident macrophage markers only after a sufficient time span dwelled in the niche (Sakai et al., 2019; Scott et al., 2016). Hence, newly-recruited macrophages first respond differently to threats than tissue-resident macrophages, but their phenotypes start to resemble those of tissue-resident macrophages after longer residency (Misharin et al., 2017).

As tissue-specific niches guide macrophage responses to specific stimuli (Svedberg et al., 2019), the permanence of macrophage tissue imprinting has been an important question. Transferring macrophages away from their original tissue niche does alter their responses to specific stimuli (Svedberg et al., 2019). For instance, transfer from the original tissue niche to *ex vivo* culture conditions causes alveolar macrophages to lose their hyporesponsiveness to IL-4, and peritoneal and pleural macrophages to lose their tolerance upon efferocytosis (Roberts et al., 2017; Svedberg et al., 2019). Strikingly, transferring peritoneal macrophages to lungs still switches 70% of tissue-specific macrophage gene expression to match the recipient tissue (Lavin et al., 2014). Yet, transferred macrophages cannot fully acquire the transcriptomic and functional profile of the recipient tissue macrophages, indicating that tissue residency limits macrophage plasticity (Lavin et al., 2014; van de Laar et al., 2016). Within these more rigid differentiation programs, macrophages can still assume distinct reversible polarization states upon encountering environmental stimuli, such as cytokines or PRR ligands, and the current polarization state modulates responses to subsequent stimuli (Cheng et al., 2020; Okabe & Medzhitov, 2016; Sheu et al., 2023; Stout et al., 2005).

As local niches significantly impact macrophage function, the relative importance of macrophage-intrinsic features, predominantly origin (yolk sac, fetal liver or bone marrow), has been investigated. First, if the original embryonic tissue-resident macrophages are depleted, monocyte-derived macrophages can carry out their tissue-specific functions (Beattie et al., 2016; van de Laar et al., 2016) – otherwise inflammation-induced macrophage suicide or tissue damage would have catastrophic consequences for the individual by compromising organ function. The newly-recruited macrophages can self-renew and acquire vastly similar, yet not identical, tissue-specific enhancer profiles and transcriptomes as original tissue-resident macrophages (Beattie et al., 2016; Ferrer et al., 2019; Gibbings et al., 2015; Gundra et al., 2014; Lavin et al., 2014; van de Laar et al., 2016). Upon direct comparison, however, local niche characteristics have been shown to dominate over

developmental origin in the regulation of macrophage chromatin state (Lavin et al., 2014). Despite this dominance of local tissue imprinting, differences in macrophage origin can still cause meaningful differences in responses to inflammatory assaults and provide competitive advantage for colonizing specific tissue environments (Shemer et al., 2018; van de Laar et al., 2016). Additional macrophage-intrinsic features contributing to their phenotype include individual's genetic variation and circadian rhythms (Keller et al., 2009; Link et al., 2018).

2.2.3 Macrophage memory

While the adaptive immune system is renowned for mounting antigen-specific memory responses, also innate immune system can recall past events (Hajishengallis et al., 2023; Netea et al., 2011). This memory has been observed in monocytes and macrophages, but also other innate immune cells, including dendritic and NK cells (Bistoni et al., 1988; Cooper et al., 2009; Hole et al., 2019; Quintin et al., 2012).

When innate immune memory generates stronger immune response upon second stimulation, the result is called trained innate immunity (Hajishengallis et al., 2023; Netea et al., 2011). After the first training stimulus, the generated memory can protect from subsequent pathogen infections (Bistoni et al., 1988; Quintin et al., 2012). Because innate immune memory is less specific, this protective effect, often depending on monocytes and macrophages, is extended beyond the original training stimulus (Aegerter et al., 2020; Barton et al., 2007; Higgins et al., 2016). For instance, viral infection in the lungs can protect from subsequent bacterial infections by training alveolar macrophages to respond with stronger chemokine secretion and neutrophil recruitment to detected bacteria (Yao et al., 2018). Innate immune memory can also be trained for weaker subsequent responses, termed tolerance, as repeated LPS exposure can downregulate macrophage pro-inflammatory gene expression (Divangahi et al., 2021; Foster et al., 2007) to potentially protect from inflammatory damage.

Unlike with the adaptive immune memory, the duration of innate immune memory varies. In murine models, the protective effects of previous pathogen encounter can disappear in a few months or even be transferred to the progeny (Aegerter et al., 2020; Katzmarski et al., 2021). In human monocytes, protective effects have been demonstrated at least after three months (Kleinnijenhuis et al., 2012). Similarly, tolerance is a dynamic state, which can be reversed by introducing a new immune-training stimulus or transferring the paralyzed macrophages from the infection-experienced host to a naïve recipient (Novakovic et al., 2016; Roquilly et al., 2020). The memory duration naturally depends on cellular life spans, and once the inflammatory cells have disappeared, the memory can be maintained by long-lived tissue-resident macrophages or HSCs (Kaufmann et al., 2018; Yao et al., 2018).

The former is termed peripheral memory, and it is sometimes propagated to a new host via macrophage transfer (Chen et al., 2014; Netea et al., 2020; Zahalka et al., 2022). The latter, central memory, relies on long-term HSCs that mount more prominent myelopoietic responses to generate epigenetically modified macrophages for enhanced immune protection (Kaufmann et al., 2018; Netea et al., 2020).

At the molecular level, innate immune memory is propagated by epigenetic modifications induced by the training (Quintin et al., 2012). The initial stimulus induces histone modifications at specific locations, such as histone H3 lysine 4 methylation (H3K4me1), trimethylation (H3K4me3), lysine 18 lactylation (H3K18la) or lysine 27 acetylation (H3K27ac), in the enhancer regions and promoters of macrophage pro-inflammatory genes (Quintin et al., 2012; Saeed et al., 2014; Ziogas et al., 2025). Some of the acquired changes can persist after the original stimulus has disappeared and leave the affected genes primed for faster and stronger trained immune responses (Ostuni et al., 2013; Quintin et al., 2012; Saeed et al., 2014). Similarly, induction and reversal of tolerance depend on loss and acquisition of histone modifications, such as histone acetylation, at specific promoters and distal regulatory elements (Novakovic et al., 2016). The introduction and persistence of these epigenetic changes are facilitated by alterations in metabolism. In particular, elevated glycolysis, glutaminolysis and cholesterol synthesis are metabolic hallmarks of trained immunity (Arts et al., 2016; Bekkering et al., 2018; Cheng et al., 2014; Zahalka et al., 2022). For instance, glutaminolysis and glycolysis promote accumulation of fumarate that inhibits a specific histone demethylase that would otherwise remove H3K4me3 marks (Arts et al., 2016).

These epigenetic mechanisms, however, represent a broader epigenetic control observed across cell types, rather than a macrophage- or innate immunity-specific feature (Allis & Jenuwein, 2016). Nevertheless, they add an important layer of regulation to macrophage responses upon recurring stimuli.

2.3 Tumor-associated macrophages (TAMs)

Malignant cells abuse their tissue environment to obtain oxygen, nutrients and growth signals and license surrounding cells to support their agenda of unrestricted growth and spread. Besides cancer cells, tumor microenvironment (TME) contains abnormally leaky vasculature, stromal cells, surrounding tissue-specific normal cells and immune cells. (Hanahan & Weinberg, 2011) While the immune system initially disrupts cancer progression, over time the immunological pressure promotes survival of immune-elusive cancer cells in a process called cancer immunoediting (Angelova et al., 2018; Schreiber et al., 2011; Shankaran et al., 2001). The immune escape is accompanied by recruitment and differentiation of tumor-promoting immune cells, including TAMs (Bohn et al., 2018; Curiel et al., 2004; Hanahan & Weinberg, 2011).

Accordingly, abundant pro-inflammatory (M1-like) TAMs, anti-tumoral $CD8^+$ T cells and lymphocyte-rich tertiary lymphoid structures mostly indicate better prognosis, while anti-inflammatory (M2-like) TAMs associate with worse prognosis (Fridman et al., 2017). Total TAM abundancy, nevertheless, predicts poorer prognosis across cancer types, with the exception of colorectal cancer (Bruni et al., 2020; Fridman et al., 2017; Zhang et al., 2012). Therefore, TAMs appear as detrimental immunosuppressive regulators of the whole TME, and their putative anti-tumoral potential often gets dampened.

2.3.1 Cancer promotion by tumor-educated macrophages

Both original tissue-resident macrophages as well as recruited monocytes contribute to the evolving TAM population (Z. Chen et al., 2017; Loyher et al., 2018; Zhu et al., 2017), but cancer drastically alters monocyte and macrophage function. To begin with, cancer alters hematopoiesis to support myeloid cell production over lymphocyte generation, and cancer cells secrete CCL2 and CCL5 chemokines to recruit an abundance of monocytes into tumors (Loberg et al., 2007; Robinson et al., 2003; Soria et al., 2008; Wu et al., 2014). Monocyte differentiation into macrophages within the TME gives rise to primarily immunosuppressive and tumor-promoting phenotypes as well as additional immature suppressive cells (DeNardo et al., 2009; Kitamura et al., 2017; Lin et al., 2001). The unique conditions in the TME result in TAM phenotypes surpassing the one-dimensional classically or alternatively activated macrophage states (Biswas et al., 2006; Cassetta et al., 2019; S. Cheng et al., 2021; Franklin et al., 2014; Szulzewsky et al., 2015). In fact, a multitude of systemic, organ-specific, TME-derived and TAM-intrinsic factors, such as specific cytokines, signaling proteins and transcription factors, contribute to macrophage phenotypes within the TME (DeNardo et al., 2009; Doedens et al., 2010; Kaneda et al., 2016; Sheban et al., 2025; Sica et al., 2000), as reviewed by Kloosterman and Akkari (Kloosterman & Akkari, 2023), but their relative importance remains to be established. Nevertheless, the end result is such an effective immunosuppressive program that introducing typically inflammatory cues only further supports anti-inflammatory TAM functions (Biswas et al., 2006; Sica et al., 2000).

Despite this strong TAM-conditioning by the TME, macrophages themselves possess limited tumoricidal functions under favorable circumstances. These include reactive radical production, phagocytosis, induction of cancer cell apoptosis and antigen presentation that can activate cytotoxic $CD8^+$ T cells and IFNγ-secreting $CD4^+$ T cells (Chen et al., 2002; Gordon et al., 2017; Hibbs et al., 1988). These functions can be reactivated with suitable therapeutic interventions, either by directly targeting TAMs, as reviewed below, or using therapies that additionally activate them. For instance, low-dose irradiation can program TAMs to recruit cytotoxic T

cells, and successful T-cell-based immunotherapy is accompanied by pro-inflammatory TAM activation (Klug et al., 2013; van Elsas et al., 2024).

Unfortunately, cancer cells adapt to the immunological attacks delivered by TAMs and other leukocytes. To avoid macrophage phagocytosis, cancer cells express "don't-eat-me" signals, such as the renowned CD47 (Jaiswal et al., 2009). To escape from T cells, cancer cells enlist assistance from TAMs by creating an acidic, hypoxic and high-lactate TME that generates immunosuppressive TAMs to inhibit T-cell responses (Bohn et al., 2018; Colegio et al., 2014; Doedens et al., 2010). TAMs can then suppress T-cell activation directly via inhibitory PD-L1 (B7-H1) and B7-H4 molecules and indirectly via anti-inflammatory IL-10 secretion (Bloch et al., 2013; Kryczek et al., 2006; Li et al., 2018; Lin et al., 2018; Ruffell et al., 2014; Wu et al., 2009). TAMs can also prevent $CD8^+$ T cells from reaching their cancer cell targets by either trapping T cells into prolonged contacts or altering extracellular matrix (ECM) composition for poorer T-cell migration (Afik et al., 2016; Peranzoni et al., 2018; Quaranta et al., 2018). Furthermore, TAMs can shut down ongoing T-cell responses by promoting T-cell apoptosis, exhausted T-cell states and CCL20/CCL22-mediated recruitment of T_{REG} cells (Curiel et al., 2004; Kersten et al., 2022; Liu et al., 2011; Saio et al., 2001; Yu et al., 2021).

TAM-mediated tumor promotion involves not only assistance in immune evasion, but direct support of cancer cell growth and spread. **Figure 3** and the text below summarize how TAMs promote cancer progression throughout the metastatic cascade, i.e. the steps from primary tumor growth to formation of metastatic lesions. For simplicity, the discussion here will focus on metastasis via blood vessels, while TAMs are similarly known to promote cancer cell metastasis via the lymphatic vessels (Bieniasz-Krzywiec et al., 2019).

In primary tumors, TAMs secrete growth factors, such as epidermal growth factor (EGF), to support cancer cell proliferation (O'Sullivan et al., 1993). To supply the expanding tumor with oxygen and nutrients, TAMs also promote expansion of the vascular network by releasing pro-angiogenic factors, including VEGFs and CCL18 (Barbera-Guillem et al., 2002; Gimbrone et al., 1972; Lin et al., 2007; Lin et al., 2015). Cancer cells of epithelial origin undergo epithelial-mesenchymal transition (EMT) to become more invasive and migratory, which TAMs promote by secreting TGFβ (Bonde et al., 2012; Giampieri et al., 2009). For easier migration, TAMs secrete ECM-modifying enzymes to degrade the underlying ECM and also induce cancer cells to do the same (Bieniasz-Krzywiec et al., 2019; L. Gao et al., 2016; Gocheva et al., 2010). Once on the move, cancer cells actually co-migrate alongside TAMs and coordinate this mutual movement with reciprocal M-CSF/CCL2 and EGF secretory loops (L. Gao et al., 2016; Goswami et al., 2005; Wyckoff et al., 2004). Upon reaching the vasculature, cancer cells are assisted by perivascular macrophages in their entry within the vessel, called intravasation. These

macrophages form contact points with endothelial cells and the intravasating cancer cell to induce transient vascular permeability and facilitate cancer cell transendothelial migration (Harney et al., 2015; Robinson et al., 2009; Roh-Johnson et al., 2014; Wyckoff et al., 2007)

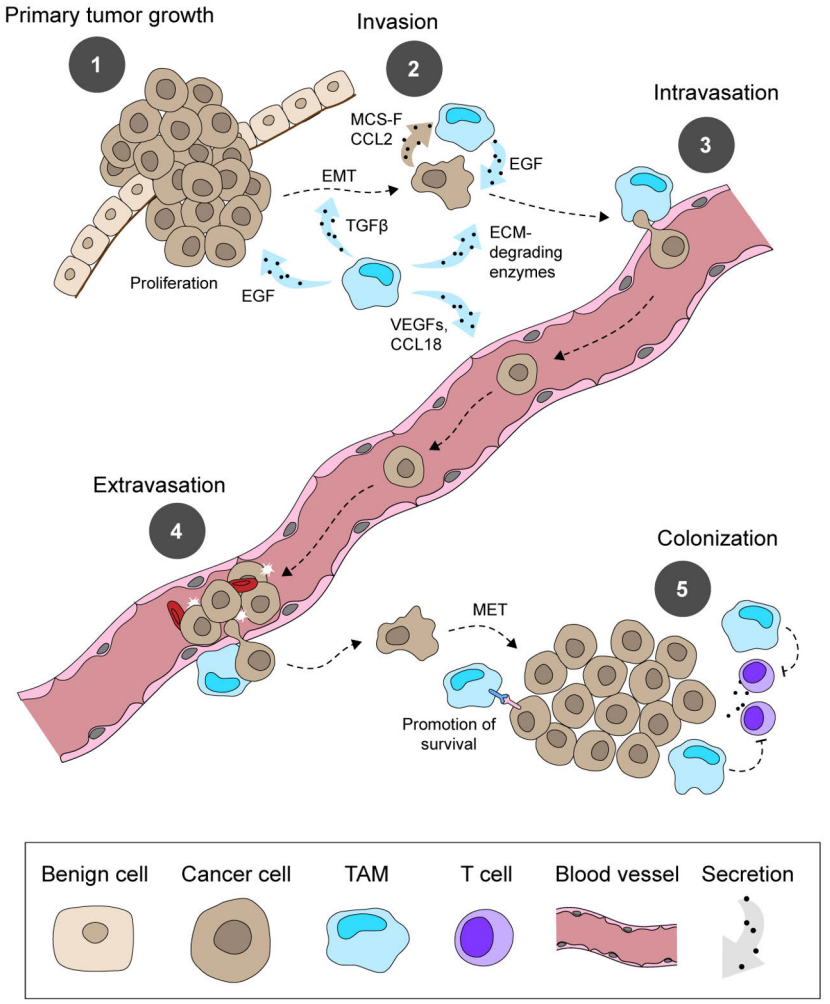


Figure 3. Macrophages support cancer progression at each step of the metastatic cascade. The schematic depicts hematogenous metastasis formation. **1,** TAMs promote cancer cell proliferation (EGF) and angiogenesis (VEGF, CCL18) to support primary tumor growth. **2,** TAMs induce EMT (TGFβ), modulate ECM and co-migrate with cancer cells (MSF-F/CCL2 and EGF) to facilitate invasion. **3,** TAMs in contact with a cancer cell and endothelial cells regulate vascular permeability and cancer cell intravasation. **4,** A clot of cancer cells at a distant site, where macrophages support cancer cell extravasation. **5,** Metastasis-associated macrophages promote metastasis seeding and growth by inducing MET, providing ligands for cancer cell survival signaling and inhibiting T-cell responses. MET, mesenchymal-epithelial transition.

At the sites of cancer cell extravasation, macrophages assist cancer cells in entering the underlying tissue (Qian et al., 2009). The macrophage population at the metastatic site increases by both enhanced recruitment of inflammatory monocytes and local proliferation (Qian et al., 2011; Sharma et al., 2015). Metastasis-associated macrophages then help the initial metastasis seeding and further growth (Qian et al., 2009; Qian et al., 2011). Cancer cells seek coverage from potential immune attacks by binding macrophage α4-integrins with their VCAM-1 molecules to obtain survival-promoting signals (Chen et al., 2011). Macrophages can also further suppress IFNγ-secreting T-cell activation and dendritic cell maturation (Sharma et al., 2015). Surviving cancer cell proliferation and metastasis growth is then accelerated by reversal of the original EMT, a process supported by myeloid cells at the metastatic sites (Gao et al., 2012).

These various roles of TAMs in cancer are by no means performed by a uniform TAM population capable of all the different functions. Instead, subpopulations of TAMs act in a context-guided manner at different tumor locations, as we will see next.

2.3.2 TAM functions defined by intratumoral localization

The exploration of macrophage diversity within tumors has been accelerated by techniques such as multiplex imaging, spatial transcriptomics and single-cell RNA-sequencing (scRNA-seq). In particular, large scRNA-seq atlases of human macrophages across various cancer and healthy tissues have been constructed to identify macrophage subtypes shared between different tissues. (S. Cheng et al., 2021; Coulton et al., 2024; Mulder et al., 2021) These atlases identify remarkably overlapping TAM subsets, which likely represent genuine TAM populations common to various cancer types and performing distinct functions regulated by their TME niches. Below, I will describe macrophage phenotypes identified by several single-cell macrophage atlases, including resident, matrix-remodeling, pro-angiogenic, lipid-associated, inflammatory, IFN-induced and proliferating macrophages. Their marker genes, functions and localization in the TME have been summarized in **Table 1**.

Resident macrophages across tissues express a core program characterized by *LYVE1*, *FOLR2* and *HES1* alongside additional tissue-specific genes, such as *PPARG* and *FABP4* in alveolar macrophages or *FTL*, *HBB* and *HMXO1* in iron-recycling macrophages (S. Cheng et al., 2021; Coulton et al., 2024; Mulder et al., 2021). Naturally, their prevalence is higher in healthy tissues (S. Cheng et al., 2021). During early cancer development, resident macrophages can regulate invasion and immune evasion, and in established tumors they often become distributed at the

tumor periphery (Casanova-Acebes et al., 2021) or trapped within the fibrotic areas between growing cancer cell islands (Matusiak et al., 2024).

Classical tumor-supporting macrophage functions, matrix remodeling and promotion of angiogenesis define specific TAM subpopulations. Matrix-remodeling TAMs express collagen genes and secreted protein acidic and rich in cysteine (*SPARC*), and associate with immune checkpoint inhibitor (ICI) therapy resistance (Coulton et al., 2024; subcluster of #13 in Mulder et al., 2021). Pro-angiogenic *SPP1*$^{+}$ TAMs are found in hypoxic and necrotic tumor areas and associate with poor prognosis. In some cancer types, however, *SPP1*$^{+}$ TAMs are missing and their functions in angiogenesis are carried out by some other TAM subset. (S. Cheng et al., 2021; Matusiak et al., 2024)

Table 1. TAM phenotypes identified across macrophage scRNA-seq atlases. The table shows TAM subset marker genes in scRNA-seq data, TAM subset function and localization in the TME, and correlation of TAM subset abundancy with ICI efficacy based on analyses of pre-treatment samples.

TAM SUBSET	MARKERS	FUNCTION	LOCALIZATION	ICI EFFICACY CORRELATION
Resident	*FOLR2, HES1, LYVE1*	?	Tumor periphery, fibrotic areas	
Matrix-remodeling	*COL1A1, COL1A2, COL6A3, SPARC*	Extracellular matrix remodeling	?	ICI resistance
Pro-angiogenic	*SPP1*	Angiogenesis	Hypoxic and necrotic areas	
Lipid-associated	*APOC1, APOE, FABP5, LIPA, MSR1, TREM2, PDCD1*	Anti-inflammatory, T-cell suppression	Invasive front	ICI resistance
Inflammatory	*CXCL1, CXCL2, IL1B, NLRP3*	Cytokine secretion, neutrophil recruitment	Neutrophil-rich areas	
IFN-induced	*CD274, CXCL9, CXCL10, CXCL11, IDO1, IL4I1*	Efferocytosis, T-cell exhaustion, T_{REG} promotion	Areas with high cell turnover	ICI response
Proliferating	*CDK1, CDKN3, MKI67*	?	?	

Lipid-associated TAMs are distinguished by their altered lipid metabolism (e.g. upregulation of *APOE*, *APOC1*, *LIPA*, *FABP5* and *PLIN2*), which results in lipid accumulation that promotes their anti-inflammatory phenotype, immunosuppressive gene expression (*TREM2*, *PDCD1*, *CD276*, *LAG3*, *MSR1*) and oxidative phosphorylation (Coulton et al., 2024; Jaitin et al., 2019; Su et al., 2020; Timperi et

al., 2022). Similar dysfunctional macrophage populations have been identified in Alzheimer's disease and obese mice (Jaitin et al., 2019). In cancer, they associate with poor prognosis and immunotherapy resistance (Donadon et al., 2020; Liu et al., 2022; Xiong et al., 2020). Depending on the depth of clustering, one or two lipid-associated macrophage subsets can be identified (Coulton et al., 2024; Mulder et al., 2021; Timperi et al., 2022; Wu et al., 2021). Predominantly tumor-associated and monocyte-derived lipid-associated macrophages (*STAB1*$^{+}$*MRC1*$^{+}$*CD163*$^{+}$*MAFB*$^{+}$) suppress T-cell responses at the invasive front, while the other subset is equally abundant in healthy and tumor tissues (Timperi et al., 2022).

TAMs subsets predominantly defined by their regulation of immune responses include inflammatory TAMs and IFN-induced TAMs. Inflammatory TAMs have activated inflammasomes and express an array of pro-inflammatory cytokines and chemokines, contributing to the neutrophil-rich niches they reside in (Coulton et al., 2024; Matusiak et al., 2024). IFN-induced TAMs express IFN-upregulated chemokines (*CXCL9*, *-10*, *-11*), immunosuppressive enzymes (*IL4I1*, *IDO1*) and immune checkpoint molecules (*CD274*) (Coulton et al., 2024; Mulder et al., 2021). They predict response to ICI therapy (Coulton et al., 2024; Matusiak et al., 2024), perform efferocytosis in areas of high cell turnover and are thought to support T-cell exhaustion and T_{REG} cells (Matusiak et al., 2024; Mulder et al., 2021).

Lastly, proliferating monocytes and macrophages are readily identified by scRNA-seq, as they distinctly express proliferation-associated genes (*MKI67*, *CDK1*, *CDKN3*) and usually cluster further away from other macrophages (Coulton et al., 2024; Mulder et al., 2021; Wu et al., 2021). Their clinical significance remains controversial, as their abundance has been associated with both better and worse outcomes (Coulton et al., 2024; Duval et al., 2025).

Altogether, these different TAM subsets residing in their distinct niches comprise a heterogeneous group of cells. The tight coupling of TAM subsets with their respective niches is demonstrated at the boundary zones between different niches, where mixed phenotypes can be found (Matusiak et al., 2024). TAM heterogeneity has important consequences for therapeutic targeting, as most promising therapeutics would disturb only TAM subsets with tumor-promoting functions. Furthermore, more sophisticated models are necessary for investigating therapeutic effects, as *in vitro* cultures and orthotopic murine tumor models (Laviron et al., 2022) fail to capture this heterogeneity.

2.4 Macrophage-targeted cancer immunotherapy

Across more than 70,000 patients with cancer and 15 analyzed immune cell types, M2-like TAMs most uniformly predict poor prognosis (Bruni et al., 2020). Yet, TAMs localized within tumor cell islands or TAMs upon therapeutic intervention

can possess anti-tumoral properties and improve patient prognosis (Di Caro et al., 2016; Wu et al., 2016). Therefore, cancer immunotherapies that could disrupt TAM-regulated cancer promotion or manipulate TAMs to unleash their anti-tumoral potential are extensively investigated.

2.4.1 Fundamentals of cancer immunotherapy

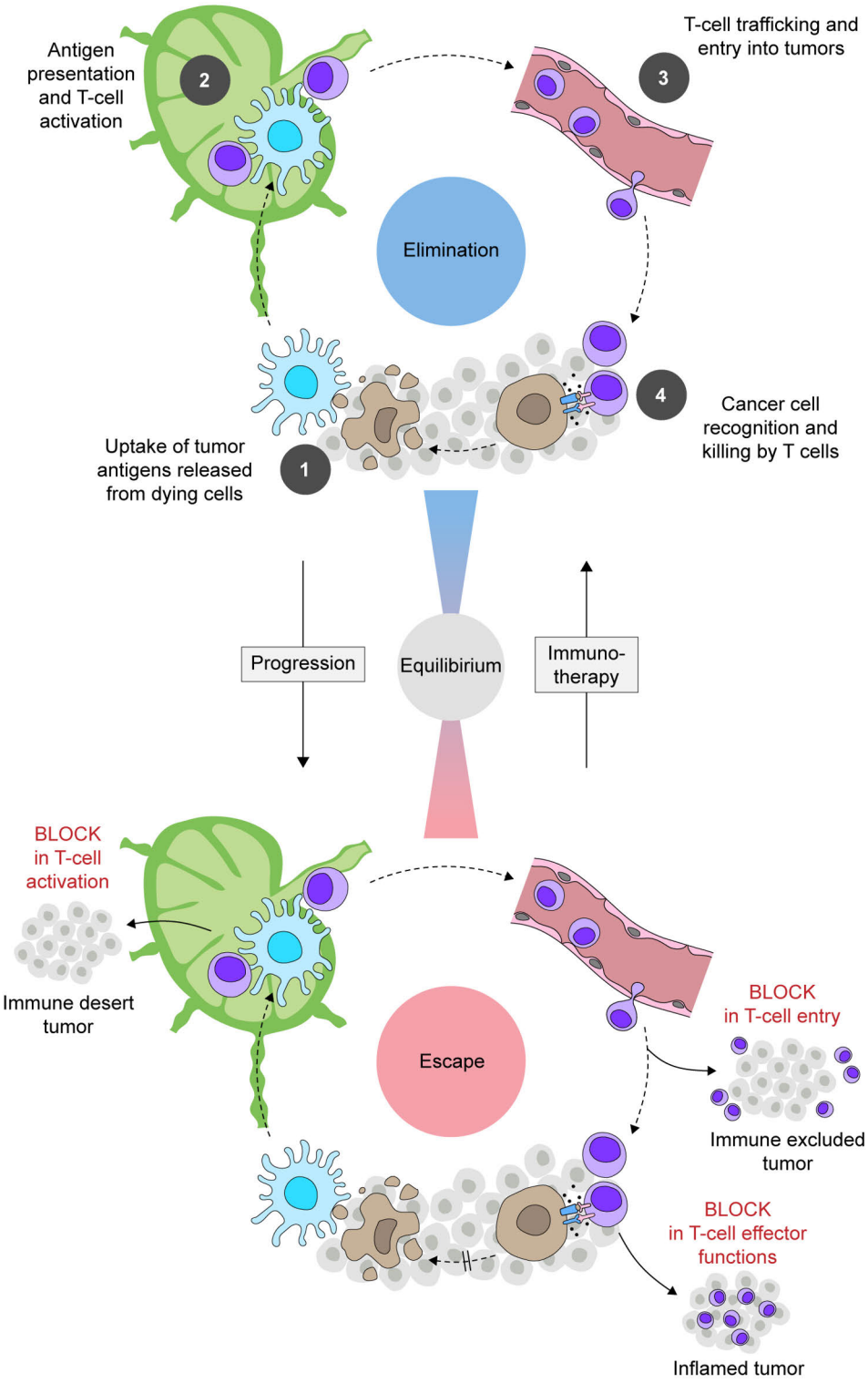
Cancer immunotherapy has various forms, and therapeutics approved for clinical use range from cancer vaccines to infused cells and antibodies (Cheever & Higano, 2011; Kazandjian et al., 2016; O'Leary et al., 2019; Prieto et al., 2012; Sul et al., 2016; Weinstock et al., 2017). These immunotherapies elicit anti-tumoral T-cell responses by infusing the patient with autologous antigen-loaded dendritic cells (sipuleucel-T), T cells engineered to recognize specific tumor antigens (chimeric antigen receptor T cells) or antibodies that block inhibitory immune checkpoint molecules (ICIs). Inhibitory immune checkpoint molecules, including CTLA-4 (cytotoxic T-lymphocyte antigen 4), PD-1 (programmed cell death protein 1) and PD-L1 (programmed cell death 1 ligand 1) would otherwise suppress T-cell priming and activation. (Burch et al., 2000; Freeman et al., 2000; Krummel & Allison, 1995; Leach et al., 1996). Yet, reactivating anti-tumor immunity for durable clinical responses remains challenging in patients with cancers that already utilize various routes for immune escape (Hegde & Chen, 2020; O'Donnell et al., 2019; Sharma et al., 2021). Objective response rates for ICIs often range from 10 to 40% depending on the indication (Hodi et al., 2010; Kazandjian et al., 2016; Larkin et al., 2018; Sul et al., 2016; Weinstock et al., 2017; Wolchok et al., 2025). Infused chimeric antigen receptor T cells show high response rates (45% - 90%) in hematological cancers, but their efficacy in solid tumors is lower (5% - 50%) (Cappell & Kochenderfer, 2023; Du et al., 2025; O'Leary et al., 2019). For some of these patients, the treatment is curative, but even patients with complete responses may relapse later on (Cappell & Kochenderfer, 2023; O'Leary et al., 2019; Robert et al., 2015).

In general, a full T-cell-mediated anti-tumor response forms after several successive steps, described as the cancer immunity cycle (**Figure 4**). (Chen & Mellman, 2013; Mellman et al., 2023) The cycle starts with the release of tumor antigens from dying cancer cells. These antigens are presented to T cells in the tumor-draining lymph nodes for their priming and activation. Then, the activated T cells are recruited into tumors, extravasate and infiltrate into cancer cell islands. Cytotoxic T cells recognize cancer cells based on their antigen presentation and within favorable tissue environment proceed with target cell killing, which releases more antigens to fuel the cycle. (Chen & Mellman, 2013; Mellman et al., 2023). This cycle can be arrested at various stages, resulting in distinct patterns of T-cell infiltration within the tumor (**Figure 4**). Completion of each step in the cycle is

affected by various stimulatory and inhibitory factors, which can be cancer-intrinsic or related to the patient's genetics, microbiome or environment. This results in a patient-specific cancer-immune profile that regulates the anti-tumor immune response (D.S. Chen & Mellman, 2017). Furthermore, based on the mechanisms that have prevented the cycle from proceeding in a specific tumor, certain types of cancer immunotherapies are more likely to confer therapeutic benefit (D. S. Chen & Mellman, 2017; Mellman et al., 2023).

Therefore, the level of immune cell infiltration provides clinically valuable information and associates with cancer prognosis (Camus et al., 2009; Galon et al., 2006). Tumors with high T-cell infiltration both within the tumor and in its periphery are called inflamed or immune hot (Galon & Bruni, 2019). Their cancer-immunity cycle, halted at the last steps of cancer cell recognition and killing, can benefit from ICIs that release PD-1/PD-L1-mediated T-cell suppression (Herbst et al., 2014; McDermott et al., 2016; Tumeh et al., 2014). On the contrary, non-inflamed or so-called immune cold tumors lack T-cell infiltration altogether due to deficiency in tumor antigens, antigen presentation, T-cell priming or activation, which causes ICI resistance (Ayers et al., 2017; D. S. Chen & Mellman, 2017; Galon & Bruni, 2019). Because of this early block in the cancer-immunity cycle, treatment combinations are investigated to both enhance T-cell priming and remove inhibitory signals from the TME (Galon & Bruni, 2019), which should support the completion of a full cancer-immunity cycle. For instance, conventional cancer therapies promote antigen release and antigen-specific T-cell activation to support ICI efficacy (Mathios et al., 2016). Intermediate levels of T-cell infiltration in immune altered tumors can result from local immunosuppression that prevents full T-cell expansion and responses. Immune excluded tumors, on the other hand, have high T-cell infiltration only at the tumor periphery, caused by poor T-cell recruitment, dysfunctional tumor vasculature or unfavorable ECM architecture. (Galon & Bruni, 2019).

Tumors can be categorized into these immunological subtypes by quantifying total ($CD3^+$) T-cell and cytotoxic ($CD8^+$) T-cell infiltration at the invasive margin and the tumor core (Marliot et al., 2020; Pagès et al., 2018). The results are combined into an Immunoscore, which has been shown to correspond or even surpass TNM staging in the power of predicting overall survival (Marliot et al., 2020; Pagès et al., 2018). Depending on the exact method, the number of resulting categories can vary from two to five (Galon & Lanzi, 2020), while biomedical research literature often uses a simplistic dual categorization into immune cold and hot tumors.


Antigen presentation and T-cell activation
2
T-cell trafficking and entry into tumors
3
Elimination
4
Cancer cell recognition and killing by T cells
Uptake of tumor antigens released from dying cells
1
Progression
Equilibirium
Immuno-therapy
BLOCK in T-cell activation
Immune desert tumor
Escape
BLOCK in T-cell entry
Immune excluded tumor
BLOCK in T-cell effector functions
Inflamed tumor

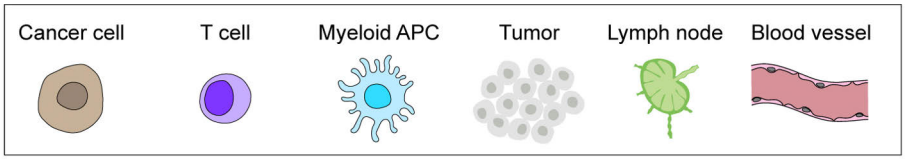


Figure 4. Fundamental concepts in cancer immunology and immunotherapy. Above, the cancer-immunity cycle illustrates the necessary steps for generating anti-tumor T-cell responses. Mid-line, the three E's of cancer immunoediting: elimination, equilibrium and escape. Immune system first mounts effective immune attacks against the tumor (elimination). The immunological pressure, however, selects for subsets of cancer cells capable of surviving the immune attacks (cancer immunoediting), generating a balance between immunological destruction and appearance of more elusive cancer cells (equilibrium). After sufficient time, the cancer progresses and escapes from the immune system (escape), while immunotherapies aim to re-activate the elimination phase. Below, T-cell infiltration within a tumor reflects, which steps of the cancer-immunity cycle were completed successfully. APC, antigen-presenting cell. The cancer-immunity cycle was adapted from Chen and Mellman, 2013; the three E's of cancer immunoediting were illustrated based on Schreiber, Old and Smyth, 2011; the association between the cancer-immunity cycle and T-cell infiltration was depicted based on D. S. Chen & Mellman, 2017 and Galon & Bruni, 2019.

TAMs fit into this paradigm by promoting tumor immune escape via T-cell exclusion, immune suppression and immune checkpoint molecule expression, as reviewed above. Yet, their anti-tumoral activation is critical for therapeutic responses to T-cell-based cancer immunotherapy (van Elsas et al., 2024). Furthermore, TAMs bind therapeutic antibodies with their FcγRs, which can either limit antibody availability for binding the intended target or induce beneficial antibody-dependent effector functions (Arlauckas et al., 2017; Richards et al., 2008). Besides, TAMs themselves pose a feasible therapeutic target with their various tumor-promoting mechanisms and unused potential for cancer cell phagocytosis and antigen presentation, as described above.

2.4.2 TAM-modulating therapeutic strategies

Already in the late 1980s, cancer patients were administered with *in vitro*-expanded and IFNγ-activated macrophages derived from patient's own monocytes (Andreesen et al., 1990; Faradji et al., 1991). At the time, the ability of monocytes and macrophages to kill cancer cells had been discovered, but the performed small-scale clinical studies commonly reported non-existing efficacy (Andreesen et al., 1998). Another early approach, PRR activation on myeloid cells, was successful in specific indications, such as imiquimod for topical use, Bacillus Calmette-Guérin vaccine for bladder cancer and mifamurtide for osteosarcoma (Ando et al., 2011; Vacchelli et al., 2012).

Since then, dual functions of TAMs within the TME have been discovered. As TAMs were shown to promote cancer progression and metastatic spread (Lin et al., 2001), subsequent strategies aimed to deplete TAMs or prevent monocytes from entering tumors. After showing that CSF1R-targeted disruption of TAMs reduces murine tumor growth, CSF1R-targeting inhibitors and antibodies entered clinical development (Butowski et al., 2016; Ries et al., 2014; Yan et al., 2017). Durable responses were seen in diffuse tenosynovial giant cell sarcomas that overexpress the CSF1R ligand M-CSF (Cassier et al., 2020; Ries et al., 2014), but otherwise the efficacy has been limited (Butowski et al., 2016), as tumors adapt to CSF1R inhibition with compensatory CSF2-driven TAM phenotypes and enhanced recruitment of tumor-promoting granulocytic cells (Klemm et al., 2021; Kumar et al., 2017). Furthermore, while CSF1R and its ligands regulate macrophage survival and population size, CSF1R inhibitors actually altered TAM phenotypes rather than depleted TAMs (Dai et al., 2002; Wei et al., 2010; Yan et al., 2017; Zhou et al., 2022). More recently, targeted approaches to deplete only specific TAM subsets, such as CD163- or TREM2-expressing TAMs, have been introduced, but they are yet to demonstrate clinical efficacy (Etzerodt et al., 2019; Yeku et al., 2025).

As a concurrently developed alternative to TAM depletion, monocyte-recruiting chemokines or their receptors were inhibited (Noel et al., 2020; Sandhu et al., 2013), since preclinical studies had shown how recruited monocytes were necessary for metastasis development (Qian et al., 2011). In the frontline, clinical studies on CCL2/CCR2 blockade failed to show efficacy due to a strikingly high (>1000-fold) adaptive increase in CCL2 levels, compensatory neutrophil recruitment and rebound metastasis promotion after therapy discontinuation (Bonapace et al., 2014; Noel et al., 2020; Nywening et al., 2018; Sandhu et al., 2013).

After the shortcomings of these two approaches, a third avenue has emerged. TAM reprogramming aims to utilize macrophage plasticity in order to transform TAM functions from tumor-promoting to anti-tumoral. Reprogramming is a very broad umbrella term, and widely speaking anything that alters TAM phenotype can be grouped under this category, including agents not specific to TAMs. In the light of section 2.2, truly specific TAM reprogramming would target central regulators of niche-specific TAM phenotypes, thereby modifying the whole TAM identity, allowing it to react differently to external stimuli. Nevertheless, both more and less specific strategies for TAM reprogramming are briefly discussed below.

Currently, most clinically investigated TAM-targeted treatments rather reprogram TAM functions than their phenotype. For functional reprogramming, TAM activation state can be modified with ligands for their PRRs or stimulatory receptors, by blocking their suppressive receptors or by altering specific TAM functions. Besides the clinically approved PRR targeting agents, a plethora of other PRR (TLRs, STING, RAGE, Dectin-2) activators have been clinically investigated

(Choi et al., 2022; Galluzzi et al., 2012; Giordano et al., 2023; Huang et al., 2023), but suffer from limited efficacy and induction of tolerance (Bourquin et al., 2011; Diab et al., 2025; Manegold et al., 2012; Meric-Bernstam et al., 2022). Currently targeted stimulatory receptors (CD40, TREM1) potentiate myeloid cell cytokine secretion and expression of MHC and costimulatory molecules to support T-cell responses, but the more studied CD40-agonism has suffered from both low efficacy and safety concerns (Caux et al., 1994; Dower et al., 2008; Salomon & Dahan, 2022; Yeku et al., 2025). Likewise, currently targeted suppressive receptors (VISTA, TREM2, Siglec-15, LILRB family) are not TAM-specific, but commonly expressed on other leukocyte subsets. Blocking their suppressive functions, such as TGFβ secretion and T-cell inhibition, supports immune responses in the TME, but their clinical efficacy is yet to be established. (Iadonato et al., 2023; Molgora et al., 2020; Siu et al., 2022; Spira et al., 2023; Takamiya et al., 2013).

More specific functional reprogramming involves promotion of phagocytosis by targeting phagocytosis checkpoints, inhibition of suppressive enzymatic activity or tolerogenic scavenging. Phagocytosis checkpoint inhibitors promote cancer cell phagocytosis by preventing interactions between cancer-cell-expressed “don’t-eat-me” molecules (CD47 and CD24) and their receptors on macrophages (Signal-regulatory protein α [SIRPα] and Siglec-10, respectively) (Barkal et al., 2019; Sikic et al., 2019; Tsai & Discher, 2008). First trials reported objective responses especially for combinatory treatment regimens, while on-target side effects, such as anemia, result from CD47 expression on normal cells and complicate their therapeutic use (Advani et al., 2018; Sikic et al., 2019). Inhibiting IDO1 enzymatic activity with small molecule inhibitors supports T-cell activation by preventing tryptophan catabolism and production of immunosuppressive kynurenine metabolites (Fallarino et al., 2006; Koblish et al., 2010; Opitz et al., 2011). Unfortunately, a large phase 3 clinical trial failed to achieve therapeutic benefit, possibly due to compensatory enzymes in the TME and inadequate dose in relation to IDO1 levels upregulated by concurrent ICI therapy (Gomes et al., 2018; Long et al., 2019; Zeitler & Murray, 2023). Therapeutics inhibiting scavenger receptors (CD163, macrophage receptor with collagenous structure [MARCO]) are in preclinical or early clinical stage, and unleash $CD8^+$ T-cell responses by pro-inflammatory conversion of immunosuppressive TAM subsets (Georgoudaki et al., 2016; La Fleur et al., 2021; Tolcher et al., 2022).

For phenotypic reprogramming, TAM-expressed transcription factors (STATs, aryl hydrocarbon receptor, C/EBPα), signaling molecules (phosphoinositide 3-kinase γ [PI3Kγ]) or enzymes (histone deacetylaces) can be blocked. Transcription factors STAT3, STAT6 and aryl hydrocarbon receptor have been targeted with small molecule inhibitors or anti-sense oligonucleotides to disrupt the immunosuppressive transcriptional programs under their regulation (Kamerkar et al., 2022; McGovern et

al., 2022; Proia et al., 2020; Reilley et al., 2018), while inducing C/EBPα with small activating RNAs impedes myeloid-cell-mediated immunoregulation in patients with hepatocellular carcinoma (Hashimoto et al., 2021). PI3Kγ is another central regulator of TAM phenotype and its inhibition allows macrophages to better support T-cell immunity, which has translated into occasional therapeutic responses in combination with nivolumab in otherwise resistant patients (Hong et al., 2023; Kaneda et al., 2016). In preclinical studies, inhibition of class IIa histone deacetylases reprograms TAMs towards immunostimulatory phenotype, while the exact treatment mechanism remains elusive due to low enzymatic activity of this histone deacetylase class (Guerriero et al., 2017; Park & Kim, 2020).

Finally, over 30 years after the first attempts of macrophage infusion, this approach has been revived by using macrophages equipped with chimeric antigen receptors to enhance their phagocytosis (Klichinsky et al., 2020). In a small phase 1 clinical trial, these macrophages targeted against human epidermal growth factor receptor 2-expressing tumors, however, failed to elicit objective therapeutic responses (Reiss et al., 2025).

The overall clinical landscape of TAM reprogramming has been reviewed in the first publication of this thesis (I), highlighting the obstacles and opportunities in developing efficacious macrophage-guided immunotherapies. We next focus on the approach investigated in this thesis, inhibition of the scavenger receptor Clever-1.

2.5 Clever-1 as a cancer immunotherapy target

Clever-1 (also known as Stabilin-1, FEEL-1, MS-1 antigen) was discovered separately by several research groups, each describing one aspect of its complex biology. First, Goerdt and colleagues identified Clever-1 as a marker of non-continuous sinusoidal endothelial cells, while generating antibodies (MS-1) against human spleen (Goerdt et al., 1991). They additionally found MS-1 antigen (Stabilin-1) to be expressed on macrophages and to contain sorting signals for shuttling between subcellular compartments (Goerdt et al., 1993; Kodelja & Goerdt, 1994; Politz et al., 2002). Then, a novel scavenger receptor (FEEL-1) was discovered to mediate the uptake of modified low-density lipoproteins (LDLs) (Adachi & Tsujimoto, 2002) and a novel adhesion molecule, expressed both on lymphatic and vascular endothelium, was identified (Irjala, Elima, et al., 2003). These three proteins turned out to be the same multifunctional receptor molecule (Adachi & Tsujimoto, 2002; Irjala, Elima, et al., 2003). Subsequent discoveries on Clever-1 functions, particularly in cancer, have paved the way for a Clever-1-blocking immunotherapy.

2.5.1 Clever-1 structure, expression and function

Since its discovery, Clever-1 expression has been identified on various types of endothelial cells, including non-continuous endothelium in the spleen, lymph nodes, liver sinusoids and lymphatics (Goerdt et al., 1991; Irjala, Elima, et al., 2003). Under homeostasis, blood vessel endothelial cells generally lack Clever-1, with the exception of lymph node high-endothelial venules (Goerdt et al., 1993; Irjala, Elima, et al., 2003). Vascular Clever-1 expression, however, is induced by different types of acute and chronic inflammatory conditions, such as cancer and rheumatoid arthritis (Goerdt et al., 1993; Goerdt et al., 1991; Irjala, Elima, et al., 2003; Szekanecz et al., 1994). Apart from endothelial cells, also classical and intermediate monocytes in the circulation as well as various macrophage populations in healthy and pathologic tissues express Clever-1 (Cupurdija et al., 2004; Goerdt et al., 1993; Kodelja & Goerdt, 1994; Mosig et al., 2009; Palani et al., 2016; Schledzewski et al., 2006; Szekanecz et al., 1994; Walsh et al., 1991). Clever-1 expression is upregulated by dexamethasone, IL-4, M-CSF and low extracellular pH, as shown by *in vitro*-cultures of monocyte-derived macrophages, while hypoxia downmodulates Clever-1 (Bosco et al., 2006; Goerdt et al., 1993; Ong et al., 2010; Park et al., 2012).

Both endothelial and myeloid Clever-1 have similar functional properties. Besides the originally identified roles in adhesion and scavenging, a plethora of research has connected Clever-1 with the regulation of immune responses, and all these three functions are mediated by both the endothelial and myeloid Clever-1 (Adachi & Tsujimoto, 2002; Irjala, Elima, et al., 2003; Karikoski et al., 2014; Kzhyshkowska, Workman, et al., 2006; Palani et al., 2016; Tadayon et al., 2021; Viitala et al., 2019). As an adhesion molecule, initial studies found endothelial Clever-1 to mediate binding of various leukocyte subsets to vascular and lymphatic endothelium, and cancer cells to lymphatic endothelium, facilitating immune cell trafficking and potentially supporting lymphatic metastasis (Irjala, Alanen, et al., 2003; Irjala, Elima, et al., 2003; Karikoski et al., 2009). Subsequent studies identified Clever-1 blockade to interfere with endothelial transmigration rather than initial adhesion (Salmi et al., 2004), and endothelial Clever-1 appeared to more strongly support immunosuppressive T_{REG} cell transmigration (Shetty et al., 2011). Furthermore, Clever-1 on monocytes can similarly mediate monocyte adhesion to endothelium (Karikoski et al., 2014).

As a scavenger receptor, Clever-1 has been shown to bind and endocytose various ligands. These include phosphatidylserine-coated dying cells, pathogens or their components (bacteria, anti-sense oligonucleotides), metabolic waste (modified LDLs, oxidized albumin and advanced glycation end products) and secreted mediators (SPARC, placental lactogen) (Adachi & Tsujimoto, 2002; Holte et al., 2023; Kzhyshkowska et al., 2008; Kzhyshkowska, Workman, et al., 2006; Lee et al., 2011; Miller et al., 2016; Park et al., 2009; Tamura et al., 2003). In the liver,

endothelial cells use Clever-1 to sequester damaged red blood cells from the circulation for nearby macrophages to phagocytose, in order to maintain body's homeostasis (Lee et al., 2011). While Clever-1 deficiency alone may not result in severe scavenging deficits due to overlapping ligand repertories with other scavenger receptors, deficiency in both Clever-1 and its only homologue Stabilin-2 causes liver fibrosis and kidney damage in mice (Leibing et al., 2023; Schledzewski et al., 2011). Clever-1-dependent endocytosis by macrophages often results in target degradation within lysosomes, but Clever-1 can alternatively sort the internalized ligands to storage or secretory granules instead (Kzhyshkowska et al., 2008; Kzhyshkowska, Workman, et al., 2006). This can importantly regulate the levels of some Clever-1 ligands, such as placental lactogen (Kzhyshkowska et al., 2008). While some of these ligands inevitably share binding sites on Clever-1 (Tamura et al., 2003), cross-regulation between specific ligand-related processes remains an uncharacterized topic.

Clever-1-mediated scavenging can additionally regulate immune responses. For instance, modified LDL suppresses chemokine CCL3 secretion in a Clever-1 dependent manner (Rantakari et al., 2016). Further suggesting an immunosuppressive role, Clever-1 expression is upregulated by anti-inflammatory stimuli and found on macrophages co-expressing TREM2 and CD163 (Goerdt et al., 1993; Kzhyshkowska et al., 2004; Timperi et al., 2022; Yu et al., 2025). Genetic deletion or antibody-mediated blockade of Clever-1 indeed induces pro-inflammatory monocyte and macrophage phenotypes characterized by elevated pro-inflammatory cytokine and chemokine secretion (TNFα, IL-12, CCL3), higher glycolysis rate, enhanced activation of NF-κB and mTOR signaling upon LPS challenge, and greater capability to support T_H1- and B-cell responses (Dunkel et al., 2018; Palani et al., 2016; Rantakari et al., 2016; Viitala et al., 2019). Similarly, genetic deletion of Clever-1 allows endothelial cells to interact with passing dendritic cells in a manner that facilitates stronger antigen-specific T-cell responses (Tadayon et al., 2021).

To perform these diverse functions, Clever-1 is a large (280-320 kDa, 69 exons) receptor protein with a diverse domain structure (Goerdt et al., 1993; Goerdt et al., 1991; Irjala, Elima, et al., 2003; Politz et al., 2002) and additional protein variants resulting from alternative splicing, glycosylation and protein cleavage (Goerdt et al., 1991; Irjala, Elima, et al., 2003). Clever-1 structure is illustrated in **Figure 5**, and its different domain types associate with distinct functions, such as fasciclin and EGF-like domains with adhesion (Politz et al., 2002). The protein's cytoplasmic tail contains sorting signals and adaptor protein binding sites for rapid trafficking between plasma membrane, trans-Golgi network and the endo-lysosomal compartment (Adachi & Tsujimoto, 2010; Hansen et al., 2005; Kzhyshkowska et al., 2004; Politz et al., 2002; Zhang et al., 2009). These properties facilitate binding and

uptake of various ligands, and their subsequent intracellular sorting for degradation, storage or secretion, as well as Clever-1 recycling back on the cell surface (Adachi & Tsujimoto, 2010; Hansen et al., 2005; Kzhyshkowska et al., 2004; Kzhyshkowska et al., 2008; Kzhyshkowska, Workman, et al., 2006; Park et al., 2010). Clever-1 can also perform similar sorting for newly-synthetized ligands via its interactions in the trans-Golgi network (Kzhyshkowska, Mamidi, et al., 2006).

2.5.2 Clever-1 in cancer

Many of the identified Clever-1 functions would appear tumor-promoting in the context of cancer, including tolerogenic scavenging, immunosuppression and promotion of cancer cell adhesion to lymphatic vessels. To investigate whether Clever-1 expression correlates with patient outcome (survival, therapeutic response, disease progression or recurrence), various studies have quantified Clever-1-expression from tumor biopsies. For endothelial Clever-1, some studies quantify Clever-1^+ blood and lymphatic vessels separately, while other studies report overall Clever-1^+ vessel quantity. High number of Clever-1^+ blood or overall vessels associates with higher number of tumor-infiltrating immune cells and better prognosis (Ålgars et al., 2012; Ammar et al., 2011; Tervahartiala et al., 2017). Conversely, high number of Clever-1^+ lymphatics predicts poorer prognosis in late-stage cancer and higher likelihood for lymphatic metastases (Ammar et al., 2011; Ålgars et al., 2021). These observations align well with the reported Clever-1 functions in cell adhesion and transmigration (Irjala, Alanen, et al., 2003; Irjala, Elima, et al., 2003; Karikoski et al., 2009; Salmi et al., 2004), as blood endothelial Clever-1 would improve prognosis by promoting immune cell trafficking into tumors and tumor-draining lymph nodes, while lymphatic endothelial Clever-1 would additionally facilitate cancer cell adhesion and metastasis.

For macrophage Clever-1, more complex associations have been reported. In several cancer types (bladder, breast, immune-infiltrated gastric, oral cavity and advanced colorectal cancer), high numbers of Clever-1^+ macrophages predict poorer prognosis or weaker treatment responses (Ålgars et al., 2012; Junttila et al., 2020; Kwon et al., 2019; Tashireva et al., 2017; Tervahartiala et al., 2017; Wang et al., 2019; Ålgars et al., 2021). Yet, opposite observations in breast and colorectal cancer have also been reported, as high intratumoral (breast cancer) or peritumoral (colorectal cancer) Clever-1^+ macrophage numbers associate with longer disease-specific survival, higher immune cell infiltration or both (Ålgars et al., 2012; Mutka et al., 2022; Ålgars et al., 2021). Different patient populations, treatment regimens and methods for macrophage identification may account for some of these conflicting associations. Nevertheless, overall conclusion would be that macrophage Clever-1 associates with poorer disease outcome. Furthermore, overall *STAB1* gene

expression, including both endothelial and myeloid Clever-1, associates with shorter survival and poorer immunotherapy responses in a large cancer cohort from The Cancer Genome Atlas (Hollmén et al., 2020).

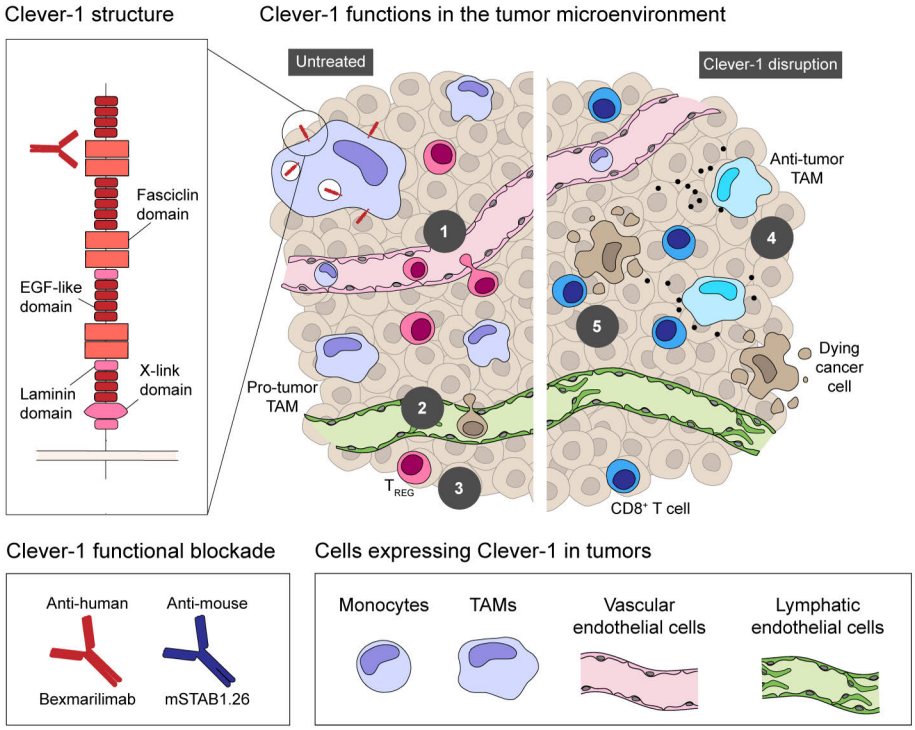


Figure 5. Clever-1 in cancer. Top left, Clever-1 domain structure reproduced from Virtakoivu et al., 2021 (II). Top right, Clever-1 functions in the TME. Clever-1 on vascular endothelial cells promotes immune cell adhesion and tumor entry (1), while Clever-1 on the lymphatic endothelium may additionally facilitate cancer cell migration into lymphatics (2). Clever-1 disruption transforms tumor immune cell infiltrate from tumor-promoting TAMs and T_{REG} cells (3) into anti-tumoral MHC-II$^+$ TAMs (4) and CD8$^+$ T cells (5). Bottom left, immunotherapeutic antibodies for disrupting Clever-1 in human cancer (bexmarilimab) or murine tumor models (mSTAB1.26). Bottom right, cell types expressing Clever-1 within the TME.

Besides endothelial cells and TAMs, also malignant myeloid cells express Clever-1 due to their hematopoietic origin (Aakko et al., 2025), and high Clever-1 expression is associated with shorter survival in acute myeloid leukemia (Lin et al., 2019). A clinical trial investigating Clever-1 blockade achieved a 45% objective response rate in patients with myelodysplastic syndrome, treatment-refractory or relapsed acute myeloid leukemia, with the majority of objective responses observed in myelodysplastic syndrome (Kontro et al., 2025).

Additionally, the role of Clever-1 in cancer has been investigated in mice carrying full or cell-type-specific genetic deletion of Clever-1, or mice treated with the murine anti-Clever-1 antibody mStab1.26 (Karikoski et al., 2014; Viitala et al., 2019). Full or macrophage-specific genetic deletion of Clever-1 suppresses the growth of several subcutaneous or orthotopic primary tumor models, but only the full genetic deletion seems to reduce metastases. Blocking Clever-1 with mStab1.26 also suppresses B16 melanoma, EL4 lymphoma and Lewis lung carcinoma primary tumor growth, but fails to control immune hot CT26.WT tumors. Depending on the method of Clever-1 disruption, slightly different changes in the TME accompany the tumor suppression. Removing Clever-1 from macrophages induces high infiltration of $CD8^+$ T cells within the tumor, and both the $CD8^+$ T cells and macrophages converted to a more pro-inflammatory phenotype are necessary for tumor growth control. Surprisingly, immunotherapeutic mStab1.26 treatment does not increase or even reduces $CD8^+$ T-cell infiltration in the TME, while still controlling tumor growth. Nevertheless, both genetic deletion and mStab1.26 reduce the number of anti-inflammatory TAMs and T_{REG} cells, and enhance pro-inflammatory cytokine secretion. (Karikoski et al., 2014; Viitala et al., 2019) These changes in the TME after Clever-1 disruption are illustrated in **Figure 5**.

3 Aims

Clever-1 in monocytes, macrophages and endothelial cells promotes immunological tolerance (Palani et al., 2016; Tadayon et al., 2021; Viitala et al., 2019), and disrupting macrophage Clever-1 function in murine tumor models enhances $CD8^+$ T-cell responses to control cancer growth (Viitala et al., 2019). To target macrophage Clever-1 in human malignancies, a mouse antibody 3-372 against human Clever-1 (Irjala, Elima, et al., 2003) was humanized into bexmarilimab (FP-1305), an immunoglobulin G4 (IgG4) antibody with limited FcγR binding (Hollmén et al., 2022). This thesis investigated bexmarilimab's efficacy in activating immune responses in the context of human cancer, generating information on mechanisms and putative biomarkers behind bexmarilimab response to assist in patient selection. The specific aims of this thesis were to:

1. Understand the overall clinical landscape of TAM reprogramming therapies, including most common challenges and potential solutions for more effective TAM-targeted cancer immunotherapy in the future (I)
2. Determine the functional significance of bexmarilimab binding site on human monocytes and macrophages (II)
3. Investigate the effects of bexmarilimab therapy on circulating and tumor-infiltrating immune cells in patients with cancer (II – III)
4. Recognize TAM phenotypes and tumor immune landscapes most sensitive to bexmarilimab therapy (IV – V)
5. Identify key changes upon bexmarilimab treatment in both tumor and cancer-free tissues (V)

4 Materials and Methods

This section briefly describes the methods for the results presented in this thesis, while the more detailed experimental and analysis methods descriptions are presented in the original publications I – V. **Figure 6** summarizes the human samples and related experimental methods used in the original publications II – V.

4.1 Systematic review (I)

To enlist past and present clinically investigated TAM-reprogramming cancer immunotherapies, a systematic literature review combined with a clinicaltrials.gov database search was performed. First, PubMed was queried for research and review articles published before 1.2.2024 that fulfilled the following search condition: "macrophage and (cancer or neoplasm or tumour or tumor or malignancies) and (phase or clinical or trial or target)", where both the word "macrophage" and the cancer-related term had to appear in the article title or abstract. The search resulted in 17,457 unique articles that were screened with Rayyan (Ouzzani et al., 2016). Rayyan's artificial intelligence-based rating of articles was used only to exclude the lowliest rated articles after manual classification of the first 6,000 articles and thorough inspection of the rating results, while remaining articles were manually sorted. Excluded articles (1) failed to discuss or identify therapeutic targets on macrophages for cancer therapy; (2) presented therapeutics that deplete TAMs or inhibit monocyte recruitment and/or (3) discussed re-purposed therapeutics. Then, TAM targets from the included articles, additional TAM targets from clinical pipelines of the related pharma companies or the word "macrophage" were queried in clinicaltrials.gov (Condition = "Cancer") to identify therapeutics entering clinical trials at the earliest on 1.1.2000. A total of 194 clinically investigated TAM-reprogramming cancer therapeutics were thus identified. Their clinical phase, development status, molecule type, combinatory treatment regimens, first clinical trial date, number of treated patients and investigated cancer types were listed from clinicaltrials.gov or additional information sources (conference abstracts, press releases, web-based company pipelines and quarterly reports). These parameters were summarized by target type to present overall trends in the clinical landscape.

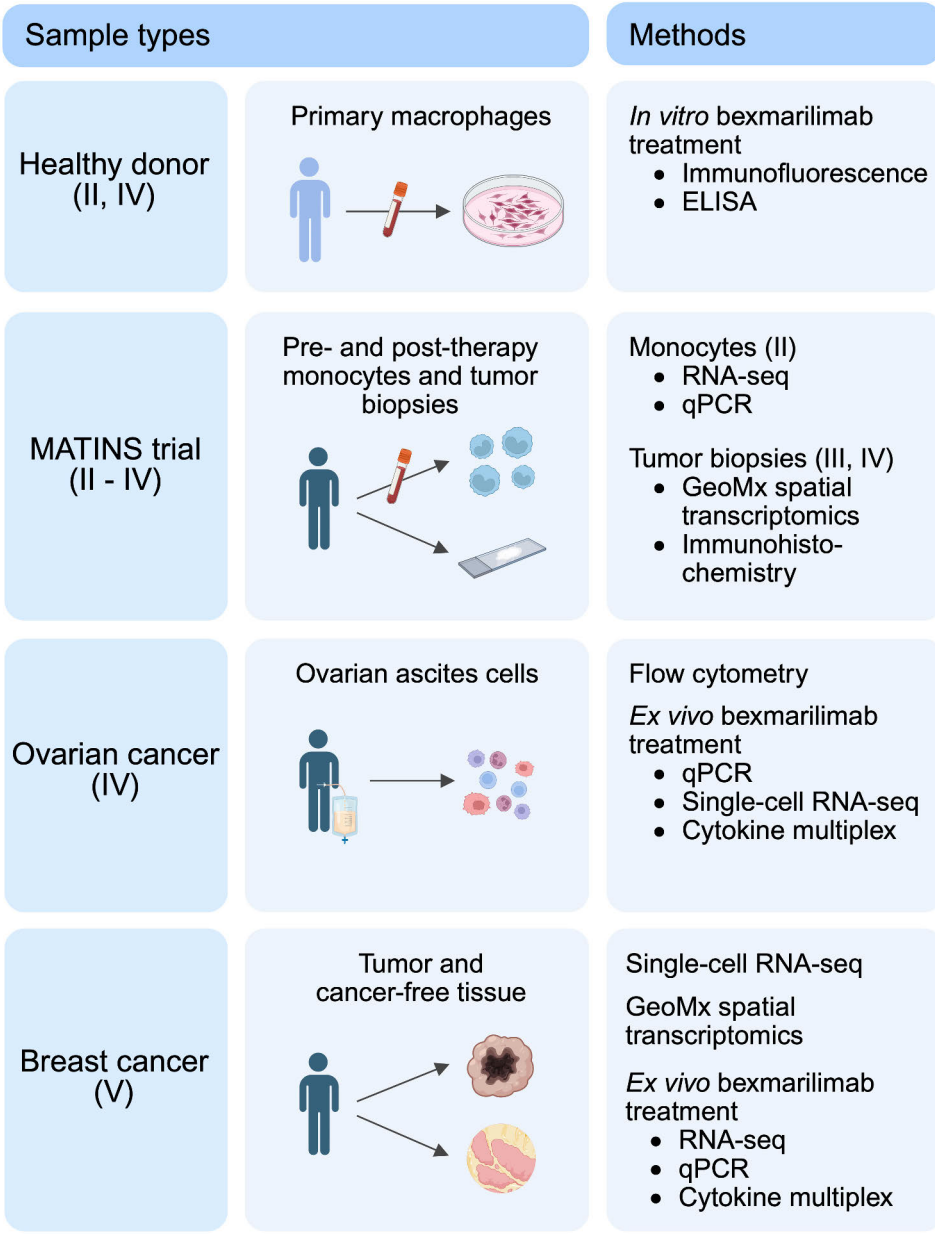


Figure 6. A summary of the human sample types and the related analysis methods used in this thesis to investigate the effects of bexmarilimab treatment on human monocytes, macrophages and the rest of the immune system. Created with Biorender.com.

4.2 Patient sample cohorts (II – V)

4.2.1 MATINS trial patient blood samples and tumor biopsies (II – IV)

MATINS (Macrophage antibody to inhibit immune suppression) trial (NCT03733990, EudraCT 2018-002732-24) was a first-in-human, open-label, non-randomized phase I/II clinical trial investigating bexmarilimab (FP-1305) therapy in patients with advanced solid malignancies. The trial was approved by the local institutional review boards and conducted following Good Clinical Practice and the Declaration of Helsinki. Eligible patients had advanced (treatment-refractory and inoperable or metastatic) gallbladder or biliary tract carcinoma (II-IV), hepatocellular cancer (II-IV), ICI-refractory cutaneous melanoma (II-IV), colorectal cancer (II-III), ovarian cancer (II-III), pancreatic ductal adenocarcinoma (II-III), estrogen–receptor-positive breast cancer (III-IV), gastric adenocarcinoma (III-IV), anaplastic thyroid cancer (III) or uveal melanoma (III), and they provided written informed consent and were not receiving any concurrent anti-neoplastic therapy. More detailed eligibility criteria can be found at https://www.clinicaltrials.gov/study/NCT03733990.

Between December 2018 and April 2021, a total of 138 patients were treated with bexmarilimab in parts 1 (dose escalation) and 2 (cohort expansion) of the trial. Bexmarilimab was administered every three weeks at dose levels 0.3, 1 or 3 mg/kg (part 2) with additional 0.1 and 10 mg/kg doses evaluated in part 1. Objective responses and disease control (DC) were defined based on Response Evaluation Criteria in Solid Tumors 1.1.

Peripheral blood mononuclear cells (PBMCs) were isolated with Ficoll-Paque (GE Healthcare) density gradient centrifugation from patient heparin blood samples. Fresh cells were used for RNA-seq, flow cytometry and LPS induction assay, and remaining cells frozen for later use. Formalin-fixed, paraffin-embedded pre-treatment (IV) or pre- and post-treatment (III) tumor biopsy sections were used for immunohistochemistry and GeoMx digital spatial profiling, respectively.

4.2.2 Ovarian cancer ascites (IV)

Malignant ascites was collected from ovarian cancer patients participating in the DECIDER project (https://www.deciderproject.eu/). The study was approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK 53/1801/2009 and ETMK 145/1801/2015) and conducted in accordance with the Declaration of Helsinki. The participants provided written informed consent.

Ascites cells were pelleted from ascites fluid with a 10-minute centrifugation at 400g and red blood cells were lysed with PharmLyse (BD Biosciences, 555899). Resulting ascites cell suspensions were stored frozen at -150°C in 10% DMSO in RPMI-1640 medium (Sigma, 5886) supplemented with 10% fetal bovine serum (FBS, Sigma, F7524), 1% GlutaMAX (Gibco, 35050-038) and penicillin-streptomycin (P/S, 12.8 U/ml, Gibco, 15140-122).

4.2.3 Breast cancer and adjacent breast tissue (V)

Tumor and adjacent cancer-free tissues were obtained from breast cancer patients undergoing mastectomy surgery at Turku University Hospital. Eligible patients had a treatment-naïve breast cancer with >2 cm tumor diameter and provided written informed consent. Matching tumor and adjacent tissue areas were examined by the pathologist to verify the presence of invasive carcinoma in the tumor tissue specimen and the absence of cancer cells in the adjacent breast tissue specimen. The study was conducted under the approval of the Ethics Committee of the Hospital District of Southwest Finland (ETMK 132/216 and ETMK 34/1801/2021) and in accordance to the Declaration of Helsinki. Tumor and adjacent tissues were primarily used freshly for *ex vivo* studies and scRNA-seq.

4.3 *Ex vivo* models (IV, V)

4.3.1 Ovarian ascites cell culture (IV)

Thawed ovarian ascites cells (5×10^5) or $CD14^+$ ascites cells (2×10^5), magnetically enriched using human CD14 MicroBeads and MS columns (both Miltenyi Biotec, 130-050-201 and 130-042-201), were cultured *ex vivo* on 96-well ultra-low attachment plates (Corning, 7007) in Iscove's modified Dulbecco's medium (IMDM, Thermo Fisher, 21980-031) supplemented with 10% FSC, 1% GlutaMAX and P/S. The cells were treated for 48h with 10 µg (all ascites cells) or 15 µg ($CD14^+$ ascites cells) bexmarilimab (clone CP12, Abzena) or its isotype control human IgG4 (human irrelevant IgG4 [S241P, L248E], Abzena). Cell culture supernatants were frozen at -70°C for subsequent cytokine analyses by multiplex or enzyme-linked immunosorbent assay (ELISA), and the cells were collected for RNA extraction or scRNA-seq.

4.3.2 Patient-derived explant culture (V)

Tumor and adjacent cancer-free tissues were cut into 1-2 mm^3 pieces. From 6 – 16 different tissue areas, neighboring pieces were divided across treatment conditions

on a 96-well ultra-low attachment plate and cultured in RPMI-1640 medium supplemented with 10% FBS, 1% GlutaMAX and P/S. These patient-derived explant cultures (PDECs) were treated with 20 µg/ml bexmarilimab or human IgG4 for 48h or 24h. After the *ex vivo* treatment, culture supernatants were frozen at -70°C for subsequent multiplex cytokine profiling and the tissue pieces were collected for RNA extraction. Matching untreated RNA was collected before the *ex vivo* culture.

Tumor and adjacent tissue-conditioned media were collected from untreated PDECs after 24h of culture. Untreated adjacent tissue PDECs were further prepared for conditioned medium treatment with a PBS wash and a 1h pre-treatment with 20 µg/ml bexmarilimab or IgG4. The collected conditioned media of the same patient were then freshly added to the pre-treated adjacent tissue PDECs at a 1:10 ratio for 48h, followed by culture supernatant collection and freezing for cytokine profiling.

4.4 *In vitro* models (II, IV)

4.4.1 KG-1 cells (II)

KG-1 cells (ATCC, CCL-246) were cultured in IMDM supplemented with 20% FBS and P/S, and differentiated into adherent macrophage-like cells by adding 300 nM phorbol 12-myristate 13-acetate (PMA, Merck, P8139) in culture medium for 3 days. Prior PMA differentiation, KG-1 cells were in some experiments transfected with 2 µM small interfering RNAs targeting Clever-1 (J-014103-05-0020 or J-014103-08-0020, Dharmacon) or pooled scramble control small interfering RNAs (D-001810-10-20, Dharmacon) in OPTI-MEM medium (Thermo Fisher, 51985026) using Nucleofector II (Amaxa Biosystems).

4.4.2 Primary human macrophages (II, IV)

For monocyte isolation, EDTA blood of healthy volunteer donors was collected under the approval of the Ethics Committee of the Hospital District of Southwest Finland (ETMK 43/1801/2015) (II, IV) or buffy coats were obtained from the Finnish Red Cross (II). PBMCs were isolated using Ficoll-Paque Plus (Cytiva, 17-1440-03) density gradient centrifugation and enriched for $CD14^+$ monocytes using magnetic human CD14 MicroBeads and MS columns (both Miltenyi Biotec, 130-050-201 and 130-042-201). For macrophage differentiation, monocytes were cultured on 8-well ibiTreat µ-Slide chambers (II: 1×10^5 cells/well; ibidi, 80826) or 6-well plates (IV: 7×10^5 cells/well; Greiner) in IMDM supplemented with 10% FBS, 1% GlutaMAX and 50 ng/ml human M-CSF (BioLegend, 574806) for 6 – 7 days with half of the medium replaced twice. Macrophages were M2 polarized by

supplementing the culture medium further with 100 nM dexamethasone (Sigma, D2915) and human IL-4 (PeproTech, 200-04) for 24h – 48h.

To measure LPS-induced TNFα secretion after IFN priming and bexmarilimab treatment (IV), macrophages were detached with a 20-minute incubation in 10 mM EDTA at 37°C and subsequent gentle scraping. Priming with 23 ng/ml IFNα2 or 20 ng/ml IFNγ (both 1.2 nM) was started at the time of monocyte plating or 2h prior to detaching the macrophages. The detached macrophages were then re-plated (2×10^4 cells/well) on flat-bottom 96-well plates in macrophage differentiation medium supplemented with 10 µg/ml bexmarilimab or human IgG4, and with or without the same IFNs. After 24h, medium was replaced with fresh macrophage differentiation medium containing 20 ng/ml LPS (InvivoGen, tlrl-3pelps) for 12h and resulting supernatants were collected for subsequent ELISA.

4.5 Experimental methods (II – V)

4.5.1 Immunofluorescence staining and imaging (II)

Immunofluorescence staining and imaging was performed on 8-well ibiTreat µ-Slide chambers (ibidi, 80826). PMA-differentiated KG-1 cells were treated with 10 µg/ml Alexa Fluor 647-conjugated (Thermo Fisher, A20173) acLDL (Bio-Rad, 5685-3404) for 3h, or M2-polarized primary human macrophages were treated with 20 µg/ml Alexa Fluor 647-conjugated bexmarilimab, Alexa Fluor 488-conjugated (Thermo Fisher, A10235) anti-Clever-1 antibody (clone 9-11, InVivo Biotech) or their conjugated isotype controls human IgG4 and rat IgG2α for 5min or 2h. For phagocytosis assay, M2-polarized primary human macrophages were pre-treated with 30 µg/ml bexmarilimab or IgG4 isotype control for 1h and incubated with CFDA SE-labeled (Thermo Fisher, V12883) and irradiated cancer cells for six hours. Treated cells were fixed with 4% paraformaldehyde, permeabilized in 0.1% triton X-100 and blocked with 5% goat serum. KG-1 cells were stained with primary antibodies for lysosomal associated membrane protein 1 (LAMP-1), ATP6V0A1 and Clever-1, or their isotype controls (**Table 2**), and secondary anti-mouse, anti-rabbit and anti-rat antibodies (all Thermo Fisher, A31556, A11030, A11006). Phagocytic macrophages were stained with Alexa Fluor 647-conjugated 9-11 and phalloidin (Thermo Fisher, A22283), and macrophage nuclei were labeled with Hoechst 33342 (Thermo Fisher, 62249). Z-stacks of KG-1 cells and phagocytic macrophages were captured with Marianas spinning disk (Intelligent Imaging Innovations) equipped with CSU-W1 scanning unit (Yokogawa), Orca Flash 4 sCMOS camera (Hamatsu) and a 63x/1.4 oil objective (Plan-Apochromat, Carl Zeiss). Z-stacks of macrophages were imaged with LSM 880 confocal microscope connected to Airyscan detector (Carl Zeiss) using the 63x/1.4 oil objective.

Table 2. Anti-human antibodies used in immunofluorescence, immunohistochemistry and flow cytometry.

ANTIBODY	CLONE	COMPANY	CAT. NUMBER	APPLICATION
ATP6V0A1	Polycl.	Abnova	H00000535-A01	Immunofluorescence (II)
Bexmarilimab AF647	CP12	Abzena + A20173*	NA	Immunofluorescence (II), Flow cytometry (IV)
CD11b AF488	ICRF44	Biolegend	301318	Flow cytometry (IV)
CD45 BV421	H130	BD	563879	Flow cytometry (IV)
CD64 BV510	10.1	BD	563459	Flow cytometry (IV)
CD68 AF647	KP-1	Santa-Cruz	sc20060	Immunofluorescence (IV)
Clever-1	9-11	InVivo Biotech	NA	Immunofluorescence (II)
Clever-1 AF488	9-11	InVivo Biotech + A10235*	NA	Immunofluorescence (II), Flow cytometry (IV)
Clever-1 AF647	9-11	InVivo Biotech + A20173*	NA	Flow cytometry (IV)
IDO1 PE	eyedio	Thermo Fisher	12-9477-42	Flow cytometry (IV)
IgG4 AF647	S241P, L248E	Abzena + A20173*	NA	Immunofluorescence (II)
IgG4 AF647	QA16215	BioLegend + A20173*	403702	Flow cytometry (IV)
IL4I1	EPR22070	Abcam	ab222102	Immunohistochemistry (IV)
LAMP-1	D2D11	Cell Signaling	9091	Immunofluorescence (II)
Mouse IgG	Polycl.	Rockland	010-0102-0005	Immunofluorescence (II)
Mouse IgG1 κ AF647	MOPC-21	BD	557783	Immunofluorescence (IV)
Mouse IgG1 κ PE	MOPC-21	BD	555749	Flow cytometry (IV)
Rabbit IgG	Polycl.	BioxCell	BE0095	Immunofluorescence (II), immunohistochemistry (IV)
Rat IgG2α	2A3	InVivoPlus	BP0089	Immunofluorescence (II)
Rat IgG2α AF488	MEL-14	InVivo Biotech + A10235*	NA	Immunofluorescence (II), Flow cytometry (IV)
Rat IgG2α AF647	R35-95	BD	557906	Flow cytometry (IV)

*, Thermo Fisher conjugation kit; AF, Alexa Fluor; NA, not applicable; polycl., polyclonal.

4.5.2 RNA extraction and quantitative PCR (II, IV, V)

For RNA extraction, MATINS patient $CD14^+$ monocytes enriched from PBMCs as described above (II) or *ex vivo*-treated ascites cells (IV) were lysed with TRIsure (Bioline, BIO-38032). Alternatively, breast cancer tumor and adjacent cancer-free tissue pieces (V) were homogenized in TRIsure using gentleMACS Dissociator (Miltenyi Biotec, 130-093-235, RNA.01_01 program) and gentleMACS M tubes (Miltenyi Biotec, 130-093-236). Lysates were frozen at -70°C until RNA extraction according to the manufacturer's protocol. RNA quantity was measured with Qubit Flex fluorometer using Qubit HS assay kit (both Thermo Fisher, Q32852) and RNA quality was analyzed using Bioanalyzer with RNA 6000 Pico kit (Agilent, 5067-1513).

Equal amounts of RNA across related samples were reverse-transcribed into cDNA with SuperScript VILO cDNA synthesis kit (Thermo Fisher, 11754-250). Quantitative PCR (qPCR) was run on QuantStudio 3 (Applied Biosystems) in triplicate or duplicate reactions (10 µl) using Taqman universal master mix II, no UNG (Thermo Fisher, 4427788) and following Taqman gene expression assays (Thermo Fisher): *CD274* (Hs01125296_m1; V), *CIITA* (Hs00172106_m1; IV-V), *CXCL9* (Hs00171065_m1; V), *FCGR1A/B* (Hs00174081_m1; V), *GAPDH* (Hs02758991_g1; II, IV), *GBP5* (Hs00369472_m1; V), *IDO1* (Hs00984148_m1; V), *IL1B* (Hs01555410_m1; II), *IL1R2* (Hs00174759_m1; II), *IL4I1* (Hs00541746_m1; V), *JUN* (Hs01103582_s1; II), *MX1* (Hs00895608_m1; IV-V), *SERPING1* (Hs00163781_m1; V), *SLAMF7* (Hs00904275; V), *TNFRSF1A* (Hs01042313_m1; II) and *RPLP0* (Hs99999902_m1; V). Quantification in relation to *GAPDH* or *RPLP0* was calculated with the ΔΔCT method.

4.5.3 RNA-seq (II, V)

RNA from MATINS patient $CD14^+$ monocytes (II) or *ex vivo*-treated breast cancer tumor and adjacent tissue (V) was extracted and quantified as described above. RNA integrity was measured with 2100 Bioanalyzer (Agilent) using RNA 6000 Pico kit (Agilent, 5067-1513). cDNA libraries were prepared and sequenced on NovaSeq 6000 (Illumina) by Novogene (Cambridge, United Kingdom).

4.5.4 GeoMx digital spatial profiling (III, V)

GeoMx digital spatial profiling was performed at FIMM Single-Cell Analytics and Sequencing units (III) or LabCorp (Morrisville, NC) (V). MATINS trial pre- and post-treatment biopsies (III) or breast cancer tumor and adjacent tissue (V) were cut into 5 µm sections, dried, deparaffinized and rehydrated. Successive heat-induced (100°C, pH 9.0) and enzymatic (0.1 µg/ml Proteinase K) antigen retrieval were

performed to retrieve both protein and RNA targets. The sections were then fixed in 10% neutral buffered formalin. RNA targets were hybridized to GeoMx human whole transcriptome atlas (NanoString, GMX-RNA-NGS-HuWTA) (III) or to GeoMx human immune pathways panel (NanoString, LBL-10747-03) complemented with custom probes, such as STAB-1 (V). Tissue morphology was visualized by staining CD68 (clone KP-1, Santa Cruz, sc-20060AF594), CD31 (clone JC/70A, Abcam, ab215912), pan-cytokeratin (clone AE-1/AE-3, NovusBio, NBP-33200AF488) and nuclei (SYTO 83, Thermo Fisher, S11364).

Using GeoMx Digital Spatial Profiler (NanoString), morphological staining was scanned, annotated for regions of interest (ROIs) and thresholded to segment the ROIs into non-overlapping areas. Within each ROI, following segments were created and separately subjected to RNA probe collection: $CD68^{+}$pan-$cytokeratin^{-}$, $CD31^{+}$ and remaining $nuclei^{+}$ area (III) or $CD68^{+}$ and $CD68^{-}$ area (V). The resulting RNA probe libraries were sequenced with NovaSeq 6000 (Illumina) (III) or quantified using nCounter (NanoString) (V).

4.5.5 scRNA-seq (IV, V)

Ex vivo-treated ovarian ascites cells were collected with 10 mM EDTA and filtered through 70µm nylon nets (Corning) (IV), while fresh breast cancer tumor and adjacent tissue were digested in 0.2 mg/ml collagenase P, 0.8 mg/ml dispase and 0.1 mg/ml DNase for 1h at 37°C (V). The resulting single-cell suspensions were prepared in 0.04% ultrapure BSA-PBS (Thermo Fisher, AM2616) and scRNA-sequenced by Single Cell Omics Facility at Turku Bioscience Centre using Chromium Next GEM Single Cell 3′ v3.1 (IV) or Chromium Single Cell 3′ v2 (V) reagent kits (10X Genomics). The resulting libraries were sequenced on NovaSeq 6000 (Illumina).

4.5.6 Cytokine multiplexing (IV, V)

Culture supernatants from *ex vivo*-treated ascites cells and $CD14^{+}$ ascites cells (II) or breast cancer PDECs (V) were analyzed for cytokine levels with Bio-Plex Pro Human Cytokine 27-plex assay (Bio-Rad, M500KCAF0Y) on Bio-Plex 200 System (Bio-Rad) following the kit's protocol. Values below the detection limit were substituted with a value corresponding to 50% of the lowest measured value, and cytokines with >10% of values outside the detection range were excluded. Cytokine-specific normalization factors for controlling plate-to-plate variation were calculated based on samples measured across different plates.

4.5.7 ELISA (IV)

TNFα was measured from macrophage culture supernatants with TNF alpha human ELISA kit (Thermo Fisher, KHC3011). Sigmoidal 4PL standard curves were fitted with Prism (GraphPad, v9.2.0), and TNFα concentrations were further normalized to combined DNA and RNA quantity, which was measured from each well with CyQUANT cell proliferation assay kit (Thermo Fisher, C7026).

4.5.8 Immunohistochemistry staining and imaging (IV)

Pre-treatment biopsy sections from MATINS trial patients were deparaffinized and their protein antigens retrieved in Tris-EDTA (pH 9.0) at 100°C. After blocking endogenous peroxidase activity with BLOXALL (Vector Laboratories, SP-6000) and non-specific antibody binding with goat serum, the sections were stained with anti-IL4I1 or its isotype control (**Table 2**) overnight at 4°C. The staining was detected using Vectastain Elite ABC-HRP kit (Vector laboratories, PK-6101) and 3,3´-diaminobenzidine (DAB, Dako, K3468). For double-staining IL4I1 and CD68, formalin-fixed, paraffin-embedded breast cancer patient tumor sections were further stained with anti-CD68 or its isotype control (**Table 2**) for 1h at RT. Stained sections were mounted in ProLong Gold antifade mountant (Thermo Fisher).

The immunohistochemistry staining was captured with Pannoramic P1000 slide scanner (3D Histech) equipped with a 20x/0.8 objective (Plan-Apochromat, Carl Zeiss). The double-staining was imaged with Nikon Eclipse Ti2-E equipped with a 20x/0.75 objective (CFI Plan Apo λ, Nikon), first in fluorescence configuration using ORCA-Flash4.0 camera (Hamatsu) and successively in brightfield configuration using DC-Fi3 (Nikon) camera.

4.5.9 Flow cytometry (IV)

Thawed ascites cells or primary human macrophages were stained with fixable viability dye eFluor 780 or eFluor 450 (Thermo Fisher, 65-0865-14 or 65-0863-14) in PBS for 20 min at 4°C. Cells were blocked with 0.2 mg/ml Kiovig (Baxter, LE-072213) for 15 min and stained with conjugated antibodies for 30 min in 2% FBS and 0.01% NaN_3 in PBS. Ascites cells were stained for CD45, CD11b, CD64 and Clever-1 (bexmarilimab Alexa Fluor 647, 9-11 Alexa Fluor 647 or their isotype controls), while macrophages were stained with antibodies for IDO1 and Clever-1 or their isotype controls (**Table 2**). Before intracellular staining of Clever-1, viability-dyed and surface-stained ascites cells or macrophages were fixed with 4% paraformaldehyde, permeabilized with 0.3% tween-20 in PBS and blocked with Kiovig and stained, as described above. Stained cells were stored in PBS supplemented with 1% formaldehyde until analysis on LSRFortessa (BD).

Fluorescence overlap was compensated using single-stained UltraComp eBeads Compensation Beads (Thermo Fisher, 01-2222-42), and data were analyzed with FlowJo (BD, v10.8.1).

4.6 Bioinformatics and statistical analyses (II – V)

4.6.1 Image analysis (II, IV)

Confocal images of immunofluorescence-stained macrophages were Airyscan processed with Zen software (Carl Zeiss, SP1 black edition, v2.3), while further processing and analysis of macrophage and KG-1 images were performed with ImageJ (NIH, v1.52p). Single-stained samples were used to confirm the absence of bleed-through signal. Background was subtracted with median filtering before subsequent analyses. Manders colocalization coefficients above Costes threshold were calculated separately for each cell using the Colocalization Threshold plug-in. Colocalization was visualized as merged channel images or as intensity profiles created with the Plot Profile function. ATP6V0A1 mean fluorescence intensity was quantified from LAMP-1$^+$ vesicles that were defined based on a fixed threshold. Proportions of Clever-1$^+$ macrophages that had ingested CFSE-labeled cancer cells were quantified manually. (II)

Scanned brightfield images of MATINS trial pre-treatment tumor biopsies were analyzed with QuPath v0.4.3 (Bankhead et al., 2017). Biopsy areas were annotated manually, and optical density sum of all RGB channels was used to represent IL4I1 staining. To detect IL4I1$^+$ stromal cells, individual IL4I1$^+$ cells were identified with the Cell detection tool (size 20-400 μm^2) and stromal area defined by excluding IL4I1$^+$ tumor cell area (> 500 μm^2) from the total biopsy area using the Create thresholder tool. Manual thresholds for these tools were set by first grouping all images into four intensity groups based on average IL4I1 staining intensity and then applying the same threshold values for the whole intensity group. Number of IL4I1$^+$ stromal cells was reported per mm^2 of stroma. Biopsies with poor quality or insufficient stromal area detection were excluded from the analysis.

To visualize CD68 and IL4I1 double staining, CD68 fluorescence images were re-scaled in ImageJ to equal pixel density with the IL4I1 brightfield images. CD68 staining images were thresholded to create binary masks, which were further adjusted by closing, filling holes and filtering out small particles. Outlines of the created masks were overlaid on the IL4I1 images to visualize colocalization. (IV)

4.6.2 RNA-seq data analysis (II, V)

Quality control was performed with fastp (https://github.com/novogene-europe/fastp, v2019/9/27) to exclude reads with low quality, short length, adapters or poly-N sequences. Remaining reads were mapped to GRCh38 reference genome using STAR (v2.6.1.d) (Dobin et al., 2013), and the reads were quantified with featureCounts (subread, v1.6.3) (Liao et al., 2014) (V). Differentially expressed genes (DEGs) were identified after size-factor normalization with DESeq2 (v1.42.1) (Love et al., 2014) using the Wald test and Patient ID as a factor (V). Alternatively, mapping with HISAT2, read quantification with HTSeq and DEG identification after trimmed mean of M-values -normalization (edgeR) with DEGseq were performed by Novogene (II).

Pathway enrichment and upstream regulators for DEGs were analyzed in Ingenuity Pathway Analysis software (Qiagen), where core analysis for each patient and subsequent comparison analysis were performed. Gene set enrichment analyses were performed with GSEA software (Broad Institute, v4.3.3) using Hallmark gene sets from the MSigDB database and size-factor-normalized read counts. To evaluate the abundance of specific TAM subsets, TAM signature scores for published TAM gene expression profiles (Coulton et al., 2024; Mulder et al., 2021; van Elsas et al., 2024; Wu et al., 2021) were calculated using R package GSVA (v1.53.3) (Hänzelmann et al., 2013). Immune cell abundancies were estimated from the bulk RNA-seq data with CIBERSORTx cell type deconvolution using the LM22 signature matrix (Newman et al., 2019).

4.6.3 GeoMx spatial transcriptomics data analysis (III, V)

Segments with insufficient staining or segmentation quality were excluded based on visual inspection. The analysis of the next-generation sequencing readout data (III, V) was performed in GeoMx DSP Control Center software (NanoString, v2.4; III) or R (GeoMx tools, v3.6.2; NanoStringNCTools, v1.10.1; V) after standard demultiplexing, adapter removal and read alignment. Segments with low signal-to-noise ratio and genes failing to surpass limit of quantitation were excluded before Q3-normalization of the remaining data. To identify DEGs after bexmarilimab therapy or between pre-treatment biopsies, linear mixed models with biopsy type (pre vs. post) or bexmarilimab response group as a fixed effect and patient ID as a random effect were fitted. Pathway enrichment against the Reactome (v78) database was visualized as an enrichment map with Cytoscape's Enrichment Map app (v3.3.4) (Merico et al., 2010).

Further analyses and visualizations were performed with R (v4.0.4, R Core team). To evaluate cell type composition within each segment, SpatialDecon (v1.0.0) and safeTME tumor-immune deconvolution cell profile matrix were used (Danaher

et al., 2022) (III). M1 and M2 macrophage gene signature scores were calculated based on published macrophage DEGs (Martinez et al., 2006) and in relation to average overall gene expression (III).

The analysis of nCounter readout data (V) was performed with R (v4.3.2). Segment quality control and probe count normalization to positive spike-in controls were performed as described in GeoMx nCounter manual (SEV-00067-07). Counts were further normalized to reference genes and background was subtracted based on negative control probe counts. To identify tumor and adjacent tissue niches, $CD68^{-}$ segments were hierarchically clustered (Euclidean distance and Ward D2 linkage) based on their expression of canonical cell type marker genes. The identified niches were then compared for differences in Clever-1 expression on $CD68^{+}$ area and immune cell subtypes.

4.6.4 scRNA-seq data analysis (IV, V)

Standard scRNA-seq data post-processing was performed with Cell Ranger package (10X Genomics) using the GRCh38 reference genome, and scRNA-seq data was analyzed with Seurat (v.4.0.1 and v5.0.3) (Hao et al., 2021). Dead cells and cell doublets were excluded by filtering out cells showing high proportion of mitochondrial RNA (>13% in IV, >15% in V), expressing high (V) or low (IV, V) number of unique genes or recognized as doublets by DoubletFinder (v2.0.3; IV) (McGinnis et al., 2019). To remove patient-to-patient heterogeneity while retaining treatment- or tissue-related variation for single-cell clustering, each patient's samples were merged and resulting patient samples integrated using Seurat's functions for SCT-transformation (IV) or log-normalization (V), integration feature selection, integration anchor identification and data integration. The integrated data were clustered by running principal component analysis, uniform manifold approximation and projection, and graph-based Louvain-optimized clustering. Cluster identities were annotated based on published single-cell or canonical cell type marker genes. Immune cell clusters were subclustered by repeating the integration and clustering to identify subtypes. To retain all biological variation, breast tissue monocytes and macrophages were subclustered without data integration (V). For visualizing the (average) expression of individual genes, log-normalized counts from the RNA assay were used.

Monocytes and macrophages, merged and log-normalized by scRNA-seq experiment batch (IV) or sample (V), were mapped into MoMac-VERSE single-cell atlas (Mulder et al., 2021) using Seurat's reference mapping functions. The resulting predicted MoMac-VERSE cluster identities were used for downstream analyses. Gene set enrichment was analyzed from DESeq2-normalized monocyte and macrophage pseudobulk samples with the GSEA software (Broad Institute) using

the Hallmarks gene sets (IV). DEGs between *STAB1*$^{+}$ tumor and adjacent tissue monocytes and macrophages were identified with Wilcoxon rank-sum test separately for each patient, followed by Ingenuity Pathway analysis (Qiagen) (V). Bexmarilimab-induced DEGs in ascites cells were analyzed with logistic regression using Patient ID as latent variable (V).

4.6.5 Statistical analyses (II – V)

Statistical testing and data visualization were performed with R (v4.0.4), Prism (v9-10, GraphPad) and JMP Pro (v16, JMP Statistical Discovery LLC) software. To select suitable data visualization methods and statistical tests, data distribution was assessed by visual inspection of histograms and Shapiro-Wilk test. Data were presented as mean ± SD or median ± IQR. Appropriate statistical tests for comparing two independent groups (Student's unpaired t test, Mann-Whitney U test), two dependent groups (Student's paired t test, Wilcoxon matched-pairs signed rank test), multiple groups of matched data (one-way analysis of variance followed by Dunnett's test, two-way analysis of variance followed by Šídák's test, Friedman test followed by Dunn's test), multiple independent groups (Kruskal-Wallis test followed by Bonferroni-corrected Dunn's test) or group's deviance from a constant (one-sample t test, Wilcoxon signed rank test) were used. P <0.05 was considered statistically significant.

Base R was complemented with suitable packages for area under the curve calculation (pROC), uniform manifold approximation and projection (uwot), principal component analysis visualization (scatterplot3d), visualization of hierarchically clustered heatmaps (ComplexHeatmap), k-means clustering (factoextra) and Venn diagrams (VennDiagram). Unsupervised hierarchical clustering was conducted using the Euclidean distance and complete or Ward D2 linkage method. Linear and non-linear correlation was evaluated by calculating Pearson and Spearman correlation coefficients, respectively. To assess the effects of tissue and cytokine type (fixed factors) on bexmarilimab-induced cytokine secretion while accounting for patient-related variation (random factor), linear mixed models were fitted using R packages lmer and lmerTest (V). For survival analysis, Kaplan-Meier curves and log-rank tests for the selected lymphocyte and TAM genes were generated with R packages survminer and survival from the TCGA-PANCAN dataset (Xena browser) (Goldman et al., 2020).

5 Results

5.1 Clinical landscape analysis identifies trends and challenges in the development of TAM-reprogramming cancer immunotherapies (I)

TAMs regulate various aspects of the immunosuppressive tumor milieu, cancer progression and therapy resistance. However, emerged challenges in clinical trials aiming to deplete TAMs and monocytes from the TME shifted focus to reprogramming TAM functions or phenotypes instead, as reviewed above. To deeply understand the clinical landscape of TAM-reprogramming cancer immunotherapies, we conducted a systematic analysis of clinically investigated TAM-reprogramming drug candidates and collected information on a total of 194 therapeutics, which entered clinical phase investigation between 2000 and early 2024. This results section summarizes major trends identified by the review.

5.1.1 Accelerating clinical development focuses on a few selected TAM targets

During the past decade, the numbers of clinically investigated TAM-reprogramming therapeutics and treatment targets have steeply increased (I: Fig. 1A-B). Highly-investigated targets included the CD47–SIRPα axis, TLRs, STING, IDO1 and STATs (I: Fig. 2A-B). Yet, only a few therapeutics have shown sufficient efficacy and safety profiles to proceed into phase 3 clinical investigation (I: Fig. 2A), which is conducted to demonstrate therapeutic efficacy prior market approval. Therefore, the accelerating clinical development is yet to deliver strictly TAM-reprogramming therapeutics approved for clinical use, as the few approved therapeutics (I: Fig. 2A), such as PI3Kγ/δ-inhibitor duvelisib (Blair, 2018), have additional modes-of-action besides TAM reprogramming.

We observed high overlap in clinical development programs, as dozens of therapeutics were directed against the same targets (I: Fig. 2A). While a subset of these therapeutics represented novel improved therapeutic strategies, clinical development appeared highly redundant. Unfortunately, many of the highly-investigated targets also had high rates of discontinuation with more than 50% of

clinical programs terminated, including TLRs, IDO1 and STATs (I: Fig. 2C). Therefore, excessive overlap in the development efforts contributes to unnecessary spending of resources and high numbers of treated patients for targets found futile after clinical investigation (I: Fig. 2B).

Our literature review revealed that most commonly the therapeutic efficacy was limited by on-target side effects or simultaneous upregulation of compensatory and counteracting pathways facilitating immune escape (I). Therefore, the vigorous clinical development focusing on selected TAM targets is yet to fulfil its promise of generating novel cancer immunotherapies.

5.1.2 Improving efficacy with re-designed therapeutics and treatment combinations

To tackle the initial efficacy- or safety-related issues, a growing body of preclinical and translational follow-up research has sparked the development of next-generation therapeutics with improved properties (I). Commonly applied strategies for enhancing target engagement, efficacy and safety include alternative administration methods, TME-targeted drug delivery, bi-specific drugs and increasingly complex re-engineered drug candidates (I: Fig. 2D and text). Therefore, many targets, including CD47–SIRPα, STING and CD40, remain extensively investigated despite numerous clinical program discontinuations (I: Fig. 2C).

Secondly, low monotherapy efficacy has prompted extensive investigation of combination treatment regimens. Less than a quarter of therapeutics were investigated as a monotherapy above phase 1 at all (I: Fig. 3A). In contrast, more than half of the TAM-reprogramming therapeutics had been investigated in combination with ICIs, which was the most common combinatory treatment approach (I: Fig. 3A). Future will show, whether these steps taken will elevate therapeutic efficacy.

Our review also aimed to find opportunities for overcoming these emerging challenges, and potential courses of action are considered in the Discussion section of this thesis. In short, many of the challenges reflect our evolving but currently incomplete understanding on TAM biology, especially upon therapeutic intervention. Therefore, selecting correct patients for TAM-targeted therapy or designing optimal combinatory treatment regimens requires more research on TAM phenotypes, specific responses to therapy and related pre- and on-treatment biomarkers. The following results sections describe our translational research on these aspects, aiming to support the clinical development of one putative TAM-reprogramming immunotherapy, Clever-1 blockade with bexmarilimab.

5.2 Clever-1 blockade elicits systemic immune activation in patients with advanced cancer (II)

Multi-functional scavenger receptor Clever-1 regulates tolerance and immune responses on subsets of monocytes, macrophages and endothelia (Dunkel et al., 2018; Palani et al., 2016; Tadayon et al., 2021; Viitala et al., 2019). To therapeutically disrupt the immunosuppressive functions of Clever-1 in cancer, a humanized Clever-1-specific and function-blocking antibody, bexmarilimab, has been developed (Hollmén et al., 2022). Previous studies have established Clever-1 blockade or deletion to suppress tumor growth in murine cancer models and to promote pro-inflammatory activation of mouse and human monocytes and macrophages *in vitro* (Karikoski et al., 2014; Palani et al., 2016; Viitala et al., 2019). To further evaluate the potential of Clever-1 blockade as a novel cancer immunotherapy, we measured systemic immune activation after bexmarilimab therapy in patients with advanced cancer.

A phase I/II clinical trial investigating the safety and preliminary efficacy of bexmarilimab therapy in patients with advanced solid malignancies (MATINS, NCT03733990) was initiated in 2018. Bexmarilimab was administered intravenously once every three weeks at dose levels ranging between 0.1 and 10 mg/kg. We measured systemic immune responses from blood samples collected before and after bexmarilimab infusion from the trial participants.

5.2.1 Bexmarilimab converts monocytes to pro-inflammatory phenotype

After intravenous administration, bexmarilimab first encounters Clever-1^+ monocytes, Clever-1^+ endothelium, such as the tumor vasculature, as well as soluble Clever-1 (Goerdt et al., 1993; Palani et al., 2016; Prince et al., 2025). The effects of Clever-1 blockade on circulating monocytes were of particular interest, as tumors recruit monocytes to differentiate into tumor-promoting TAMs. As expected, we observed bexmarilimab to mainly bind $CD14^{high}$ classical monocytes, and not lymphocytes or granulocytes in the circulation (II: Fig. S4A). Clever-1 is mainly found in intracellular compartments, but a small portion of Clever-1 is localized on the plasma membrane to facilitate receptor-mediated endocytosis (Kzhyshkowska et al., 2004; Kzhyshkowska, Workman, et al., 2006). Upon binding Clever-1, bexmarilimab is swiftly taken inside monocytes/macrophages (II: Fig. S1A). Regardless, we detected more than 50% receptor occupancy on the surface of $CD14^{high}$ monocytes one day after dosing with at least 1 mg/kg bexmarilimab (II: Fig. 3D; III: Fig. S2B). The occupancy had declined by day 7 (II: Fig. 3D; III: Fig. S2B), as classical monocytes have short half-lives in the circulation and are constantly replenished by new monocytes from the bone marrow (Patel et al., 2017).

We investigated how bexmarilimab therapy altered circulating monocyte phenotypes by RNA-seq and cytometry by time-of-flight (CyTOF). Bexmarilimab reduced anti-inflammatory macrophage markers CD206 and CD163 on $CD14^{high}$ monocytes by day 7 post-treatment (II: Fig. 3C), and downregulated LXR/RXR and PPAR signaling pathways on $CD14^{+}$ monocytes (II: Fig. 3G). Simultaneously, inflammation-related genes, such as *IL1B* (pro-inflammatory cytokine), *IL1R2* (IL1 decoy receptor), *JUN* (AP-1 transcription factor subunit) and *TNFRSF1A* (TNF receptor) were upregulated (II: Fig. 3G-H and Suppl. Data File 2). Such pro-inflammatory monocyte conversion was observed only in patients with sufficiently high Clever-1 receptor occupancy (II: Fig. 3G and S4D). Despite rapid bexmarilimab internalization and transient receptor occupancy, these changes in monocyte transcriptome were observed on day 7 and not yet on day 1 post-treatment (II: Fig. 3E, F and H).

5.2.2 Bexmarilimab activates circulating T cells

The observed pro-inflammatory monocyte conversion was accompanied by increases in circulating NK, B and $CD8^{+}$ T cells and a decrease in T_{REG} cells (II: Fig. 5A). More detailed analyses with CyTOF identified most T-cell subsets to express markers associated with T-cell proliferation (CD25), activation and recruitment (C-X-C chemokine receptor type 3, CXCR3) more highly after bexmarilimab therapy, with the exception of T_{REG} cells and two naïve $CD4^{+}$ T-cell subsets not upregulating CD25 (II: Fig. 4D and S5). Furthermore, immune checkpoint molecules were downregulated on $CD8^{+}$ T cells (inhibitory LAG-3) and $CD4^{+}$ T cells (inhibitory CTLA-4 and PD-L1, stimulatory CD28, OX40 and ICOS) (II: Fig. 4D). We also evaluated how peripheral immune cells react to TLR activation by LPS *ex vivo*, and observed that patient PBMCs produced stronger type I IFN responses already one day after the first bexmarilimab dosing (II: Fig. 5D). Therefore, bexmarilimab therapy promotes both T-cell expansion and activation.

5.2.3 Clever-1 blockade impairs lysosomal acidification to promote antigen presentation

To investigate how blocking monocyte Clever-1 with bexmarilimab associates with the observed T-cell activation, we treated whole blood from healthy donors with a bexmarilimab concentration corresponding to intermediate MATINS trial dose, and analyzed the treatment-induced changes by CyTOF (II: Fig. S3). We observed similar CD206 downregulation on classical monocytes as in the trial patients. Furthermore, proportions of both $CD4^{+}$ and $CD8^{+}$ effector cells increased and the

CTLA-4 checkpoint molecule was downregulated on $CD4^+$ T cells, suggesting a link between monocyte Clever-1 blockade and T-cell activation.

In search of a putative mechanism, we investigated the functionality of bexmarilimab binding site on Clever-1. When human macrophages were treated with bexmarilimab and a non-function-blocking anti-Clever-1 antibody (9-11), the antibodies were internalized into slightly different intracellular vesicles with more single-stained vesicles lacking bexmarilimab than 9-11 (II: Fig. 1A), suggesting that bexmarilimab binds only a portion of Clever-1 molecules. Upon scavenging (e.g. acetylated LDL), Clever-1 is known to deliver its cargo into lysosomes for degradation (Kzhyshkowska, Workman, et al., 2006). We further showed that bexmarilimab binding site on Clever-1 interacts with vacuolar ATPase, a proton pump, which was recruited into LAMP-1^+ lysosomes by Clever-1-mediated scavenging (II: Fig. 1B-G and 2F-H). By inhibiting this interaction bexmarilimab impaired lysosomal acidification (II: Fig. 2I). This has immunological consequences, as slower antigen degradation alters antigen processing and facilitates cross-presentation for $CD8^+$ T cells (Alloatti et al., 2015; Delamarre et al., 2005; Tang-Huau et al., 2018). Therefore, our results suggest that bexmarilimab binding site supports enhanced antigen degradation in more acidic lysosomes, while bexmarilimab treatment reverses this Clever-1-mediated suppression of antigen presentation. Importantly, bexmarilimab does not impair uptake of tumor antigens, as we detected unaltered macrophage phagocytosis of dying cancer cells by confocal microscopy (II: Fig. S1C).

5.3 DC during bexmarilimab therapy associates with TAM phenotype conversion and IFN responses (III)

Having observed monocyte and T-cell activation after bexmarilimab therapy in the peripheral blood, we went on to investigate immunological changes within the treated tumors and their association with therapeutic response. Upon near completion of the MATINS trial, with one of the cancer cohorts still recruiting, a total of 138 patients with advanced cancer had been treated with bexmarilimab. Partial response (n = 1) or stable disease (n = 18) according to Response Evaluation Criteria in Solid Tumors 1.1 was observed in 14% of the treated patients. Highest DC rates (25-40%) were reported in cutaneous melanoma, hepatocellular, gastric, estrogen-positive breast and biliary tract cancer cohorts. (III: Table 3) A considerable proportion of DC patients (42%) also achieved progression-free survival time that exceeded the duration of their previous line of therapy (III: Table S4). DC patients were also characterized by increasing IFNγ serum levels during therapy (III: Fig. 1F) and

higher pre-treatment intratumoral Clever-1 expression, especially in conjunction with lower pre-treatment tumor PD-L1 expression (III: Table 4).

To investigate DC-associated immunological changes within patient tumors, we performed spatial transcriptomics profiling of available paired pre- and post-treatment tumor biopsies from patients with (DC, n = 3) and without (non-DC, n = 3) DC during bexmarilimab therapy. A total of 180 tumor areas, including macrophages ($CD68^+$), vessels ($CD31^+$) and remaining tumor area ($CD68^-CD31^-$) were subjected to next-generation sequencing, which allowed us to analyze bexmarilimab therapy effects on Clever-1 expressing cell subsets (macrophages and endothelial cells) separately from the rest of the tumor area.

5.3.1 Bexmarilimab activates TAMs in patients with DC

In DC patient macrophages, we observed upregulation of both type I and type II IFN signaling and pathways related to adaptive immune system activation (III: Fig. 2C). This was accompanied by changes in macrophage metabolic pathways, such as downregulation of respiratory electron transport (III: Fig. 2C). In contrast, non-DC patients failed to show similar immune activation and even downregulated these same pathways (III: Fig. 2C), while demonstrating remarkably fewer treatment-induced transcriptional changes (III: Fig. 2D). As we had previously observed a pro-inflammatory switch in circulating monocytes during bexmarilimab therapy, we also quantified average expression of pro-inflammatory (M1) and anti-inflammatory (M2) macrophage genes to evaluate, whether similar phenotype conversion occurs in intratumoral macrophages. While non-DC macrophages had unchanging M1 and M2 macrophage scores despite bexmarilimab therapy, DC patients' TAMs showed a clear induction in M1 scores and downregulation of the M2/M1 ratio (III: Fig. 2F-G).

In comparison to macrophages, changes in the vessels upon bexmarilimab therapy were more similar between DC and non-DC patients (III: Fig. S10A-D). Interestingly, both DC and non-DC patient vessels showed downregulation in pathways involving integrin interactions and ECM synthesis (III: Fig. S10B and D). Previous work has shown Clever-1 blockade to interfere with leukocyte binding to the vascular endothelium and subsequent extravasation (Irjala, Elima, et al., 2003; Karikoski et al., 2009), but in addition to mediating leukocyte adhesion, integrins also regulate angiogenesis and vascular integrity (Foubert & Varner, 2012; Vestweber, 2015). Observing equal downregulation regardless of therapy response suggests that therapeutic efficacy depends more on Clever-1 blockade in monocytes and TAMs.

5.3.2 Bexmarilimab activates adaptive immunity within tumors

Next, we analyzed how the pro-inflammatory macrophage switch in DC patients was reflected in the surrounding TME. Expectedly, upregulation in pathways related to adaptive immune activation was observed, including CD3 and TCR ζ-chain phosphorylation, IFNγ signaling and PD-1 signaling (III: Fig. 3B). We additionally observed metabolic reprogramming characterized by strong downregulation of pathways related to mitochondrial function and respiratory electron transport in the DC patient TME (III: Fig. 3B). Unsurprisingly, these effects were observed only in DC patients, while non-DC patients showed downregulation of IFN signaling (III: Fig. S10F).

Having analyzed all three tumor region transcriptomes, we looked for compartmentalization of the observed responses. For instance, changes related to lymphocyte activation, inflammatory signaling pathways and mitochondrial function were exclusive to macrophages and TME, while ECM and integrin pathways were specifically downregulated in the Clever-1-expressing cell types, TAMs and vessels (III: Fig. 3D).

While these patterns suggested successful annotation of macrophages and vessels in the biopsies, we further confirmed the specificity of our $CD68^+$ and $CD31^+$ area transcriptomes by deconvolution, which estimates cell type abundancies based on known single-cell transcriptomes of tumor-infiltrating cells. The deconvolution analyses confirmed that our $CD68^+$ and $CD31^+$ region transcriptomes were mainly derived from macrophages and endothelial cells (III: Fig. S8A-B). Furthermore, deconvolution allowed us to compare immune cell abundancies in the TME before and after bexmarilimab therapy. In accordance with analyses from patient peripheral blood (II: Fig. 5A), we observed increases in NK cells, $CD8^+$ and $CD4^+$ T cells, regardless of DC status (III: Fig. 3C). Interestingly, B cells and macrophages were increased only in DC patient TME, while upon bexmarilimab therapy their abundance in non-DC TME decreased (III: Fig. 3C).

In search for patient characteristics that could potentially explain the different responses observed in DC and non-DC patients, we compared their pre-treatment transcriptomes. Before bexmarilimab, DC patients' TME showed stronger activation of pathways related to mitochondrial function (III: Fig. 3F). Surprisingly, when comparing macrophage transcriptomes, the resemblance between DC and non-DC patients was high, with mainly only ECM remodeling pathways expressed more highly in DC patient TAMs (III: Fig. 2H).

5.4 Bexmarilimab activates TAMs in IFN-poor microenvironments (IV)

MATINS trial revealed patient heterogeneity in bexmarilimab responses, as the progression of advanced cancer was curbed in a subset of patients, while dampened intratumoral immune responses were seen in others (III). For successful patient selection, mechanisms regulating therapeutic responses and predictive biomarkers need to be uncovered. Therefore, we next investigated, which TME- and TAM-related characteristics would associate with bexmarilimab response.

5.4.1 *Ex vivo* bexmarilimab elicits IFN responses and T-cell recruitment in malignant ascites

We selected ovarian cancer patient ascites cells as an *ex vivo* model to evaluate how tumor-infiltrating immune cell phenotypes regulate bexmarilimab responses, as ascites cell suspensions were rich in Clever-1-expressing TAMs and immune cells (IV: Fig. 1B). Ascites cells were treated *ex vivo* with bexmarilimab for 48h before analyzing bexmarilimab-elicited immune activation. Bexmarilimab response was defined as upregulation of IFN signaling, as we had previously observed elevated serum IFN levels and intratumoral IFN signaling in MATINS trial DC patients during bexmarilimab therapy (III: Fig. 1F, 2C and 3B). When both type I (*MX1*) and type II (*CIITA*) IFN response genes were measured from bexmarilimab-treated ascites cells by qPCR, a subset of ascites samples showed upregulation of both type I and type II IFN responses (IV: Fig. 1D). As *MX1* was more strongly upregulated, we defined bexmarilimab-responsive ascites samples based on *MX1* upregulation (IV: Fig. 1D), resulting in 36% of responsive samples, a proportion similar to MATINS trial DC rates in the most promising cancer cohorts (III).

Bexmarilimab responses were then investigated more closely by scRNA-seq of bexmarilimab-responsive and non-responsive ascites samples. We confirmed upregulation of both IFNα and IFNγ signaling in monocytes/macrophages and $CD8^+$ T cells of the responsive ascites samples. Opposite effects, namely downregulation of these same pathways, was observed in the non-responsive ascites cells (IV: Fig. 2A-B). The elevated IFN responses were associated with increased secretion of CXCL10 and CCL5 (IV: Fig. 1E), which mediate $CD8^+$ T-cell and pro-inflammatory macrophage infiltration and co-operation in the TME for enhanced anti-tumor immune responses (Dangaj et al., 2019; Han et al., 2023; House et al., 2020; van Elsas et al., 2024). High *CXCL10* mRNA expression was observed specifically in monocytes/macrophages after bexmarilimab treatment (IV: Fig 2F and S4F). This bexmarilimab-induced CXCL10 secretion depended on monocyte/macrophage interactions with other ascites cell types, as treating ascites $CD14^+$ monocytes and macrophages alone was not sufficient to elevate CXCL10 secretion (IV: Fig. 2G).

5.4.2 IL4I1 macrophages and high baseline IFN signaling associate with bexmarilimab resistance

After observing similar bexmarilimab responses in *ex vivo*-treated ascites samples as in patients receiving bexmarilimab therapy, we searched for characteristics associating with bexmarilimab sensitivity. Based on the scRNA-seq of non-responsive and responsive ascites samples, we observed the non-responsive monocytes/macrophages to have higher baseline IFN signaling levels (IV: Fig. 3E). Furthermore, after annotating ascites immune cell subtypes at the single-cell level, we found that non-responsive ascites samples had more IL4I1 macrophages, T_{REG} cells and proliferating NK cells (IV: Fig. 3A, C and D). Notably, IL4I1 macrophages exhibit an immunosuppressive phenotype driven by prolonged exposure to IFNs (Mulder et al., 2021). These observations suggested macrophage IFN exposure as a key factor determining their sensitivity to bexmarilimab.

5.4.3 Chronic IFN priming impairs macrophage activation by bexmarilimab

To validate that IFN exposure regulates bexmarilimab sensitivity, we treated primary human macrophages with IFNs and measured subsequent bexmarilimab-induced macrophage activation. Macrophage activation was measured as LPS-induced secretion of TNFα, which is a pro-inflammatory cytokine known to be elevated upon Clever-1 blockade (Hollmén et al., 2022; Palani et al., 2016). We observed both IFNγ and IFNα2 to inhibit bexmarilimab-induced TNFα secretion, but notably chronic exposure to IFNs was necessary for the full inhibitory effect (IV: Fig. 4B-D).

We next investigated the relevance of these findings in patients receiving bexmarilimab therapy. First, lower serum IFNγ and CXCL10 levels pre-treatment associated with upregulation of these same cytokines during subsequent bexmarilimab therapy (II: Fig. 5C). Secondly, a trend for higher $IL4I1^+$ stromal cell abundance, representing mostly TAMs (Carbonnelle-Puscian et al., 2009; Ramspott et al., 2018), was observed in pre-treatment tumor biopsies of patients not achieving DC during bexmarilimab therapy (IV: Fig. 4G). These results suggest IFNγ exposure to regulate bexmarilimab sensitivity in patients with advanced cancer.

5.5 Breast tissue niches determine distinct bexmarilimab responses (V)

We continued our investigation of mechanisms regulating bexmarilimab sensitivity, but switched on to study an *ex vivo* model with a complete TME, breast cancer PDECs. Despite differing from the real TME in terms of new immune cell recruitment, circulation and longevity, PDECs can accurately model and predict

cancer immunotherapy responses (Voabil et al., 2021). We treated breast cancer PDECs with bexmarilimab for 48h and measured early immunological responses with RNA-seq, qPCR and cytokine profiling.

5.5.1 A five-gene signature identifies bexmarilimab-sensitive PDECs

To recognize bexmarilimab-sensitive PDECs, we first defined a core bexmarilimab-elicited gene expression response by identifying DEGs common to MATINS trial DC patient tumors (III) and bexmarilimab-responsive ascites cells (IV) (V: Fig. 1A-C). Bexmarilimab-induced changes in these genes were measured from a discovery set of 13 breast cancer PDECs, which resulted in the recognition of bexmarilimab-responsive PDECs (38%) and top 5 genes characterizing these PDECs (*GBP5*, *FCGR1A*, *SERPING1*, *SLAMF7* and *CXCL9*) (V: Fig. 1D-E). Upon validation in a set of 24 PDECs, these five genes clustered patients into two groups showing either upregulation of at least 4 of the genes (responsive PDECs) or overall downregulation (non-responsive PDECs) (V: Fig. 1F-H). Each individual gene classified the PDECs with excellent power, as demonstrated by AUC values between 0.76 and 1.0 (V: Fig. 1G).

The resulting two PDEC groups captured the dual nature in bexmarilimab responses, as observed in MATINS patient tumors (III) and ovarian ascites cells (IV). Bexmarilimab upregulated IFNγ signaling (V: Fig. 2G-H), pathways involved in cytokine-mediated immune activation (TNFα, IL-2) and lymphocyte regulation in the responsive PDECs, with IFNγ and TNFα identified as the foremost regulators upstream of these transcriptional changes (V: Fig. 2A and E-G). Bexmarilimab also elevated chemokine secretion, predominantly CCL4 and CCL5, in the responsive PDECs (V: Fig. 2I-K). In contrast, bexmarilimab treatment of the non-responsive PDECs strongly downregulated their existing IFN signaling, chemokine secretion and pathways related to adaptive immunity (V: Fig. 2B, E and I-K). Both PDEC groups responded to bexmarilimab with upregulated ECM remodeling (V: Fig. 2C and E), reflecting changes in MATINS patient TME rather than macrophages or vessels (III: Fig. 3D).

This PDEC study also independently validated our previous observations on IFNγ exposure determining bexmarilimab sensitivity, as non-responsive PDECs showed higher immune activation status, regulated foremost by IFNγ and IL-2, and more active IFNγ and IFNα signaling (V: Fig. 3A-C). Furthermore, signatures for IL4I1 macrophages and other IFN-regulated TAM subsets were markedly higher in non-responsive PDECs (V: Fig. 3D and S2E). Higher pre-treatment levels of serum IFNγ also associated with a trend for shorter survival in the small breast cancer cohort of the MATINS trial (V: Fig. 3G). In a much larger pan-cancer analysis,

STAB1 expression associated with poorer survival across several tumor immune landscape subtypes, but not in IFNγ-dominant tumors (V: Fig. 3E-F).

5.5.2 Bexmarilimab activates B cells in cancer-free tissues

We compared bexmarilimab responses between tumor and adjacent cancer-free breast tissues of the same patients to gain insight on how bexmarilimab may regulate tissue-resident macrophages across the body. PDECs of adjacent cancer-free breast tissues were treated *ex vivo* with bexmarilimab alongside the corresponding tumor PDECs and analyzed by RNA-seq and cytokine multiplexing. Most notably, bexmarilimab responses in the adjacent tissues were mainly unaffected by the bexmarilimab sensitivity in the matching tumor. In both responsive and non-responsive patients' adjacent tissue PDECs, bexmarilimab-induced inflammatory responses resembled those of the responsive tumors (V: Fig. 4B and E). In comparison to responsive tumor PDECs, bexmarilimab failed to elevate chemokine and cytokine secretion in the adjacent tissue PDECs (V: Fig. 4E and H-J), and upregulated immunoglobulin gene transcription and B-cell signaling instead (V: Fig. 4C, D and E). The observed changes in B-cell activation appeared stronger in the adjacent tissue PDECs of responsive patients (V: Fig. 4C, D and E).

5.5.3 Tumor secretome confers bexmarilimab sensitivity

Altogether, three distinct bexmarilimab responses were thereby identified: cytokine- and chemokine-dominated inflammatory response (responsive tumors), B-cell activation (adjacent tissue) and dampening of the existing inflammation (non-responsive tumors). We next asked, whether macrophages themselves or their surrounding tissue niches determine the type of bexmarilimab response.

We compared scRNA-sequenced *STAB1*$^{+}$ monocytes and macrophages between tumor and cancer-free tissues to reveal differences in ontogeny and activation state (V: Fig. 5). While *STAB1*$^{+}$ TAMs mapped to monocyte-derived TAM populations in the MoMac-VERSE scRNA-seq atlas, *STAB1*$^{+}$ macrophages from the adjacent tissues resembled tissue-resident HES1 macrophages expressing *FOLR2*, *LYVE1* and *CD206* (Matusiak et al., 2024; Mulder et al., 2021; Nalio Ramos et al., 2022) (V: Fig. 5C-F and S4H). TME had also stimulated *STAB1*$^{+}$ monocytes and TAMs for elevated expression of MHC molecules and IFNγ signaling, which was offset by their higher expression of immunosuppressive mediators, such as TREM2 and IL4I1 (V: Fig. 5G-I). These *STAB1*$^{+}$ monocytes and TAMs were found in several types of leukocyte-rich tumor niches (T_{REG}-, B-cell- and myeloid cell-dominated) based on spatial transcriptomics analyses (V: Fig. 6C, E and G). Among tumor niches, *STAB1* expression also correlated with B-cell (*MS4A1*) and T-cell (*CD3E*, *CD4*, *CD8A*)

marker gene expression (V: Fig. 6I). In contrast, only B-cell dominated healthy tissue niches were rich in *STAB1*$^{+}$ macrophages, suggesting that bexmarilimab targets Clever-1^{+} resident macrophages in these niches for the observed B-cell activation.

In summary, tissue type determined Clever-1^{+} macrophage ontogeny, activation status and lymphocyte subtypes under their control. Still, culturing adjacent tissue PDECs in tumor-conditioned medium of the same patient allowed bexmarilimab to elicit similar cytokine responses, either increased or decreased secretion, as in the corresponding tumors (V: Fig. 7A-B). Therefore, the soluble tumor secretome is sufficient to convey bexmarilimab sensitivity or resistance to healthy tissue macrophages that remain reprogrammable despite their different ontogeny and neighboring cell types.

6 Discussion

6.1 The future of targeting TAMs (I)

Our review on TAM-reprogramming immunotherapies aimed to uncover big trends across past and current clinically developed therapeutics. We observed an increasingly high number of therapeutics entering clinical investigation, but also a high rate of clinical program discontinuations, which had excited translational research for designing more potent and safer therapeutics against the original TAM targets. Furthermore, low monotherapy efficacy has made combinatory treatment regimens, especially with ICIs, more appealing than investigating the TAM-reprogramming therapeutics alone.

As we observed high redundancy in clinical studies, we suggested that similar therapeutics should be compared early on, possibly by utilizing patient-derived explant models that can offer predictive information on therapeutic responses in patients (Voabil et al., 2021). With valid end-point measures that acknowledge the complex nature of tumor immunobiology, such analyses could focus the efforts of clinical investigation on the most promising therapeutic candidates. Additionally, open sharing of clinical trial results and designing clinical trials that facilitate comparison with other therapeutics could elevate the impact of conducted trials.

To improve modest efficacy, a frequent cause for terminating clinical development (NCT4313881, NCT04778397, NCT03937141) (Bekaii-Saab et al., 2023; Fayad et al., 2015; Galluzzi et al., 2012; Long et al., 2019), patient selection should be optimized. This can be done by identifying optimal cancer types, disease stages, treatment timings, therapeutic combinations and predictive biomarkers, and designing the clinical trials accordingly. While current knowledge on these issues seems quite scarce for most of the therapeutics undergoing clinical investigation, discovering the determinants of treatment sensitivity for one TAM-reprogramming agent can benefit the development of others, especially if the treatments target similar TAM subsets.

Another route for enhancing treatment efficacy is to utilize treatment combinations, and we observed routine investigation of TAM-targeted agents in conjunction with T-cell-activating ICIs. In general, coordinated activation of TAMs and T cells does facilitate strong anti-tumor immunity and tumor rejection (House et

al., 2020; Klug et al., 2013; van Elsas et al., 2024; Vermare et al., 2022). Therefore, well-designed and mechanism-driven combinatory approaches will undoubtedly enhance treatment efficacy. Nevertheless, design of successful combinatory strategies additionally calls for deep understanding on the complex interactions between TAMs, T cells and the administered therapeutics.

Furthermore, we highlighted the already initiated translational research that has generated a vast repertoire of novel approaches and considerations for enhanced efficacy and safety, including improved molecular entities and delivery methods (Bukhalid et al., 2025; Dahan et al., 2016; Eremina et al., 2020; Hägerbrand et al., 2022; Kamerkar et al., 2022; Thisted et al., 2024; Ye et al., 2019), which will benefit future drug development. Additionally, as the majority of currently explored treatment targets are not specific to TAMs, identifying novel, truly TAM-specific targets or utilizing TAM-targeted delivery strategies can enhance both efficacy and safety. Single-cell and spatial transcriptomics have identified common TAM phenotypes across cancers, as reviewed in this thesis, possibly exposing novel targetable TAM vulnerabilities that allow more specific control of TAM function.

In the future, therapeutic manipulation of TAMs should be combined with methods that deeply probe the existing TAM phenotypes and their therapeutic responses, preferably at the TAM subset level. Likewise, other recent reviews on therapeutic targeting of TAMs emphasize the understanding of complex TAM populations present in clinical samples and targeting treatments for functionally distinct TAM subsets (Cassetta & Pollard, 2023; Nasir et al., 2023). To generate knowledge on the determinants of treatment efficacy, observed responses should be further associated with patient's clinical history, tumor immune landscape and pre-treatment localization and functionality of TAMs. Alongside these powerful single-cell and spatially focused methods, future therapeutic targeting still needs to conform with fundamentals of biology, ensuring sufficient solid tumor access, non-redundancy in targeted processes and strategies for overcoming negative feedback activation. All in all, knowledge accumulated from the past trials and therapeutic candidates will accelerate the development of TAM-targeted treatments that could significantly improve the care of cancer.

6.2 Clever-1 blockade in human cancer (II – III)

In patients with advanced cancer, therapeutic responses to bexmarilimab-mediated Clever-1 blockade were manifested as disease stabilization, which was observed in 14% of all treated patients and in 25 – 40% of patients from the most promising cancer cohorts. The duration of disease stabilization often exceeded the duration of the previous line-of-therapy, which has been suggested to indicate therapeutic benefit (Massard et al., 2017; Von Hoff, 1998).

Since Clever-1 exists as a membrane-bound receptor on monocytes, macrophages and endothelial cells, and as a secreted protein in plasma and lymph (Goerdt et al., 1993; Goerdt et al., 1991; Irjala, Elima, et al., 2003; Palani et al., 2016; Politz et al., 2002; Prince et al., 2025), the observed therapeutic effects may stem from blocking some or all of these Clever-1 sources. Preclinical studies have investigated the contribution of different Clever-1 sources to tumor growth in mice with total or cell-type-specific deletion of Clever-1 (Karikoski et al., 2014; Viitala et al., 2019). Deleting Clever-1 from macrophages rather than all cell types suppresses primary tumor growth more strongly in most murine tumor models (Lewis lung carcinoma, EL4 lymphoma and E0771 breast cancer) (Viitala et al., 2019), but not all (B16 melanoma) (Karikoski et al., 2014), while lymphatic Clever-1 supports the growth of metastatic lesions (Karikoski et al., 2014).

Building on to these results, the overarching aim of this thesis was to investigate immunotherapeutic blockade of Clever-1 on human monocytes and macrophages to understand bexmarilimab therapy responses and sensitivity in patients with cancer. As immunotherapeutic blockade differs from genetic deletion by targeting a specific epitope on Clever-1, I first consider here the clinical evidence on how bexmarilimab interferes with the established Clever-1 functions, scavenging, immunosuppression and adhesion, on different Clever-1^{+} cell types. Future research will elucidate the relative therapeutic contribution of inhibiting secreted Clever-1 with bexmarilimab, as the ability of secreted Clever-1 to suppress T-cell responses was discovered very recently (Prince et al., 2025).

First, Clever-1 mediates scavenging of various ligands, such as modified LDLs, apoptotic cells and SPARC (Adachi & Tsujimoto, 2002; Kzhyshkowska, Workman, et al., 2006; Lee et al., 2011; Park et al., 2009). We did not observe bexmarilimab to impair apoptotic cell uptake inside human macrophages, suggesting that TAMs remain capable of scavenging tumor-derived material. However, bexmarilimab does inhibit scavenging of acetylated LDL (Hollmén et al., 2020), and Clever-1 can mediate the uptake of other modified LDLs, such as malondialdehyde-modified and oxidized LDLs (Adachi & Tsujimoto, 2002; Rantakari et al., 2016; Tamura et al., 2003). Enhanced lipid uptake via modified LDL scavenging can induce LXRα and PPARγ in human macrophages *in vitro* (Huang et al., 1999; Ignatova et al., 2013; Laffitte et al., 2001; Nagy et al., 1998; Nelson et al., 2017). Hence, inhibition of modified LDL scavenging by bexmarilimab *in vivo* would provide a potential mechanism for the observed LXR/RXR and PPARγ signaling downregulation in MATINS trial patient monocytes. Therefore, scavenging of selected Clever-1 ligands appears diminished after bexmarilimab treatment, likely depending on their binding site on Clever-1. We did not directly investigate, whether bexmarilimab similarly alters scavenging on endothelial cells, but evident adverse events from impaired endothelial scavenging were not observed in the MATINS trial (III).

Importantly for anti-tumor immunity, both myeloid cell and endothelial Clever-1 perform immunosuppressive functions (Dunkel et al., 2018; Palani et al., 2016; Tadayon et al., 2021; Viitala et al., 2019). Our spatial transcriptomics analyses revealed activation of several immune-related pathways on MATINS trial DC patient TAMs. Tumor vasculature, comprising of CD31-identified blood and lymphatic vessels (Podgrabinska et al., 2002), lacked similar bexmarilimab-induced pro-inflammatory changes that would associate with patient DC. This suggests that immunotherapeutic blockade of macrophage Clever-1 has a greater role in controlling tumor growth, as was suggested by previous studies in mice (Viitala et al., 2019). Notably, the immunostimulatory effects on MATINS patient monocytes were more pronounced seven days rather than one day after the first treatment dose, despite a short monocyte half-life in the circulation (Patel et al., 2017). This would suggest that the initial Clever-1 blockade elicits systemic immunological changes outside monocytes, thereby maintaining the pro-inflammatory conversion of the circulating inflammatory monocyte pool.

Cell adhesion to tumor vascular and lymphatic endothelium can be indirectly evaluated from our studies by post-therapy transcriptional changes in endothelial cells and altered densities of tumor-infiltrating immune cells. The parent antibody of bexmarilimab, 3-372, inhibits adhesion and transmigration on the vascular and lymphatic endothelium (Irjala, Alanen, et al., 2003; Irjala, Elima, et al., 2003; Shetty et al., 2011). Accordingly, we observed decreased integrin signaling in the tumor endothelium region. However, integrin signaling is known to regulate other processes besides adhesion within the vasculature (Foubert & Varner, 2012; Vestweber, 2015). While 3-372 also reduces immune cell trafficking into inflammatory sites (Karikoski et al., 2009), we observed increased intratumoral macrophage, NK and B-cell infiltration in patients with DC, and elevated intratumoral T-cell infiltration regardless of bexmarilimab therapy response. Therefore, blockade of endothelial Clever-1 does not significantly interfere with tumor immune cell infiltration, and any inhibitory effects may have been counterbalanced by local immune cell proliferation, alternative adhesion molecules or immune cell retention caused by lymphatic Clever-1 inhibition.

6.3 Bexmarilimab therapy engages adaptive immunity (II – V)

Effective cancer immunotherapy depends on the activation of both innate and adaptive arms of immunity, even with T-cell-targeted therapeutics (House et al., 2020; Klug et al., 2013; van Elsas et al., 2024; Vermare et al., 2022). While bexmarilimab activated patient monocytes and TAMs, we also reported enhanced T-cell responses. Elevated and proliferating $CD8^+$ and $CD4^+$ T cells were observed in

the patient circulation after bexmarilimab therapy, and increased $CD4^+$ and $CD8^+$ effector T cells were measured from *ex vivo*-treated healthy blood. In DC patient tumors, bexmarilimab increased T-cell infiltration as well as CD3 and TCRζ chain phosphorylation. Therefore, control of tumor growth during bexmarilimab therapy likely depends on both macrophages and T cells, as has been reported in murine tumor models (Viitala et al., 2019).

Our research also revealed putative mechanisms linking monocyte and macrophage Clever-1 blockade with elevated T-cell responses. *In vitro*, we observed blockade of bexmarilimab epitope on Clever-1 to halt lysosomal acidification. Since slower antigen degradation caused by increased lysosomal pH promotes alternative antigen processing for antigen presentation (Alloatti et al., 2015; Delamarre et al., 2005), this observation could explain the enhanced stimulation of T cells after bexmarilimab treatment. Concurrently, Clever-1 disruption alters monocyte and TAM phenotypes by supporting pro-inflammatory cytokine secretion (TNFα, IL-12) and signaling (NF-κB, JUN) (II, IV; Palani et al., 2016; Viitala et al., 2019) over immunosuppressive signaling pathways (LXR/RXR, PPARγ, CD206, CD163; II). Both elevated antigen presentation and pro-inflammatory mediators will support T-cell activation, while the relative importance of these events for bexmarilimab mode-of-action remains to be investigated.

Another consistent effect of bexmarilimab treatment across our studies was upregulation of IFN signaling. All of the five genes in our core bexmarilimab response signature were IFNγ-stimulated genes (V), demonstrating their activation in PDECs, ascites cells and DC patient tumors. Such activated IFN signaling is essential for ICI therapy efficacy, whereas chronic IFN exposure can facilitate tumor immune escape and ICI resistance by inducing the expression of immunosuppressive IFN-stimulated genes (Ayers et al., 2017; Benci et al., 2016; J. Gao et al., 2016; Garris et al., 2018). As treating TAMs alone with bexmarilimab did not elevate the IFN-induced cytokine CXCL10, the induction of IFN responses also appears to depend on TAM interactions with other immune cells, likely T cells (Gocher et al., 2022).

As yet another mechanism for bexmarilimab-mediated interactions between the innate and adaptive arms of immunity, we observed elevated immune cell recruitment. Bexmarilimab treatment induced TAMs to secrete CXCL10 *ex vivo* and upregulated another CXCR3 ligand, CXCL9, in patient tumors. These changes alongside elevated CXCR3 expression in circulating patient T cells strongly suggest that bexmarilimab enhances T-cell recruitment into tumors (House et al., 2020). Indeed, MATINS trial patients showed higher T-cell infiltration after bexmarilimab therapy. Furthermore, ovarian ascites cells and breast cancer PDECs sensitive to bexmarilimab-mediated induction of IFN signaling concurrently upregulated their secretion of CCR5-ligands CCL3, CCL4 and CCL5. These two cytokine groups

together can elicit beneficial co-recruitment of monocytes and T cells into tumors, as described by others (Dangaj et al., 2019; van Elsas et al., 2024) and observed in MATINS DC patient tumors. In particular, activated IFNγ-secreting $CD8^+$ T cells can use CCR5-ligands to recruit monocytes into their vicinity for immunostimulatory macrophage differentiation (van Elsas et al., 2024).

Altogether, these results suggest a following chain of events: Bexmarilimab treatment would first directly alter monocyte and macrophage function, including impaired scavenging, enhanced pro-inflammatory cytokine and chemokine secretion, and elevated antigen presentation. These changes would then promote anti-tumoral T-cell recruitment and activation, as evidenced by elevated T-cell infiltration, proliferation, receptor signaling and IFN responses after bexmarilimab treatment. In turn, activated T cells would support a broader second wave of immune responses, including further monocyte and macrophage recruitment and activation by IFNs.

Several of these bexmarilimab-induced changes also predict better ICI therapy response, such as elevated CXCL9 levels, T-cell infiltration and IFN signaling (Ayers et al., 2017; Herbst et al., 2014). Therefore, a combinatory treatment strategy consisting of both bexmarilimab and an ICI may best work, if started with bexmarilimab to sensitize the TME for subsequent ICI therapy. However, the effect of bexmarilimab therapy on immune checkpoint molecule levels within the TME should be first investigated, as bexmarilimab downregulated PD-L1 and CTLA-4 in patient blood.

All in all, this thesis investigated bexmarilimab mode-of-action using several research settings, including primary human macrophages, patient-derived immune cells, breast cancer PDECs as well as patient blood samples and tumor biopsies from the MATINS trial. **Table 3** summarizes our observations on bexmarilimab immunobiology and compares the obtained results across different sample types. Overall, different approaches showed highly similar results. For instance, bexmarilimab-elicited T-cell recruitment and IFN responses were observed in all research sample types containing both T cells and monocytes or macrophages. The related **Figure 7** illustrates these results in the context of previous research, mainly performed *in vivo* and *in vitro* using mice with genetic deletion of Clever-1, murine anti-Clever-1 antibody mSTAB1.26 or bexmarilimab's parent antibody 3-372.

Table 3. Bexmarilimab treatment effects observed in this thesis and categorized by research sample types. *, *Ex vivo* treatment of cells isolated from patients with cancer; **, Treatment of blood or cells from healthy individuals.

BEXMARILIMAB TREATMENT EFFECT	PATIENT TUMOR (III)	PATIENT BLOOD (II-III)	*EX VIVO* PDEC (V)	*EX VIVO* CELLS* (II, IV)	*IN VITRO* CELLS** (II, IV)
1a. Pro-inflammatory conversion of macrophages					
TNFα secretion			X		X
IL-1β production		X	X		
CD163 downregulation		X			
CD206 downregulation		X			X
Decreased M2/M1 ratio	X				
Inhibition of LXR/PPARγ signaling		X			
Suppression of mitochondrial respiration	X				
1b. Modulation of macrophage endocytosis and cargo processing					
Lysosomal de-acidification					X
Antigen presentation					X
2. T-cell activation					
CD3 and TCR signaling	X				
Immune checkpoint downregulation		X			X
CXCR3 and CD25 upregulation		X			X
Enhanced LPS response (type I IFNs)				X	
Enhanced IFN signalling	X	X	X	X	
3. Immune cell recruitment					
Increased T-cell numbers	X	X			X
Increased B-cell numbers	X	X			
Increased macrophage numbers	X				
Secretion of CXCR3 ligands for T cells	X	X	X	X	
Secretion of CCL3, -4 and -5			X	X	
Downregulation of vessel integrin signaling (association with immune cell trafficking not established)	X				
4. Suppression of cancer growth					
Halted tumor growth	X				

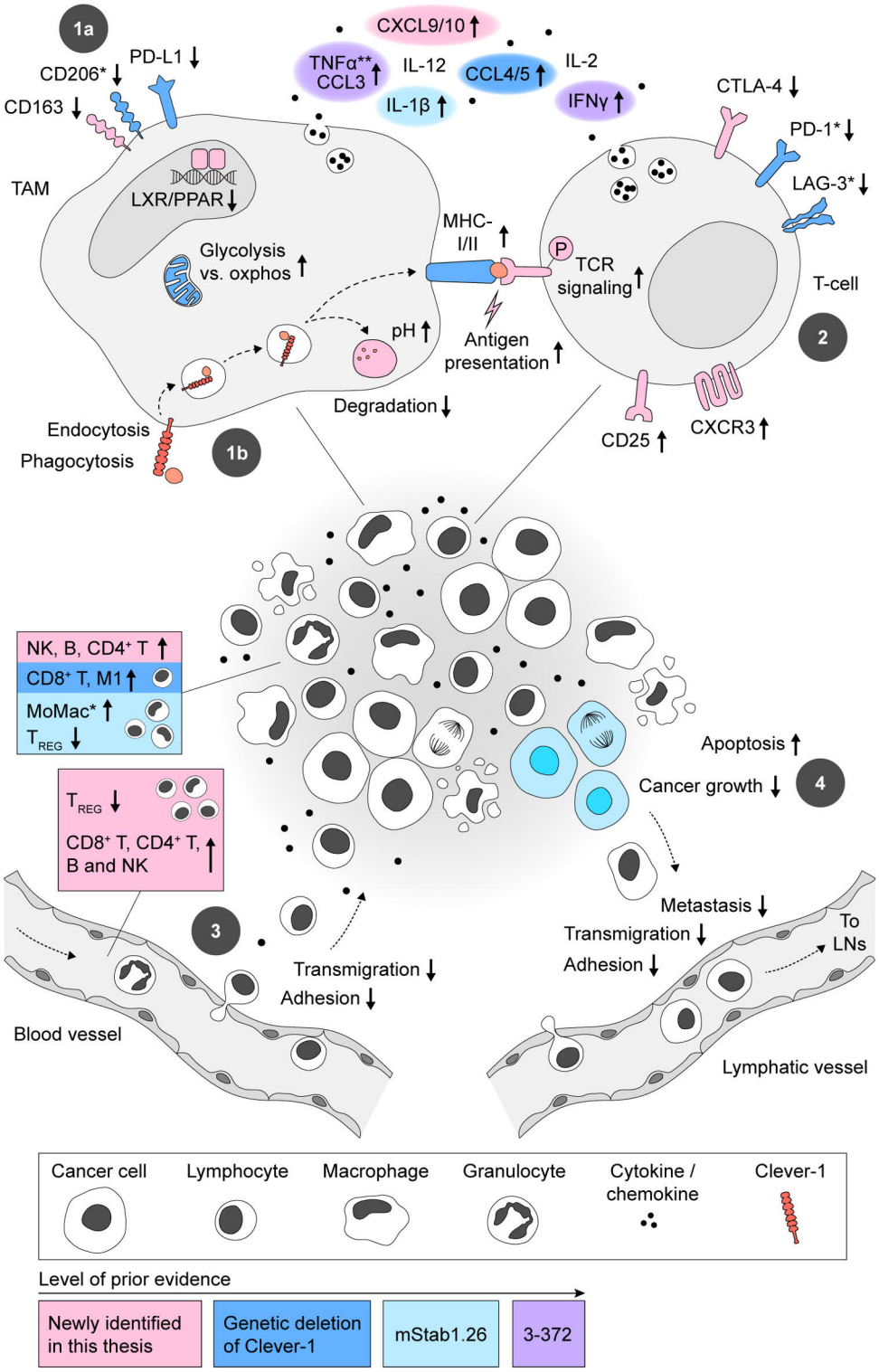

1a
CD206* ↓
CD163 ↓
PD-L1 ↓
CXCL9/10 ↑
TNFα** CCL3 ↑
IL-12
IL-1β ↑
CCL4/5 ↑
IL-2
IFNγ ↑
CTLA-4 ↓
PD-1* ↓
LAG-3* ↓
TAM
LXR/PPAR ↓
Glycolysis vs. oxphos ↑
MHC-I/II ↑
P
TCR signaling ↑
T-cell
2
pH ↑
Antigen presentation ↑
Degradation ↓
Endocytosis
Phagocytosis
1b
CD25 ↑
CXCR3 ↑
NK, B, CD4+ T ↑
CD8+ T, M1 ↑
MoMac* ↑
TREG ↓
Apoptosis ↑
Cancer growth ↓
4
TREG ↓
CD8+ T, CD4+ T, B and NK ↑
3
Metastasis ↓
Transmigration ↓
Adhesion ↓
To LNs
Transmigration ↓
Adhesion ↓
Blood vessel
Lymphatic vessel
Cancer cell
Lymphocyte
Macrophage
Granulocyte
Cytokine / chemokine
Clever-1
Level of prior evidence
Newly identified in this thesis
Genetic deletion of Clever-1
mStab1.26
3-372

Figure 7. Bexmarilimab treatment effects observed in this thesis. Bexmarilimab treatment converts TAMs towards a pro-inflammatory phenotype (1a) and alters their processing of endocytosed cargo to enhance antigen presentation (1b). Both of these processes support T-cell activation leading to stronger IFN responses (2) that further activate TAMs. These interactions result in enhanced secretion of cytokines (TNFα, IL-1β, IL-2) and chemokines (CXCL9, -10, CCL3, -4, -5) that further potentiate immune responses and recruit more immune cells to the TME (3). While bexmarilimab can also block Clever-1 on tumor vessels to limit circulating leukocyte adhesion and transmigration, we observed increased immune cell infiltration in the cancer patient TME after bexmarilimab therapy. Improved inflammatory responses can ultimately limit cancer growth (4), and blockade of Clever-1 on tumor lymphatics, not investigated in this thesis, may restrict cancer cell spread. Changes observed in this thesis are highlighted with colors. The coloring also indicates the highest level of prior knowledge, i.e. whether the highlighted effect of Clever-1 disruption was observed for the first time in this thesis (pink), previously in mice with genetic deletion of Clever-1 (blue), previously in mice treated with mStab1.26 antibody (light blue) or previously with bexmarilimab's parent antibody 3-372 (lilac). *, genetic deletion or immunotherapeutic blockade caused an opposite effect than reported in this thesis with bexmarilimab; **, elevated TNFα secretion has been previously reported with bexmarilimab.

6.4 Bexmarilimab breaches tissue tolerance (III, V)

Cancer immunotherapies strengthen the body's immune response against cancer cells, but not exclusively. Therefore, different autoimmune reactions against non-malignant tissues occur relatively commonly as immune-related adverse effects, and their occurrence associates with treatment benefit to anti-PD-1 ICIs (Okada et al., 2019; Rogado et al., 2019; Sato et al., 2018). In the MATINS trial, ten patients (7.2%) developed immune-related adverse events, which were mostly of lower grade (1 – 2), except for three cases of grade 4 hepatitis that were resolved by therapy discontinuation or corticosteroid treatment (III: Table S3).

Our *ex vivo* studies with PDECs allowed us to explore, how cancer-free tissues respond to bexmarilimab (V). While tumor PDECs were readily categorized in two groups based on their bexmarilimab sensitivity, adjacent cancer-free PDECs from both groups responded to bexmarilimab with moderate B-cell-dominated immune responses. It remains to be investigated, whether such B-cell activation would promote treatment-emergent autoimmune reactions or even support therapeutic efficacy. Nevertheless, the ability of bexmarilimab to activate the immune system outside the TME would explain, why we observed rather uniform immunological changes in the MATINS trial patient circulation. The TME in non-responsive patients would then actively dampen any immune responses within the tumor and prohibit treatment benefit, leading us to detect beneficial immune responses only in the tumor biopsies of patients with DC.

The unique bexmarilimab responses in cancer-free tissues were likely elicited via tissue-resident Clever-1^+ macrophages residing in B-cell-rich niches. Recent single-cell and spatial transcriptomics studies have described this macrophage subset and other Clever-1^+ macrophages in more detail. In the breast and colon tissue

(Matusiak et al., 2024), *STAB1* expression can be found on two FOLR2$^+$ tissue-resident macrophage populations (FOLR2$^+$LYVE1$^+$ and FOLR2$^+$LYVE1$^-$; my unpublished observation from Matusiak et al., scRNA-seq data). These macrophages were shown to reside either in plasma cell-rich, perivascular, lymphocyte-rich or fibrous stromal niches. The authors suggested FOLR2$^+$LYVE1$^-$ tissue-resident macrophages to actually maintain the plasma cell niches they reside in (Matusiak et al., 2024), associating with our report of enhanced B-cell activation in cancer-free tissues by bexmarilimab. The FOLR2$^+$LYVE1$^+$ macrophage population, on the other hand, additionally expresses CD163, MRC1 and scavenger receptors (Matusiak et al., 2024), and therefore resembles S*TAB1*$^+$*LYVE1*$^+$*FOLR2*$^+$*CD163*$^+$ lipid-associated macrophages identified in another scRNAseq study (Timperi et al., 2022). These macrophages are more prevalent in tumor tissues, suppress T-cell responses and associate with poor responses to anti-PD-1 therapy (Timperi et al., 2022), implying that Clever-1 inhibition on them could improve anti-PD-1 therapy efficacy.

Importantly, these unique Clever-1$^+$ macrophage subsets, residing in their local niches, appeared to remain malleable to environment- and bexmarilimab-mediated reprogramming, as evidenced by our studies on cancer-free tissues treated with tumor-conditioned media and bexmarilimab.

6.5 Determinants of bexmarilimab sensitivity (III – V)

TAM-reprogramming therapeutics commonly show modest overall objective response rates (I), which encourages to identify biomarkers for treatment-sensitive patients. Even though high individual heterogeneity in patient TMEs and therapeutic responses complicate finding useful predictive biomarkers, they are important for achieving sufficient overall treatment efficacy and reducing unnecessary side effects and treatment costs.

The first-in-human MATINS trial recruited patients with several types of advanced solid tumors, while high tumor Clever-1 expression was not yet among the inclusion criteria. In search for biomarkers, we first evaluated how target expression and occupancy associate with treatment responses. In patient circulation, sufficient monocyte Clever-1 occupancy with bexmarilimab was required for observing the changes in monocyte transcriptome (II). The level of receptor occupancy in circulation can be impacted by various factors, such as bexmarilimab pharmacokinetics, soluble Clever-1 levels and Clever-1 expression levels on monocytes and endothelial cells. In tumors, intratumoral Clever-1$^+$ cells significantly associated with DC during bexmarilimab therapy, while stromal Clever 1$^+$ cells did not (II: Table 4). This association may reflect a direct requirement for Clever-1$^+$

TAMs to reside near cancer cells, or alternatively, an overall need for intratumoral TAMs or specific TAM subsets.

Among the treated patients, five cancer types showed prominent (25 – 40%) DC rates: cutaneous melanoma, biliary tract cancers, hepatocellular, gastric and estrogen receptor-positive breast cancer. While apparent similarities in the immune contextures of these cancer types does not emerge (Bruni et al., 2020), quantification of PD-L1 alongside intratumoral Clever-1 separated patients with and without DC better, as patients with DC had a lower PD-L1/Clever-1 ratio. Furthermore, patients with DC had lower TNFα ($P = 0.011$) and IFNγ ($P = 0.067$) serum concentrations before bexmarilimab treatment. These exploratory findings connect therapeutic responses with sufficient intratumoral Clever-1 expression and circulating monocyte Clever-1 occupancy as well as non-inflamed immunological state. Future clinical studies with less heterogeneous patient populations in terms of cancer types, previous lines-of-therapies and varying levels of immune system fitness may possess higher power for validating our findings and discovering additional biomarkers.

Most notably, this thesis discovered prior, especially chronic, IFN exposure to prevent bexmarilimab-elicited immune activation in primary human macrophages, patient-derived cells and explant cultures (IV – V). Finding the exact mechanism for this inhibitory effect has proven to be difficult due to the various signaling pathways regulated by IFNs (Bhat et al., 2018; Ivashkiv, 2018; Kang et al., 2019), even though we know that the negative regulation can be mediated by both type I and II IFNs (IV). Curiously, prior IFN exposure not only inhibits bexmarilimab-mediated TNFα release, but actually decreases it (IV). Similarly, bexmarilimab downregulates inflammatory responses and IFN signaling in IFN-rich TMEs of bexmarilimab-resistant patient-derived cells and explant cultures (IV – V). Such downregulation can positively or negatively impact the overall prognosis of bexmarilimab-resistant patients, as chronic IFN signaling can be detrimental (Benci et al., 2019; Benci et al., 2016), but IFN responses before and upon ICI therapy are also a prerequisite for their therapeutic efficacy (Ayers et al., 2017; J. Gao et al., 2016; Garris et al., 2018; Herbst et al., 2014). In any case, these results suggest patient selection for future bexmarilimab trials in solid tumors based on baseline IFN levels.

Other possible characteristics affecting bexmarilimab response could relate to existing TAM phenotypes. We observed higher prevalence of $IL4I1^+$ TAMs and other IFN(γ)-regulated TAM phenotypes in ascites cells and PDECs not sensitive to *ex vivo* bexmarilimab treatment. Additionally, a trend for higher $IL4I1^+$ stromal cell abundancy was observed in the tumor biopsies of patients not achieving DC. As $IL4I1^+$ TAM phenotype has been associated with ICI responses (Matusiak et al., 2024; van Elsas et al., 2024), these findings suggest ICI and bexmarilimab to benefit distinct tumor types.

To summarize, bexmarilimab sensitivity associates with abundant Clever-1$^+$ TAMs (III), less IL4I1$^+$ TAMs and T_{REG} cells (IV – V), lower PD-L1 expression (III) and lower IFNγ, IL2 and TNFα signaling (II – V). These are features of non-inflamed and ICI-resistant tumors, while anti-PD-1 and anti-PD-L1 therapy require T-cell-inflamed and IFNγ-rich TME for clinical benefit (Ayers et al., 2017; Coulton et al., 2024; J. Gao et al., 2016; Herbst et al., 2014; Matusiak et al., 2024; Spranger et al., 2013). These findings suggest that non-inflamed (immune cold) tumors, rich in immunosuppressive macrophages, would more likely benefit from bexmarilimab, while inflamed (immune hot) tumors would more likely benefit from ICIs than bexmarilimab. Patient selection for either therapy would benefit from similar biomarkers, as patients with low Immunoscore, PD-L1 and IFN score would be non-eligible for ICI therapy and eligible for bexmarilimab therapy. Therefore, these results propose ICI and bexmarilimab to target different patient groups.

7 Conclusions

This thesis investigated the immune responses to Clever-1 blockade and macrophage reprogramming with bexmarilimab. Collectively, our studies on bexmarilimab treatment in patients with advanced cancer and *ex vivo*-treated patient-derived cells and tissues provide the first functional characterization of Clever-1 blockade in human cancer. The conclusions of this thesis can be summarized as follows:

1. Successful clinical targeting of TAMs requires deep understanding on TAM responses to therapeutic intervention under various tumor immune landscapes
2. Clever-1 blockade with bexmarilimab activates IFN-mediated immune responses and stabilizes disease progression in subsets of advanced cancer patients
3. Bexmarilimab activates circulating monocytes and TAMs in advanced cancer to enhance T-cell recruitment and activation
4. Clever-1 disruption breaches tissue tolerance to elicit B-cell responses in cancer-free tissues
5. Patients more likely to benefit from bexmarilimab therapy are characterized by high intratumoral Clever-1 but low PD-L1, IL4I1^{+} TAMs and IFN signaling before treatment, i.e., immune cold tumors

Overall, these results pave the way forward for the clinical development of bexmarilimab therapy by establishing preliminary efficacy, immune correlates of bexmarilimab response and putative markers for patients likely to respond. Future clinical studies with controlled design can formally measure treatment efficacy, validate the best methods for patient selection and investigate putative treatment combinations to enhance efficacy. Additional translational studies focusing on non-immune cell types within the TME, in particular cancer and endothelial cells, will further deepen the understanding on bexmarilimab treatment effects within tumors.

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References

A-Gonzalez, N., Bensinger, S. J., Hong, C., Beceiro, S., Bradley, M. N., Zelcer, N., et al. (2009). Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. *Immunity*, 31(2), 245-258.

A-Gonzalez, N., Guillen, J. A., Gallardo, G., Diaz, M., de la Rosa, J. V., Hernandez, I. H., et al. (2013). The nuclear receptor LXRα controls the functional specialization of splenic macrophages. *Nat Immunol*, 14(8), 831-839.

A-Gonzalez, N., Quintana, J. A., García-Silva, S., Mazariegos, M., González de la Aleja, A., Nicolás-Ávila, J. A., et al. (2017). Phagocytosis imprints heterogeneity in tissue-resident macrophages. *J Exp Med*, 214(5), 1281-1296.

Aakko, S., Ylitalo, A., Kuusanmäki, H., Rannikko, J. H., Björkman, M., Mandelin, J., et al. (2025). CLEVER-1 targeting antibody, bexmarilimab, supports HLA-DR expression and alters ex vivo responsiveness to azacitidine and venetoclax in myeloid malignancies. *Sci Rep*, 15(1), 16775.

Adachi, H., & Tsujimoto, M. (2002). FEEL-1, a novel scavenger receptor with in vitro bacteria-binding and angiogenesis-modulating activities. *J Biol Chem*, 277(37), 34264-34270.

Adachi, H., & Tsujimoto, M. (2010). Adaptor protein sorting nexin 17 interacts with the scavenger receptor FEEL-1/stabilin-1 and modulates its expression on the cell surface. *Biochim Biophys Acta*, 1803(5), 553-563.

Advani, R., Flinn, I., Popplewell, L., Forero, A., Bartlett, N. L., Ghosh, N., et al. (2018). CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. *N Engl J Med*, 379(18), 1711-1721.

Aegerter, H., Kulikauskaite, J., Crotta, S., Patel, H., Kelly, G., Hessel, E. M., et al. (2020). Influenza-induced monocyte-derived alveolar macrophages confer prolonged antibacterial protection. *Nat Immunol*, 21(2), 145-157.

Afik, R., Zigmond, E., Vugman, M., Klepfish, M., Shimshoni, E., Pasmanik-Chor, M., et al. (2016). Tumor macrophages are pivotal constructors of tumor collagenous matrix. *J Exp Med*, 213(11), 2315-2331.

Aichele, P., Zinke, J., Grode, L., Schwendener, R. A., Kaufmann, S. H., & Seiler, P. (2003). Macrophages of the splenic marginal zone are essential for trapping of blood-borne particulate antigen but dispensable for induction of specific T cell responses. *J Immunol*, 171(3), 1148-1155.

Ait Ahmed, Y., Fu, Y., Rodrigues, R. M., He, Y., Guan, Y., Guillot, A., et al. (2021). Kupffer cell restoration after partial hepatectomy is mainly driven by local cell proliferation in IL-6-dependent autocrine and paracrine manners. *Cell Mol Immunol*, 18(9), 2165-2176.

Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M., & Rossi, F. M. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci*, 14(9), 1142-1149.

Akashi, K., Traver, D., Miyamoto, T., & Weissman, I. L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature*, 404(6774), 193-197.

Alexander, W. S., Starr, R., Fenner, J. E., Scott, C. L., Handman, E., Sprigg, N. S., et al. (1999). SOCS1 is a critical inhibitor of interferon gamma signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell*, 98(5), 597-608.

Alexopoulou, L., Holt, A. C., Medzhitov, R., & Flavell, R. A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*, 413(6857), 732-738.

Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nat Rev Genet*, 17(8), 487-500.

Alloatti, A., Kotsias, F., Pauwels, A. M., Carpier, J. M., Jouve, M., Timmerman, E., et al. (2015). Toll-like Receptor 4 Engagement on Dendritic Cells Restrains Phago-Lysosome Fusion and Promotes Cross-Presentation of Antigens. *Immunity*, 43(6), 1087-1100.

Ammar, A., Mohammed, R. A., Salmi, M., Pepper, M., Paish, E. C., Ellis, I. O., et al. (2011). Lymphatic expression of CLEVER-1 in breast cancer and its relationship with lymph node metastasis. *Anal Cell Pathol (Amst)*, 34(1-2), 67-78.

An, H., Hou, J., Zhou, J., Zhao, W., Xu, H., Zheng, Y., et al. (2008). Phosphatase SHP-1 promotes TLR- and RIG-I-activated production of type I interferon by inhibiting the kinase IRAK1. *Nat Immunol*, 9(5), 542-550.

Ando, K., Mori, K., Corradini, N., Redini, F., & Heymann, D. (2011). Mifamurtide for the treatment of nonmetastatic osteosarcoma. *Expert Opin Pharmacother*, 12(2), 285-292.

Andreesen, R., Hennemann, B., & Krause, S. W. (1998). Adoptive immunotherapy of cancer using monocyte-derived macrophages: rationale, current status, and perspectives. *J Leukoc Biol*, 64(4), 419-426.

Andreesen, R., Scheibenbogen, C., Brugger, W., Krause, S., Meerpohl, H. G., Leser, H. G., et al. (1990). Adoptive transfer of tumor cytotoxic macrophages generated in vitro from circulating blood monocytes: a new approach to cancer immunotherapy. *Cancer Res*, 50(23), 7450-7456.

Angelova, M., Mlecnik, B., Vasaturo, A., Bindea, G., Fredriksen, T., Lafontaine, L., et al. (2018). Evolution of Metastases in Space and Time under Immune Selection. *Cell*, 175(3), 751-765.e716.

Aouadi, M., Vangala, P., Yawe, J. C., Tencerova, M., Nicoloro, S. M., Cohen, J. L., et al. (2014). Lipid storage by adipose tissue macrophages regulates systemic glucose tolerance. *Am J Physiol Endocrinol Metab*, 307(4), E374-383.

Ara, T., Tokoyoda, K., Sugiyama, T., Egawa, T., Kawabata, K., & Nagasawa, T. (2003). Long-term hematopoietic stem cells require stromal cell-derived factor-1 for colonizing bone marrow during ontogeny. *Immunity*, 19(2), 257-267.

Arlauckas, S. P., Garris, C. S., Kohler, R. H., Kitaoka, M., Cuccarese, M. F., Yang, K. S., et al. (2017). In vivo imaging reveals a tumor-associated macrophage-mediated resistance pathway in anti-PD-1 therapy. *Sci Transl Med*, 9(389), eaal3604.

Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., van Rooijen, N., Plonquet, A., et al. (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med*, 204(5), 1057-1069.

Arts, R. J., Novakovic, B., Ter Horst, R., Carvalho, A., Bekkering, S., Lachmandas, E., et al. (2016). Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. *Cell Metab*, 24(6), 807-819.

Asano, K., Miwa, M., Miwa, K., Hanayama, R., Nagase, H., Nagata, S., et al. (2004). Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. *J Exp Med*, 200(4), 459-467.

Atabai, K., Jame, S., Azhar, N., Kuo, A., Lam, M., McKleroy, W., et al. (2009). Mfge8 diminishes the severity of tissue fibrosis in mice by binding and targeting collagen for uptake by macrophages. *J Clin Invest*, 119(12), 3713-3722.

Atcha, H., Jairaman, A., Holt, J. R., Meli, V. S., Nagalla, R. R., Veerasubramanian, P. K., et al. (2021). Mechanically activated ion channel Piezo1 modulates macrophage polarization and stiffness sensing. *Nat Commun*, 12(1), 3256.

Auffray, C., Fogg, D., Garfa, M., Elain, G., Join-Lambert, O., Kayal, S., et al. (2007). Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*, 317(5838), 666-670.

Ayers, M., Lunceford, J., Nebozhyn, M., Murphy, E., Loboda, A., Kaufman, D. R., et al. (2017). IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest*, 127(8), 2930-2940.

Babior, B. M. (1999). NADPH oxidase: an update. *Blood*, 93(5), 1464-1476.

Bain, C. C., Bravo-Blas, A., Scott, C. L., Perdiguero, E. G., Geissmann, F., Henri, S., et al. (2014). Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol*, 15(10), 929-937.

Banaei-Bouchareb L., Gouon-Evans V., Samara-Boustani D., Claire Castellotti, M., Czernichow P., Pollard J. W., et al. (2004). Insulin cell mass is altered in Csf1op/Csf1op macrophage-deficient mice. *J Leukoc Biol*, 76(2):359-67.

Bankhead, P., Loughrey, M. B., Fernández, J. A., Dombrowski, Y., McArt, D. G., Dunne, P. D., et al. (2017). QuPath: Open source software for digital pathology image analysis. *Sci Rep*, 7(1), 16878.

Baranska, A., Shawket, A., Jouve, M., Baratin, M., Malosse, C., Voluzan, O., et al. (2018). Unveiling skin macrophage dynamics explains both tattoo persistence and strenuous removal. *J Exp Med*, 215(4), 1115-1133.

Barbera-Guillem, E., Nyhus, J. K., Wolford, C. C., Friece, C. R., & Sampsel, J. W. (2002). Vascular endothelial growth factor secretion by tumor-infiltrating macrophages essentially supports tumor angiogenesis, and IgG immune complexes potentiate the process. *Cancer Res*, 62(23), 7042-7049.

Barkal, A. A., Brewer, R. E., Markovic, M., Kowarsky, M., Barkal, S. A., Zaro, B. W., et al. (2019). CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature*, 572(7769), 392-396.

Barton, E. S., White, D. W., Cathelyn, J. S., Brett-McClellan, K. A., Engle, M., Diamond, M. S., et al. (2007). Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature*, 447(7142), 326-329.

Beattie, L., Sawtell, A., Mann, J., Frame, T. C. M., Teal, B., de Labastida Rivera, F., et al. (2016). Bone marrow-derived and resident liver macrophages display unique transcriptomic signatures but similar biological functions. *J Hepatol*, 65(4), 758-768.

Bekaii-Saab, T., Okusaka, T., Goldstein, D., Oh, D. Y., Ueno, M., Ioka, T., et al. (2023). Napabucasin plus nab-paclitaxel with gemcitabine versus nab-paclitaxel with gemcitabine in previously untreated metastatic pancreatic adenocarcinoma: an adaptive multicentre, randomised, open-label, phase 3, superiority trial. *EClinicalMedicine*, 58, 101897.

Bekkering, S., Arts, R. J. W., Novakovic, B., Kourtzelis, I., van der Heijden, C. D. C. C., Li, Y., et al. (2018). Metabolic Induction of Trained Immunity through the Mevalonate Pathway. ci, 172(1-2), 135-146.e139.

Benci, J. L., Johnson, L. R., Choa, R., Xu, Y., Qiu, J., Zhou, Z., et al. (2019). Opposing Functions of Interferon Coordinate Adaptive and Innate Immune Responses to Cancer Immune Checkpoint Blockade. *Cell*, 178(4), 933-948.e914.

Benci, J. L., Xu, B., Qiu, Y., Wu, T. J., Dada, H., Twyman-Saint Victor, C., et al. (2016). Tumor Interferon Signaling Regulates a Multigenic Resistance Program to Immune Checkpoint Blockade. *Cell*, 167(6), 1540-1554.e1512.

Bertrand, J. Y., Chi, N. C., Santoso, B., Teng, S., Stainier, D. Y., & Traver, D. (2010). Haematopoietic stem cells derive directly from aortic endothelium during development. *Nature*, 464(7285), 108-111.

Bhat, M. Y., Solanki, H. S., Advani, J., Khan, A. A., Keshava Prasad, T. S., Gowda, H., et al. (2018). Comprehensive network map of interferon gamma signaling. *J Cell Commun Signal*, 12(4), 745-751.

Bian, Z., Gong, Y., Huang, T., Lee, C. Z. W., Bian, L., Bai, Z., et al. (2020). Deciphering human macrophage development at single-cell resolution. *Nature*, 582(7813), 571-576.

Bieghs, V., Verheyen, F., van Gorp, P. J., Hendrikx, T., Wouters, K., Lütjohann, D., et al. (2012). Internalization of modified lipids by CD36 and SR-A leads to hepatic inflammation and lysosomal cholesterol storage in Kupffer cells. *PLoS One*, 7(3), e34378.

Bieniasz-Krzywiec, P., Martín-Pérez, R., Ehling, M., García-Caballero, M., Pinioti, S., Pretto, S., et al. (2019). Podoplanin-Expressing Macrophages Promote Lymphangiogenesis and Lymphoinvasion in Breast Cancer. *Cell Metab*, 30(5), 917-936.e910.

Bistoni, F., Verducci, G., Perito, S., Vecchiarelli, A., Puccetti, P., Marconi, P., et al. (1988). Immunomodulation by a low-virulence, agerminative variant of Candida albicans. Further evidence for macrophage activation as one of the effector mechanisms of nonspecific anti-infectious protection. *J Med Vet Mycol*, 26(5), 285-299.

Biswas, S. K., Gangi, L., Paul, S., Schioppa, T., Saccani, A., Sironi, M., et al. (2006). A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). *Blood,* 107(5), 2112-2122.

Blair, H. A. (2018). Duvelisib: First Global Approval. *Drugs*, 78(17), 1847-1853.

Bloch, O., Crane, C. A., Kaur, R., Safaee, M., Rutkowski, M. J., & Parsa, A. T. (2013). Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. *Clin Cancer Res*, 19(12), 3165-3175.

Blériot, C., Chakarov, S., & Ginhoux, F. (2020). Determinants of Resident Tissue Macrophage Identity and Function. *Immunity*, 52(6), 957-970.

Blériot, C., Dupuis, T., Jouvion, G., Eberl, G., Disson, O., & Lecuit, M. (2015). Liver-resident macrophage necroptosis orchestrates type 1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. *Immunity*, 42(1), 145-158.

Bohn, T., Rapp, S., Luther, N., Klein, M., Bruehl, T. J., Kojima, N., et al. (2018). Tumor immunoevasion via acidosis-dependent induction of regulatory tumor-associated macrophages. *Nat Immunol*, 19(12), 1319-1329.

Boisset, J. C., van Cappellen, W., Andrieu-Soler, C., Galjart, N., Dzierzak, E., & Robin, C. (2010). In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature*, 464(7285), 116-120.

Bonapace, L., Coissieux, M. M., Wyckoff, J., Mertz, K. D., Varga, Z., Junt, T., et al. (2014). Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis. *Nature*, 515(7525), 130-133.

Bonde, A. K., Tischler, V., Kumar, S., Soltermann, A., & Schwendener, R. A. (2012). Intratumoral macrophages contribute to epithelial-mesenchymal transition in solid tumors. *BMC Cancer*, 12, 35.

Bonnardel, J., T'Jonck, W., Gaublomme, D., Browaeys, R., Scott, C. L., Martens, L., et al. (2019). Stellate Cells, Hepatocytes, and Endothelial Cells Imprint the Kupffer Cell Identity on Monocytes Colonizing the Liver Macrophage Niche. *Immunity*, 51(4), 638-654.e639.

Boring, L., Gosling, J., Chensue, S. W., Kunkel, S. L., Farese, R. V., Jr., Broxmeyer, H. E., et al. (1997). Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. *J Clin Invest*, 100(10), 2552-2561.

Borisenko, G. G., Matsura, T., Liu, S. X., Tyurin, V. A., Jianfei, J., Serinkan, F. B., et al. (2003). Macrophage recognition of externalized phosphatidylserine and phagocytosis of apoptotic Jurkat cells--existence of a threshold. *Arch Biochem Biophys*, 413(1), 41-52.

Bosco, M. C., Puppo, M., Santangelo, C., Anfosso, L., Pfeffer, U., Fardin, P., et al. (2006). Hypoxia modifies the transcriptome of primary human monocytes: modulation of novel immune-related genes and identification of CC-chemokine ligand 20 as a new hypoxia-inducible gene. *J Immunol*, 177(3), 1941-1955.

Boulter, L., Govaere, O., Bird, T. G., Radulescu, S., Ramachandran, P., Pellicoro, A., et al. (2012). Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nat Med*, 18(4), 572-579.

Bourquin, C., Hotz, C., Noerenberg, D., Voelkl, A., Heidegger, S., Roetzer, L. C., et al. (2011). Systemic cancer therapy with a small molecule agonist of toll-like receptor 7 can be improved by circumventing TLR tolerance. *Cancer Res*, 71(15), 5123-5133.

Bruni, D., Angell, H. K., & Galon, J. (2020). The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer*, 20(11), 662-680.

Buechler, M. B., Kim, K. W., Onufer, E. J., Williams, J. W., Little, C. C., Dominguez, C. X., et al. (2019). A Stromal Niche Defined by Expression of the Transcription Factor WT1 Mediates Programming and Homeostasis of Cavity-Resident Macrophages. *Immunity*, 51(1), 119-130.e115.

Bukhalid, R. A., Duvall, J. R., Lancaster, K., Catcott, K. C., Malli Cetinbas, N., Monnell, T., et al. (2025). XMT-2056, a HER2-Directed STING Agonist Antibody-Drug Conjugate, Induces Innate Antitumor Immune Responses by Acting on Cancer Cells and Tumor-Resident Immune Cells. *Clin Cancer Res*, 31(9), 1766-1782.

Burch, P. A., Breen, J. K., Buckner, J. C., Gastineau, D. A., Kaur, J. A., Laus, R. L., et al. (2000). Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin Cancer Res*, 6(6), 2175-2182.

Butowski, N., Colman, H., De Groot, J. F., Omuro, A. M., Nayak, L., Wen, P. Y., et al. (2016). Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy Foundation Early Phase Clinical Trials Consortium phase II study. *Neuro Oncol*, 18(4), 557-564.

Byrne, A., & Reen, D. J. (2002). Lipopolysaccharide induces rapid production of IL-10 by monocytes in the presence of apoptotic neutrophils. *J Immunol*, 168(4), 1968-1977.

Bürckstümmer, T., Baumann, C., Blüml, S., Dixit, E., Dürnberger, G., Jahn, H., et al. (2009). An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat Immunol*, 10(3), 266-272.

Calderon, B., Carrero, J. A., Ferris, S. T., Sojka, D. K., Moore, L., Epelman, S., et al. (2015). The pancreas anatomy conditions the origin and properties of resident macrophages. *J Exp Med*, 212(10), 1497-1512.

Caldwell, A. B., Cheng, Z., Vargas, J. D., Birnbaum, H. A., & Hoffmann, A. (2014). Network dynamics determine the autocrine and paracrine signaling functions of TNF. *Genes Dev*, 28(19), 2120-2133.

Camus, M., Tosolini, M., Mlecnik, B., Pagès, F., Kirilovsky, A., Berger, A., et al. (2009). Coordination of intratumoral immune reaction and human colorectal cancer recurrence. *Cancer Res*, 69(6), 2685-2693.

Canton, J., Neculai, D., & Grinstein, S. (2013). Scavenger receptors in homeostasis and immunity. *Nat Rev Immunol*, 13(9), 621-634.

Cappell, K. M., & Kochenderfer, J. N. (2023). Long-term outcomes following CAR T cell therapy: what we know so far. *Nat Rev Clin Oncol*, 20(6), 359-371.

Carbonnelle-Puscian, A., Copie-Bergman, C., Baia, M., Martin-Garcia, N., Allory, Y., Haioun, C., et al. (2009). The novel immunosuppressive enzyme IL4I1 is expressed by neoplastic cells of several B-cell lymphomas and by tumor-associated macrophages. *Leukemia*, 23(5), 952-960.

Carlin, L. M., Stamatiades, E. G., Auffray, C., Hanna, R. N., Glover, L., Vizcay-Barrena, G., et al. (2013). Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal. *Cell*, 153(2), 362-375.

Casanova-Acebes, M., Dalla, E., Leader, A. M., LeBerichel, J., Nikolic, J., Morales, B. M., et al. (2021). Tissue-resident macrophages provide a pro-tumorigenic niche to early NSCLC cells. *Nature*, 595(7868), 578-584.

Cassetta, L., Fragkogianni, S., Sims, A. H., Swierczak, A., Forrester, L. M., Zhang, H., et al. (2019). Human Tumor-Associated Macrophage and Monocyte Transcriptional Landscapes Reveal Cancer-Specific Reprogramming, Biomarkers, and Therapeutic Targets. *Cancer Cell*, 35(4), 588-602.e510.

Cassetta, L., & Pollard, J. W. (2023). A timeline of tumour-associated macrophage biology. *Nat Rev Cancer*, 23(4), 238-257.

Cassier, P. A., Italiano, A., Gomez-Roca, C., Le Tourneau, C., Toulmonde, M., D'Angelo, S. P., et al. (2020). Long-term clinical activity, safety and patient-reported quality of life for emactuzumab-treated patients with diffuse-type tenosynovial giant-cell tumour. *Eur J Cancer*, 141, 162-170.

Caux, C., Massacrier, C., Vanbervliet, B., Dubois, B., Van Kooten, C., Durand, I., et al. (1994). Activation of human dendritic cells through CD40 cross-linking. *J Exp Med*, 180(4), 1263-1272.

Chakarov, S., Lim, H. Y., Tan, L., Lim, S. Y., See, P., Lum, J., et al. (2019). Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. *Science*, 363(6432).

Cheever, M. A., & Higano, C. S. (2011). PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. *Clin Cancer Res*, 17(11), 3520-3526.

Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. *Immunity*, 39(1), 1-10.

Chen, D. S., & Mellman, I. (2017). Elements of cancer immunity and the cancer-immune set point. *Nature*, 541(7637), 321-330.

Chen, F., Wu, W., Millman, A., Craft, J. F., Chen, E., Patel, N., et al. (2014). Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. *Nat Immunol*, 15(10), 938-946.

Chen, G. G., Chu, Y. S., Chak, E. C., Leung, B. C., & Poon, W. S. (2002). Induction of apoptosis in glioma cells by molecules released from activated macrophages. *J Neurooncol*, 57(3), 179-186.

Chen, J., & Ivashkiv, L. B. (2010). IFN-γ abrogates endotoxin tolerance by facilitating Toll-like receptor-induced chromatin remodeling. *Proc Natl Acad Sci U S A*, 107(45), 19438-19443.

Chen, Q., Zhang, X. H., & Massagué, J. (2011). Macrophage binding to receptor VCAM-1 transmits survival signals in breast cancer cells that invade the lungs. *Cancer Cell*, 20(4), 538-549.

Chen, Z., Feng, X., Herting, C. J., Garcia, V. A., Nie, K., Pong, W. W., et al. (2017). Cellular and Molecular Identity of Tumor-Associated Macrophages in Glioblastoma. *Cancer Res*, 77(9), 2266-2278.

Cheng, C. S., Behar, M. S., Suryawanshi, G. W., Feldman, K. E., Spreafico, R., & Hoffmann, A. (2017). Iterative Modeling Reveals Evidence of Sequential Transcriptional Control Mechanisms. *Cell Syst*, 4(3), 330-343.e335.

Cheng, H., Sun, L., Shen, D., Ren, A., Ma, F., Tai, G., et al. (2020). Beta-1,6 glucan converts tumor-associated macrophages into an M1-like phenotype. *Carbohydr Polym*, 247, 116715.

Cheng, Q. J., Ohta, S., Sheu, K. M., Spreafico, R., Adelaja, A., Taylor, B., et al. (2021). NF-κB dynamics determine the stimulus specificity of epigenomic reprogramming in macrophages. *Science*, 372(6548), 1349-1353.

Cheng, S., Li, Z., Gao, R., Xing, B., Gao, Y., Yang, Y., et al. (2021). A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. *Cell*, 184(3), 792-809.e723.

Cheng, S. C., Quintin, J., Cramer, R. A., Shepardson, K. M., Saeed, S., Kumar, V., et al. (2014). mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science*, 345(6204), 1250684.

Childs, B. G., Baker, D. J., Wijshake, T., Conover, C. A., Campisi, J., & van Deursen, J. M. (2016). Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science,* 354(6311), 472-477.

Choi, Y. I., Kim, K. Y., Jung, N. J., Lee, J. J., & Park, S. B. (2022). Abstract 5599: A novel orally available anti-cancer drug candidate, SB17170, represses myeloid-derived suppressor cells by targeting HMGB1. *Cancer Res*, 82(12_Supplement), 5599.

Chow, A., Lucas, D., Hidalgo, A., Méndez-Ferrer, S., Hashimoto, D., Scheiermann, C., et al. (2011). Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. *J Exp Med*, 208(2), 261-271.

Chua, A. C., Hodson, L. J., Moldenhauer, L. M., Robertson, S. A., & Ingman, W. V. (2010). Dual roles for macrophages in ovarian cycle-associated development and remodelling of the mammary gland epithelium. *Development*, 137(24), 4229-4238.

Chuang, T. H., & Ulevitch, R. J. (2004). Triad3A, an E3 ubiquitin-protein ligase regulating Toll-like receptors. *Nat Immunol*, 5(5), 495-502.

Claus, V., Jahraus, A., Tjelle, T., Berg, T., Kirschke, H., Faulstich, H., et al. (1998). Lysosomal enzyme trafficking between phagosomes, endosomes, and lysosomes in J774 macrophages. Enrichment of cathepsin H in early endosomes. *J Biol Chem*, 273(16), 9842-9851.

Cohen, M., Giladi, A., Gorki, A. D., Solodkin, D. G., Zada, M., Hladik, A., et al. (2018). Lung Single-Cell Signaling Interaction Map Reveals Basophil Role in Macrophage Imprinting. *Cell*, 175(4), 1031-1044.e1018.

Colegio, O. R., Chu, N. Q., Szabo, A. L., Chu, T., Rhebergen, A. M., Jairam, V., et al. (2014). Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature*, 513(7519), 559-563.

Cooper, M. A., Elliott, J. M., Keyel, P. A., Yang, L., Carrero, J. A., & Yokoyama, W. M. (2009). Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A*, 106(6), 1915-1919.

Cope, A. P., Liblay, R. S., Yang, X. D., Congia, M., Laudanna, C., Schreiber, R. D., et al. (1997). Chronic tumor necrosis factor alters T cell responses by attenuating T cell receptor signaling. *J Exp Med*, 185(9), 1573-1584.

Coulton, A., Murai, J., Qian, D., Thakkar, K., Lewis, C. E., & Litchfield, K. (2024). Using a pan-cancer atlas to investigate tumour associated macrophages as regulators of immunotherapy response. *Nat Commun*, 15(1), 5665.

Cros, J., Cagnard, N., Woollard, K., Patey, N., Zhang, S. Y., Senechal, B., et al. (2010). Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity*, 33(3), 375-386.

Culemann, S., Grüneboom, A., Nicolás-Ávila, J., Weidner, D., Lämmle, K. F., Rothe, T., et al. (2019). Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature*, 572(7771), 670-675.

Cummings, R. J., Barbet, G., Bongers, G., Hartmann, B. M., Gettler, K., Muniz, L., et al. (2016). Different tissue phagocytes sample apoptotic cells to direct distinct homeostasis programs. *Nature*, 539(7630), 565-569.

Cupurdija, K., Azzola, D., Hainz, U., Gratchev, A., Heitger, A., Takikawa, O., et al. (2004). Macrophages of human first trimester decidua express markers associated to alternative activation. *Am J Reprod Immunol*, 51(2), 117-122.

Curiel, T. J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., et al. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med*, 10(9), 942-949.

Dahan, R., Barnhart, B. C., Li, F., Yamniuk, A. P., Korman, A. J., & Ravetch, J. V. (2016). Therapeutic Activity of Agonistic, Human Anti-CD40 Monoclonal Antibodies Requires Selective FcγR Engagement. *Cancer Cell*, 29(6), 820-831.

Dai, X. M., Ryan, G. R., Hapel, A. J., Dominguez, M. G., Russell, R. G., Kapp, S., et al. (2002). Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood*, 99(1), 111-120.

Dalton, D. K., Pitts-Meek, S., Keshav, S., Figari, I. S., Bradley, A., & Stewart, T. A. (1993). Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science*, 259(5102), 1739-1742.

Danaher, P., Kim, Y., Nelson, B., Griswold, M., Yang, Z., Piazza, E., et al. (2022). Advances in mixed cell deconvolution enable quantification of cell types in spatial transcriptomic data. *Nat Commun*, 13(1), 385.

Dangaj, D., Bruand, M., Grimm, A. J., Ronet, C., Barras, D., Duttagupta, P. A., et al. (2019). Cooperation between Constitutive and Inducible Chemokines Enables T Cell Engraftment and Immune Attack in Solid Tumors. *Cancer Cell*, 35(6), 885-900.e810.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci*, 8(6), 752-758.

Davies, L. C., Rosas, M., Jenkins, S. J., Liao, C. T., Scurr, M. J., Brombacher, F., et al. (2013). Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. *Nat Commun*, 4, 1886.

Davies, L. C., Rosas, M., Smith, P. J., Fraser, D. J., Jones, S. A., & Taylor, P. R. (2011). A quantifiable proliferative burst of tissue macrophages restores homeostatic macrophage populations after acute inflammation. *Eur J Immunol*, 41(8), 2155-2164.

Dawson, C. A., Pal, B., Vaillant, F., Gandolfo, L. C., Liu, Z., Bleriot, C., et al. (2020). Tissue-resident ductal macrophages survey the mammary epithelium and facilitate tissue remodelling. *Nat Cell Biol*, 22(5), 546-558.

de Bruin, A. M., Libregts, S. F., Valkhof, M., Boon, L., Touw, I. P., & Nolte, M. A. (2012). IFNγ induces monopoiesis and inhibits neutrophil development during inflammation. *Blood*, 119(6), 1543-1554.

De Filippo, K., Henderson, R. B., Laschinger, M., & Hogg, N. (2008). Neutrophil chemokines KC and macrophage-inflammatory protein-2 are newly synthesized by tissue macrophages using distinct TLR signaling pathways. *J Immunol*, 180(6), 4308-4315.

De Schepper, S., Verheijden, S., Aguilera-Lizarraga, J., Viola, M. F., Boesmans, W., Stakenborg, N., et al. (2018). Self-Maintaining Gut Macrophages Are Essential for Intestinal Homeostasis. *Cell*, 175(2), 400-415.e413.

deCathelineau, A. M., & Henson, P. M. (2003). The final step in programmed cell death: phagocytes carry apoptotic cells to the grave. *Essays Biochem*, 39, 105-117.

Delamarre, L., Pack, M., Chang, H., Mellman, I., & Trombetta, E. S. (2005). Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science*, 307(5715), 1630-1634.

DeNardo, D. G., Barreto, J. B., Andreu, P., Vasquez, L., Tawfik, D., Kolhatkar, N., et al. (2009). CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell*, 16(2), 91-102.

Deppermann C., Kratofil R. M., Peiseler M., David B. A., Zinderl J., Silva Castanheira F. V. E., et al. (2020). Macrophage galactose lectin is critical for Kupffer cells to clear aged platelets. *J Exp Med*, 217(4):e20190723.

Di Caro, G., Cortese, N., Castino, G. F., Grizzi, F., Gavazzi, F., Ridolfi, C., et al. (2016). Dual prognostic significance of tumour-associated macrophages in human pancreatic adenocarcinoma treated or untreated with chemotherapy. *Gut*, 65(10), 1710-1720.

Di Micco, A., Frera, G., Lugrin, J., Jamilloux, Y., Hsu, E. T., Tardivel, A., et al. (2016). AIM2 inflammasome is activated by pharmacological disruption of nuclear envelope integrity. *Proc Natl Acad Sci U S A*, 113(32), E4671-4680.

Diab, A., Ascierto, P. A., Maio, M., Abdel-Wahab, R., Negrier, S., Mortier, L., et al. (2025). Randomized, Open-Label, Phase III Study of Tilsotolimod in Combination With Ipilimumab Versus Ipilimumab Alone in Patients With Advanced Refractory Melanoma (ILLUMINATE-301). *J Clin Oncol*, 43(15), 1800-1809.

Divangahi, M., Aaby, P., Khader, S. A., Barreiro, L. B., Bekkering, S., Chavakis, T., et al. (2021). Trained immunity, tolerance, priming and differentiation: distinct immunological processes. *Nat Immunol*, 22(1), 2-6.

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., et al. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29(1), 15-21.

Doedens, A. L., Stockmann, C., Rubinstein, M. P., Liao, D., Zhang, N., DeNardo, D. G., et al. (2010). Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. *Cancer Res*, 70(19), 7465-7475.

Donadon, M., Torzilli, G., Cortese, N., Soldani, C., Di Tommaso, L., Franceschini, B., et al. (2020). Macrophage morphology correlates with single-cell diversity and prognosis in colorectal liver metastasis. *J Exp Med*, 217(11), e20191847.

Dower, K., Ellis, D. K., Saraf, K., Jelinsky, S. A., & Lin, L. L. (2008). Innate immune responses to TREM-1 activation: overlap, divergence, and positive and negative cross-talk with bacterial lipopolysaccharide. *J Immunol*, 180(5), 3520-3534.

Doyle, A. G., Herbein, G., Montaner, L. J., Minty, A. J., Caput, D., Ferrara, P., et al. (1994). Interleukin-13 alters the activation state of murine macrophages in vitro: comparison with interleukin-4 and interferon-gamma. *Eur J Immunol*, 24(6), 1441-1445.

Du, B., Qin, J., Lin, B., Zhang, J., Li, D., & Liu, M. (2025). CAR-T therapy in solid tumors. *Cancer Cell*, 43(4), 665-679.

Dunkel, J., Viitala, M., Karikoski, M., Rantakari, P., Virtakoivu, R., Elima, K., et al. (2018). Enhanced Antibody Production in Clever-1/Stabilin-1-Deficient Mice. *Front Immunol*, 9, 2257.

Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J., & Schreiber, R. D. (2002). Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol*, 3(11), 991-998.

Dunne, D. W., Resnick, D., Greenberg, J., Krieger, M., & Joiner, K. A. (1994). The type I macrophage scavenger receptor binds to gram-positive bacteria and recognizes lipoteichoic acid. *Proc Natl Acad Sci U S A*, 91(5), 1863-1867.

Duval, F., Lourenco, J., Hicham, M., Boivin, G., Guichard, A., Wyser-Rmili, C., et al. (2025). Trajectories of macrophage ontogeny and reprogramming in cancer. *iScience*, 28(5), 112498.

Ecker, J., Liebisch, G., Englmaier, M., Grandl, M., Robenek, H., & Schmitz, G. (2010). Induction of fatty acid synthesis is a key requirement for phagocytic differentiation of human monocytes. *Proc Natl Acad Sci U S A*, 107(17), 7817-7822.

Edwards, J. P., Zhang, X., Frauwirth, K. A., & Mosser, D. M. (2006). Biochemical and functional characterization of three activated macrophage populations. *J Leukoc Biol*, 80(6), 1298-1307.

Elliott, M. R., Chekeni, F. B., Trampont, P. C., Lazarowski, E. R., Kadl, A., Walk, S. F., et al. (2009). Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature*, 461(7261), 282-286.

Elomaa, O., Kangas, M., Sahlberg, C., Tuukkanen, J., Sormunen, R., Liakka, A., et al. (1995). Cloning of a novel bacteria-binding receptor structurally related to scavenger receptors and expressed in a subset of macrophages. *Cell*, 80(4), 603-609.

Epelman, S., Lavine, K. J., Beaudin, A. E., Sojka, D. K., Carrero, J. A., Calderon, B., et al. (2014). Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity*, 40(1), 91-104.

Eremina, N. V., Kazey, V. I., Mishugin, S. V., Leonenkov, R. V., Pushkar, D. Y., Mett, V. L., et al. (2020). First-in-human study of anticancer immunotherapy drug candidate mobilan: safety, pharmacokinetics and pharmacodynamics in prostate cancer patients. *Oncotarget*, 11(14), 1273-1288.

Etzerodt, A., Tsalkitzi, K., Maniecki, M., Damsky, W., Delfini, M., Baudoin, E., et al. (2019). Specific targeting of CD163^{+} TAMs mobilizes inflammatory monocytes and promotes T cell-mediated tumor regression. *J Exp Med*, 216(10), 2394-2411.

Fadok, V. A., Bratton, D. L., Konowal, A., Freed, P. W., Westcott, J. Y., & Henson, P. M. (1998). Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest*, 101(4), 890-898.

Fadok, V. A., Voelker, D. R., Campbell, P. A., Cohen, J. J., Bratton, D. L., & Henson, P. M. (1992). Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol*, 148(7), 2207-2216.

Fallarino, F., Grohmann, U., You, S., McGrath, B. C., Cavener, D. R., Vacca, C., et al. (2006). The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell

receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J Immunol*, 176(11), 6752-6761.

Fallowfield, J. A., Mizuno, M., Kendall, T. J., Constandinou, C. M., Benyon, R. C., Duffield, J. S., et al. (2007). Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J Immunol*, 178(8), 5288-5295.

Faradji, A., Bohbot, A., Schmitt-Goguel, M., Roeslin, N., Dumont, S., Wiesel, M. L., et al. (1991). Phase I trial of intravenous infusion of ex-vivo-activated autologous blood-derived macrophages in patients with non-small-cell lung cancer: toxicity and immunomodulatory effects. *Cancer Immunol Immunother*, 33(5), 319-326.

Fayad, L., Ansell, S. M., Advani, R., Coiffier, B., Stuart, R., Bartlett, N. L., et al. (2015). Dacetuzumab plus rituximab, ifosfamide, carboplatin and etoposide as salvage therapy for patients with diffuse large B-cell lymphoma relapsing after rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone: a randomized, double-blind, placebo-controlled phase 2b trial. *Leuk Lymphoma*, 56(9), 2569-2578.

Feinberg, M. W., Wara, A. K., Cao, Z., Lebedeva, M. A., Rosenbauer, F., Iwasaki, H., et al. (2007). The Kruppel-like factor KLF4 is a critical regulator of monocyte differentiation. *EMBO J*, 26(18), 4138-4148.

Feng, R., Desbordes, S. C., Xie, H., Tillo, E. S., Pixley, F., Stanley, E. R., et al. (2008). PU.1 and C/EBPalpha/beta convert fibroblasts into macrophage-like cells. *Proc Natl Acad Sci U S A*, 105(16), 6057-6062.

Ferrer, I. R., West, H. C., Henderson, S., Ushakov, D. S., Santos E Sousa, P., Strid, J., et al. (2019). A wave of monocytes is recruited to replenish the long-term Langerhans cell network after immune injury. *Sci Immunol*, 4(38).

Fogg, D. K., Sibon, C., Miled, C., Jung, S., Aucouturier, P., Littman, D. R., et al. (2006). A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science*, 311(5757), 83-87.

Foster, S. L., Hargreaves, D. C., & Medzhitov, R. (2007). Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature*, 447(7147), 972-978.

Foubert, P., & Varner, J. A. (2012). Integrins in tumor angiogenesis and lymphangiogenesis. *Methods Mol Biol*, 757, 471-486.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Özören, N., Jagirdar, R., et al. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat Immunol*, 7(6), 576-582.

Franklin, R. A., Liao, W., Sarkar, A., Kim, M. V., Bivona, M. R., Liu, K., et al. (2014). The cellular and molecular origin of tumor-associated macrophages. *Science*, 344(6186), 921-925.

Frasch, S. C., Fernandez-Boyanapalli, R. F., Berry, K. Z., Leslie, C. C., Bonventre, J. V., Murphy, R. C., et al. (2011). Signaling via macrophage G2A enhances efferocytosis of dying neutrophils by augmentation of Rac activity. *J Biol Chem*, 286(14), 12108-12122.

Freeman, G. J., Long, A. J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., et al. (2000). Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*, 192(7), 1027-1034.

Freire-de-Lima, C. G., Xiao, Y. Q., Gardai, S. J., Bratton, D. L., Schiemann, W. P., & Henson, P. M. (2006). Apoptotic cells, through transforming growth factor-beta, coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages. *J Biol Chem*, 281(50), 38376-38384.

Fridman, W. H., Zitvogel, L., Sautès-Fridman, C., & Kroemer, G. (2017). The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol*, 14(12), 717-734.

Galluzzi, L., Vacchelli, E., Eggermont, A., Fridman, W. H., Galon, J., Sautès-Fridman, C., et al. (2012). Trial Watch: Experimental Toll-like receptor agonists for cancer therapy. *Oncoimmunology*, 1(5), 699-716.

Galon, J., & Bruni, D. (2019). Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov*, 18(3), 197-218.

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pagès, C., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*, 313(5795), 1960-1964.

Galon, J., & Lanzi, A. (2020). Immunoscore and its introduction in clinical practice. *Q J Nucl Med Mol Imaging*, 64(2), 152-161.

Ganesan, S., & Roy, C. R. (2019). Host cell depletion of tryptophan by IFNγ-induced Indoleamine 2,3-dioxygenase 1 (IDO1) inhibits lysosomal replication of Coxiella burnetii. *PLoS Pathog*, 15(8), e1007955.

Gao, D., Joshi, N., Choi, H., Ryu, S., Hahn, M., Catena, R., et al. (2012). Myeloid progenitor cells in the premetastatic lung promote metastases by inducing mesenchymal to epithelial transition. *Cancer Res*, 72(6), 1384-1394.

Gao, J., Shi, L. Z., Zhao, H., Chen, J., Xiong, L., He, Q., et al. (2016). Loss of IFN-γ Pathway Genes in Tumor Cells as a Mechanism of Resistance to Anti-CTLA-4 Therapy. *Cell*, 167(2), 397-404.e399.

Gao, L., Wang, F. Q., Li, H. M., Yang, J. G., Ren, J. G., He, K. F., et al. (2016). CCL2/EGF positive feedback loop between cancer cells and macrophages promotes cell migration and invasion in head and neck squamous cell carcinoma. *Oncotarget*, 7(52), 87037-87051.

Garber, M., Yosef, N., Goren, A., Raychowdhury, R., Thielke, A., Guttman, M., et al. (2012). A high-throughput chromatin immunoprecipitation approach reveals principles of dynamic gene regulation in mammals. *Mol Cell*, 47(5), 810-822.

Garris, C. S., Arlauckas, S. P., Kohler, R. H., Trefny, M. P., Garren, S., Piot, C., et al. (2018). Successful Anti-PD-1 Cancer Immunotherapy Requires T Cell-Dendritic Cell Crosstalk Involving the Cytokines IFN-γ and IL-12. *Immunity*, 49(6), 1148-1161.e1147.

Gautier, E. L., Shay, T., Miller, J., Greter, M., Jakubzick, C., Ivanov, S., et al. (2012). Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol*, 13(11), 1118-1128.

Geng, J., Sun, X., Wang, P., Zhang, S., Wang, X., Wu, H., et al. (2015). Kinases Mst1 and Mst2 positively regulate phagocytic induction of reactive oxygen species and bactericidal activity. *Nat Immunol*, 16(11), 1142-1152.

Georgoudaki, A. M., Prokopec, K. E., Boura, V. F., Hellqvist, E., Sohn, S., Östling, J., et al. (2016). Reprogramming Tumor-Associated Macrophages by Antibody Targeting Inhibits Cancer Progression and Metastasis. *Cell Rep*, 15(9), 2000-2011.

Ghisletti, S., Barozzi, I., Mietton, F., Polletti, S., De Santa, F., Venturini, E., et al. (2010). Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. *Immunity*, 32(3), 317-328.

Giampieri, S., Manning, C., Hooper, S., Jones, L., Hill, C. S., & Sahai, E. (2009). Localized and reversible TGFbeta signalling switches breast cancer cells from cohesive to single cell motility. *Nat Cell Biol*, 11(11), 1287-1296.

Gibbings, S. L., Goyal, R., Desch, A. N., Leach, S. M., Prabagar, M., Atif, S. M., et al. (2015). Transcriptome analysis highlights the conserved difference between embryonic and postnatal-derived alveolar macrophages. *Blood*, 126(11), 1357-1366.

Gimbrone, M. A., Leapman, S. B., Cotran, R. S., & Folkman, J. (1972). Tumor dormancy in vivo by prevention of neovascularization. *J Exp Med*, 136(2), 261-276.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*, 330(6005), 841-845.

Giordano, A., Dumbrava, E., Parikh, A., Kenkel, J., Cai, D., Demady, D., et al. (2023). Abstract 720: A phase 1/2 study of BDC-3042, a novel Dectin-2 agonistic antibody, in patients with advanced cancers. *J Immunother Cancer*, 11(Suppl 1), A815.

Gocher, A. M., Workman, C. J., & Vignali, D. A. A. (2022). Interferon-γ: teammate or opponent in the tumour microenvironment? *Nat Rev Immunol*, 22(3), 158-172.

Gocheva, V., Wang, H. W., Gadea, B. B., Shree, T., Hunter, K. E., Garfall, A. L., et al. (2010). IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes Dev*, 24(3), 241-255.

Goerdt, S., Bhardwaj, R., & Sorg, C. (1993). Inducible expression of MS-1 high-molecular-weight protein by endothelial cells of continuous origin and by dendritic cells/macrophages in vivo and in vitro. *Am J Pathol*, 142(5), 1409-1422.

Goerdt, S., Walsh, L. J., Murphy, G. F., & Pober, J. S. (1991). Identification of a novel high molecular weight protein preferentially expressed by sinusoidal endothelial cells in normal human tissues. *J Cell Biol*, 113(6), 1425-1437.

Goldman, M. J., Craft, B., Hastie, M., Repečka, K., McDade, F., Kamath, A., et al. (2020). Visualizing and interpreting cancer genomics data via the Xena platform *Nat Biotechnol*, 38(6), 675-678.

Goldstein, J. L., Ho, Y. K., Basu, S. K., & Brown, M. S. (1979). Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci U S A*, 76(1), 333-337.

Gomes, B., Driessens, G., Bartlett, D., Cai, D., Cauwenberghs, S., Crosignani, S., et al. (2018). Characterization of the Selective Indoleamine 2,3-Dioxygenase-1 (IDO1) Catalytic Inhibitor EOS200271/PF-06840003 Supports IDO1 as a Critical Resistance Mechanism to PD-(L)1 Blockade Therapy. *Mol Cancer Ther*, 17(12), 2530-2542.

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., et al. (2015). Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*, 518(7540), 547-551.

Gordon, S. R., Maute, R. L., Dulken, B. W., Hutter, G., George, B. M., McCracken, M. N., et al. (2017). PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature*, 545(7655), 495-499.

Goren, I., Allmann, N., Yogev, N., Schürmann, C., Linke, A., Holdener, M., et al. (2009). A transgenic mouse model of inducible macrophage depletion: effects of diphtheria toxin-driven lysozyme M-specific cell lineage ablation on wound inflammatory, angiogenic, and contractive processes. *Am J Pathol*, 175(1), 132-147.

Gosselin, D., Link, V. M., Romanoski, C. E., Fonseca, G. J., Eichenfield, D. Z., Spann, N. J., et al. (2014). Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell*, 159(6), 1327-1340.

Goswami, S., Sahai, E., Wyckoff, J. B., Cammer, M., Cox, D., Pixley, F. J., et al. (2005). Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. *Cancer Res*, 65(12), 5278-5283.

Gottschalk, R. A., Martins, A. J., Angermann, B. R., Dutta, B., Ng, C. E., Uderhardt, S., et al. (2016). Distinct NF-κB and MAPK Activation Thresholds Uncouple Steady-State Microbe Sensing from Anti-pathogen Inflammatory Responses. *Cell Syst*, 2(6), 378-390.

Gouon-Evans, V., Rothenberg, M. E., & Pollard, J. W. (2000). Postnatal mammary gland development requires macrophages and eosinophils. *Development*, 127(11), 2269-2282.

Grajchen, E., Wouters, E., van de Haterd, B., Haidar, M., Hardonnière, K., Dierckx, T., et al. (2020). CD36-mediated uptake of myelin debris by macrophages and microglia reduces neuroinflammation. *J Neuroinflammation*, 17(1), 224.

Green, D. R., Ferguson, T., Zitvogel, L., & Kroemer, G. (2009). Immunogenic and tolerogenic cell death. *Nat Rev Immunol*, 9(5), 353-363.

Greenberg, M. E., Sun, M., Zhang, R., Febbraio, M., Silverstein, R., & Hazen, S. L. (2006). Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J Exp Med*, 203(12), 2613-2625.

Griffin, G. K., Newton, G., Tarrio, M. L., Bu, D. X., Maganto-Garcia, E., Azcutia, V., et al. (2012). IL-17 and TNF-α sustain neutrophil recruitment during inflammation through synergistic effects on endothelial activation. *J Immunol*, 188(12), 6287-6299.

Gude, D. R., Alvarez, S. E., Paugh, S. W., Mitra, P., Yu, J., Griffiths, R., et al. (2008). Apoptosis induces expression of sphingosine kinase 1 to release sphingosine-1-phosphate as a "come-and-get-me" signal. *FASEB J*, 22(8), 2629-2638.

Guerriero, J. L., Sotayo, A., Ponichtera, H. E., Castrillon, J. A., Pourzia, A. L., Schad, S., et al. (2017). Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages. *Nature*, 543(7645), 428-432.

Guilliams, M., & Scott, C. L. (2017). Does niche competition determine the origin of tissue-resident macrophages? *Nat Rev Immunol*, 17(7), 451-460.

Guilliams, M., Thierry, G. R., Bonnardel, J., & Bajenoff, M. (2020). Establishment and Maintenance of the Macrophage Niche. *Immunity*, 52(3), 434-451.

Gundra, U. M., Girgis, N. M., Ruckerl, D., Jenkins, S., Ward, L. N., Kurtz, Z. D., et al. (2014). Alternatively activated macrophages derived from monocytes and tissue macrophages are phenotypically and functionally distinct. *Blood*, 123(20), e110-122.

Guo, L., Bertola, D. R., Takanohashi, A., Saito, A., Segawa, Y., Yokota, T., et al. (2019). Bi-allelic CSF1R Mutations Cause Skeletal Dysplasia of Dysosteosclerosis-Pyle Disease Spectrum and Degenerative Encephalopathy with Brain Malformation. *Am J Hum Genet*, 104(5), 925-935.

Hackam, D. J., Rotstein, O. D., Zhang, W., Gruenheid, S., Gros, P., & Grinstein, S. (1998). Host resistance to intracellular infection: mutation of natural resistance-associated macrophage protein 1 (Nramp1) impairs phagosomal acidification. *J Exp Med*, 188(2), 351-364.

Hajishengallis, G., Netea, M. G., & Chavakis, T. (2023). Innate immune memory, trained immunity and nomenclature clarification. *Nat Immunol*, 24(9), 1393-1394.

Haldar, M., Kohyama, M., So, A. Y., Kc, W., Wu, X., Briseño, C. G., et al. (2014). Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. *Cell*, 156(6), 1223-1234.

Han, Y., Guo, Z., Jiang, L., Li, X., Chen, J., Ouyang, L., et al. (2023). CXCL10 and CCL5 as feasible biomarkers for immunotherapy of homologous recombination deficient ovarian cancer. *Am J Cancer Res*, 13(5), 1904-1922.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, 144(5), 646-674.

Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K., Koike, M., Uchiyama, Y., et al. (2004). Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science*, 304(5674), 1147-1150.

Hansen, B., Longati, P., Elvevold, K., Nedredal, G. I., Schledzewski, K., Olsen, R., et al. (2005). Stabilin-1 and stabilin-2 are both directed into the early endocytic pathway in hepatic sinusoidal endothelium via interactions with clathrin/AP-2, independent of ligand binding. *Exp Cell Res*, 303(1), 160-173.

Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W. M., Zheng, S., Butler, A., et al. (2021). Integrated analysis of multimodal single-cell data. *Cell*, 184(13), 3573-3587.e3529.

Harding, C. V., & Geuze, H. J. (1992). Class II MHC molecules are present in macrophage lysosomes and phagolysosomes that function in the phagocytic processing of Listeria monocytogenes for presentation to T cells. *J Cell Biol*, 119(3), 531-542.

Harney, A. S., Arwert, E. N., Entenberg, D., Wang, Y., Guo, P., Qian, B. Z., et al. (2015). Real-Time Imaging Reveals Local, Transient Vascular Permeability, and Tumor Cell Intravasation Stimulated by TIE2hi Macrophage-Derived VEGFA. *Cancer Discov*, 5(9), 932-943.

Hashimoto, A., Sarker, D., Reebye, V., Jarvis, S., Sodergren, M. H., Kossenkov, A., et al. (2021). Upregulation of C/EBPα Inhibits Suppressive Activity of Myeloid Cells and Potentiates Antitumor Response in Mice and Patients with Cancer. *Clin Cancer Res*, 27(21), 5961-5978.

Hashimoto, D., Chow, A., Noizat, C., Teo, P., Beasley, M. B., Leboeuf, M., et al. (2013). Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity*, 38(4), 792-804.

Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., et al. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*, 410(6832), 1099-1103.

Hegde, P. S., & Chen, D. S. (2020). Top 10 Challenges in Cancer Immunotherapy. *Immunity*, 52(1), 17-35.

Hegde, S. P., Zhao, J., Ashmun, R. A., & Shapiro, L. H. (1999). c-Maf induces monocytic differentiation and apoptosis in bipotent myeloid progenitors. *Blood*, 94(5), 1578-1589.

Helmy, K. Y., Katschke, K. J., Gorgani, N. N., Kljavin, N. M., Elliott, J. M., Diehl, L., et al. (2006). CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell*, 124(5), 915-927.

Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., et al. (2000). A Toll-like receptor recognizes bacterial DNA. *Nature*, 408(6813), 740-745.

Herbst, R. S., Soria, J. C., Kowanetz, M., Fine, G. D., Hamid, O., Gordon, M. S., et al. (2014). Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*, 515(7528), 563-567.

Hettinger, J., Richards, D. M., Hansson, J., Barra, M. M., Joschko, A. C., Krijgsveld, J., et al. (2013). Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol*, 14(8), 821-830.

Hibbs, J. B., Taintor, R. R., Vavrin, Z., & Rachlin, E. M. (1988). Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun*, 157(1), 87-94.

Higgins, J. P., Soares-Weiser, K., López-López, J. A., Kakourou, A., Chaplin, K., Christensen, H., et al. (2016). Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ*, 355, i5170.

Hodi, F. S., O'Day, S. J., McDermott, D. F., Weber, R. W., Sosman, J. A., Haanen, J. B., et al. (2010). Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*, 363(8), 711-723.

Hoeffel, G., Chen, J., Lavin, Y., Low, D., Almeida, F. F., See, P., et al. (2015). C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity*, 42(4), 665-678.

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., et al. (2012). Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med*, 209(6), 1167-1181.

Hole, C. R., Wager, C. M. L., Castro-Lopez, N., Campuzano, A., Cai, H., Wozniak, K. L., et al. (2019). Induction of memory-like dendritic cell responses in vivo. *Nat Commun*, 10(1), 2955.

Hollmén, M., Figueiredo, C. R., & Jalkanen, S. (2020). New tools to prevent cancer growth and spread: a 'Clever' approach. *Br J Cancer*, 123(4), 501-509.

Hollmén, M., Maksimow, M., Rannikko, J. H., Karvonen, M. K., Vainio, M., Jalkanen, S., et al. (2022). Nonclinical characterization of bexmarilimab, a Clever-1-targeting antibody for supporting immune defense against cancers. *Mol Cancer Ther*, 21(7), 1207-1218.

Holte, C., Szafranska, K., Kruse, L., Simon-Santamaria, J., Li, R., Svistounov, D., et al. (2023). Highly oxidized albumin is cleared by liver sinusoidal endothelial cells via the receptors stabilin-1 and -2. *Sci Rep*, 13(1), 19121.

Hong, D. S., Postow, M., Chmielowski, B., Sullivan, R., Patnaik, A., Cohen, E. E. W., et al. (2023). Eganelisib, a First-in-Class PI3Kγ Inhibitor, in Patients with Advanced Solid Tumors: Results of the Phase 1/1b MARIO-1 Trial. *Clin Cancer Res*, 29(12), 2210-2219.

Hori, O., Brett, J., Slattery, T., Cao, R., Zhang, J., Chen, J. X., et al. (1995). The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. *J Biol Chem*, 270(43), 25752-25761.

House, I. G., Savas, P., Lai, J., Chen, A. X. Y., Oliver, A. J., Teo, Z. L., et al. (2020). Macrophage-Derived CXCL9 and CXCL10 Are Required for Antitumor Immune Responses Following Immune Checkpoint Blockade. *Clin Cancer Res*, 26(2), 487-504.

Huang, C., Shao, N., Huang, Y., Chen, J., Wang, D., Hu, G., et al. (2023). Overcoming challenges in the delivery of STING agonists for cancer immunotherapy: A comprehensive review of strategies and future perspectives. *Mater Today Bio*, 23, 100839.

Huang, J. T., Welch J. S., Ricote M., Binder C. J., Willson T. M., Kelly C., et al. (1999). Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature*, 400(6742), 378-382.

Huang, S. C., Everts, B., Ivanova, Y., O'Sullivan, D., Nascimento, M., Smith, A. M., et al. (2014). Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat Immunol*, 15(9), 846-855.

Hulsmans, M., Clauss, S., Xiao, L., Aguirre, A. D., King, K. R., Hanley, A., et al. (2017). Macrophages Facilitate Electrical Conduction in the Heart. *Cell*, 169(3), 510-522.e520.

Huynh, M. L., Fadok, V. A., & Henson, P. M. (2002). Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. *J Clin Invest*, 109(1), 41-50.

Hägerbrand, K., Varas, L., Deronic, A., Nyesiga, B., Sundstedt, A., Ljung, L., et al. (2022). Bispecific antibodies targeting CD40 and tumor-associated antigens promote cross-priming of T cells resulting in an antitumor response superior to monospecific antibodies. *J Immunother Cancer*, 10(11).

Hänzelmann, S., Castelo, R., & Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics*, 14, 7.

Iadonato, S., Ovechkina, Y., Lustig, K., Cross, J., Eyde, N., Frazier, E., et al. (2023). A highly potent anti-VISTA antibody KVA12123 - a new immune checkpoint inhibitor and a promising therapy against poorly immunogenic tumors. *Front Immunol*, 14, 1311658.

Ignatova, I. D., Angdisen, J., Moran, E., & Schulman, I. G. (2013). Differential regulation of gene expression by LXRs in response to macrophage cholesterol loading. *Mol Endocrinol*, 27(7), 1036-1047.

Ingman, W. V., Wyckoff, J., Gouon-Evans, V., Condeelis, J., & Pollard, J. W. (2006). Macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland. *Dev Dyn*, 235(12), 3222-3229.

Irjala, H., Alanen, K., Grénman, R., Heikkilä, P., Joensuu, H., & Jalkanen, S. (2003). Mannose receptor (MR) and common lymphatic endothelial and vascular endothelial receptor (CLEVER)-1 direct the binding of cancer cells to the lymph vessel endothelium. *Cancer Res*, 63(15), 4671-4676.

Irjala, H., Elima, K., Johansson, E. L., Merinen, M., Kontula, K., Alanen, K., et al. (2003). The same endothelial receptor controls lymphocyte traffic both in vascular and lymphatic vessels. *Eur J Immunol*, 33(3), 815-824.

Ito, A., Hong, C., Rong, X., Zhu, X., Tarling, E. J., Hedde, P. N., et al. (2015). LXRs link metabolism to inflammation through Abca1-dependent regulation of membrane composition and TLR signaling. *Elife*, 4, e08009.

Ivashkiv, L. B. (2018). IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. *Nat Rev Immunol*, 18(9), 545-558.

Iwasaki, A., & Medzhitov, R. (2015). Control of adaptive immunity by the innate immune system. *Nat Immunol*, 16(4), 343-353.

Iwasaki, H., Somoza, C., Shigematsu, H., Duprez, E. A., Iwasaki-Arai, J., Mizuno, S., et al. (2005). Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. *Blood*, 106(5), 1590-1600.

Jaiswal, S., Jamieson, C. H., Pang, W. W., Park, C. Y., Chao, M. P., Majeti, R., et al. (2009). CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell*, 138(2), 271-285.

Jaitin, D. A., Adlung, L., Thaiss, C. A., Weiner, A., Li, B., Descamps, H., et al. (2019). Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner. *Cell*, 178(3), 686-698.e614.

Jakubzick, C., Gautier, E. L., Gibbings, S. L., Sojka, D. K., Schlitzer, A., Johnson, T. E., et al. (2013). Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity*, 39(3), 599-610.

Jenkins, S. J., Ruckerl, D., Thomas, G. D., Hewitson, J. P., Duncan, S., Brombacher, F., et al. (2013). IL-4 directly signals tissue-resident macrophages to proliferate beyond homeostatic levels controlled by CSF-1. *J Exp Med*, 210(11), 2477-2491.

Jiang, Z., Mak, T. W., Sen, G., & Li, X. (2004). Toll-like receptor 3-mediated activation of NF-kappaB and IRF3 diverges at Toll-IL-1 receptor domain-containing adapter inducing IFN-beta. *Proc Natl Acad Sci U S A*, 101(10), 3533-3538.

Jorgensen, I., Zhang, Y., Krantz, B. A., & Miao, E. A. (2016). Pyroptosis triggers pore-induced intracellular traps (PITs) that capture bacteria and lead to their clearance by efferocytosis. *J Exp Med*, 213(10), 2113-2128.

Junt, T., Moseman, E. A., Iannacone, M., Massberg, S., Lang, P. A., Boes, M., et al. (2007). Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature*, 450(7166), 110-114.

Junttila, A., Helminen, O., Väyrynen, J. P., Ahtiainen, M., Kenessey, I., Jalkanen, S., et al. (2020). Immunophenotype based on inflammatory cells, PD-1/PD-L1 signalling pathway and M2 macrophages predicts survival in gastric cancer. *Br J Cancer*, 123(11), 1625-1632.

Jäppinen, N., Félix, I., Lokka, E., Tyystjärvi, S., Pynttäri, A., Lahtela, T., et al. (2019). Fetal-derived macrophages dominate in adult mammary glands. *Nat Commun*, 10(1), 281.

Kagan, J. C., Su, T., Horng, T., Chow, A., Akira, S., & Medzhitov, R. (2008). TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. *Nat Immunol*, 9(4), 361-368.

Kamerkar, S., Leng, C., Burenkova, O., Jang, S. C., McCoy, C., Zhang, K., et al. (2022). Exosome-mediated genetic reprogramming of tumor-associated macrophages by exoASO-STAT6 leads to potent monotherapy antitumor activity. *Sci Adv*, 8(7), eabj7002.

Kaneda, M. M., Messer, K. S., Ralainirina, N., Li, H., Leem, C. J., Gorjestani, S., et al. (2016). PI3Kγ is a molecular switch that controls immune suppression. *Nature*, 539(7629), 437-442.

Kang, K., Bachu, M., Park, S. H., Bae, S., Park-Min, K. H., & Ivashkiv, L. B. (2019). IFN-γ selectively suppresses a subset of TLR4-activated genes and enhancers to potentiate macrophage activation. *Nat Commun*, 10(1), 3320.

Karikoski, M., Irjala, H., Maksimow, M., Miiluniemi, M., Granfors, K., Hernesniemi, S., et al. (2009). Clever-1/Stabilin-1 regulates lymphocyte migration within lymphatics and leukocyte entrance to sites of inflammation. *Eur J Immunol*, 39(12), 3477-3487.

Karikoski, M., Marttila-Ichihara, F., Elima, K., Rantakari, P., Hollmén, M., Kelkka, T., et al. (2014). Clever-1/stabilin-1 controls cancer growth and metastasis. *Clin Cancer Res*, 20(24), 6452-6464.

Katzmarski, N., Domínguez-Andrés, J., Cirovic, B., Renieris, G., Ciarlo, E., Le Roy, D., et al. (2021). Transmission of trained immunity and heterologous resistance to infections across generations. *Nat Immunol*, 22(11), 1382-1390.

Kaufmann, E., Sanz, J., Dunn, J. L., Khan, N., Mendonça, L. E., Pacis, A., et al. (2018). BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis. *Cell*, 172(1-2), 176-190.e119.

Kawai, T., Adachi, O., Ogawa, T., Takeda, K., & Akira, S. (1999). Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity*, 11(1), 115-122.

Kawamura, S., Onai, N., Miya, F., Sato, T., Tsunoda, T., Kurabayashi, K., et al. (2017). Identification of a Human Clonogenic Progenitor with Strict Monocyte Differentiation Potential: A Counterpart of Mouse cMoPs. *Immunity*, 46(5), 835-848.e834.

Kawane, K., Fukuyama, H., Kondoh, G., Takeda, J., Ohsawa, Y., Uchiyama, Y., et al. (2001). Requirement of DNase II for definitive erythropoiesis in the mouse fetal liver. *Science*, 292(5521), 1546-1549.

Kayagaki, N., Kornfeld, O. S., Lee, B. L., Stowe, I. B., O'Rourke, K., Li, Q., et al. (2021). NINJ1 mediates plasma membrane rupture during lytic cell death. *Nature*, 591(7848), 131-136.

Kazandjian, D., Khozin, S., Blumenthal, G., Zhang, L., Tang, S., Libeg, M., et al. (2016). Benefit-Risk Summary of Nivolumab for Patients With Metastatic Squamous Cell Lung Cancer After Platinum-Based Chemotherapy: A Report From the US Food and Drug Administration. *JAMA Oncol*, 2(1), 118-122.

Kearns, J. D., Basak, S., Werner, S. L., Huang, C. S., & Hoffmann, A. (2006). IkappaBepsilon provides negative feedback to control NF-kappaB oscillations, signaling dynamics, and inflammatory gene expression. *J Cell Biol*, 173(5), 659-664.

Keller, M., Mazuch, J., Abraham, U., Eom, G. D., Herzog, E. D., Volk, H. D., et al. (2009). A circadian clock in macrophages controls inflammatory immune responses. *Proc Natl Acad Sci U S A*, 106(50), 21407-21412.

Kelly, L. M., Englmeier, U., Lafon, I., Sieweke, M. H., & Graf, T. (2000). MafB is an inducer of monocytic differentiation. *EMBO J*, 19(9), 1987-1997.

Kersten, K., Hu, K. H., Combes, A. J., Samad, B., Harwin, T., Ray, A., et al. (2022). Spatiotemporal co-dependency between macrophages and exhausted $CD8^+$ T cells in cancer. *Cancer Cell*, 40(6), 624-638.e629.

Kieusseian, A., Brunet de la Grange, P., Burlen-Defranoux, O., Godin, I., & Cumano, A. (2012). Immature hematopoietic stem cells undergo maturation in the fetal liver. *Development*, 139(19), 3521-3530.

Kitamura, T., Doughty-Shenton, D., Cassetta, L., Fragkogianni, S., Brownlie, D., Kato, Y., et al. (2017). Monocytes Differentiate to Immune Suppressive Precursors of Metastasis-Associated Macrophages in Mouse Models of Metastatic Breast Cancer. *Front Immunol*, 8, 2004.

Kleinnijenhuis, J., Quintin, J., Preijers, F., Joosten, L. A., Ifrim, D. C., Saeed, S., et al. (2012). Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc Natl Acad Sci U S A*, 109(43), 17537-17542.

Klemm, F., Möckl, A., Salamero-Boix, A., Alekseeva, T., Schäffer, A., Schulz, M., et al. (2021). Compensatory CSF2-driven macrophage activation promotes adaptive resistance to CSF1R inhibition in breast-to-brain metastasis. *Nat Cancer*, 2(10), 1086-1101.

Klichinsky, M., Ruella, M., Shestova, O., Lu, X. M., Best, A., Zeeman, M., et al. (2020). Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol*, 38(8), 947-953.

Kloosterman, D. J., & Akkari, L. (2023). Macrophages at the interface of the co-evolving cancer ecosystem. *Cell*, 186(8), 1627-1651.

Klug, F., Prakash, H., Huber, P. E., Seibel, T., Bender, N., Halama, N., et al. (2013). Low-dose irradiation programs macrophage differentiation to an $iNOS^+$/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell*, 24(5), 589-602.

Koblish, H. K., Hansbury, M. J., Bowman, K. J., Yang, G., Neilan, C. L., Haley, P. J., et al. (2010). Hydroxyamidine inhibitors of indoleamine-2,3-dioxygenase potently suppress systemic tryptophan catabolism and the growth of IDO-expressing tumors. *Mol Cancer Ther*, 9(2), 489-498.

Kodelja, V., & Goerdt, S. (1994). Dissection of macrophage differentiation pathways in cutaneous macrophage disorders and in vitro. *Exp Dermatol*, 3(6), 257-268.

Kohyama, M., Ise, W., Edelson, B. T., Wilker, P. R., Hildner, K., Mejia, C., et al. (2009). Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. *Nature*, 457(7227), 318-321.

Kolter, J., Feuerstein, R., Zeis, P., Hagemeyer, N., Paterson, N., d'Errico, P., et al. (2019). A Subset of Skin Macrophages Contributes to the Surveillance and Regeneration of Local Nerves. *Immunity*, 50(6), 1482-1497.e1487.

Kontro, M., Stein, A. S., Pyörälä, M., Rimpiläinen, J., Siitonen, T., Ylitalo, A., et al. (2025). Bexmarilimab plus azacitidine for high-risk myelodysplastic syndrome and relapsed or refractory acute myeloid leukaemia: results from the dose-escalation part of a multicentre, single-arm, phase 1/2 trial. *Lancet Haematol*, 12(7), e516-e528.

Kraakman, M. J., Lee, M. K., Al-Sharea, A., Dragoljevic, D., Barrett, T. J., Montenont, E., et al. (2017). Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. *J Clin Invest*, 127(6), 2133-2147.

Krummel, M. F., & Allison, J. P. (1995). CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med*, 182(2), 459-465.

Kryczek, I., Zou, L., Rodriguez, P., Zhu, G., Wei, S., Mottram, P., et al. (2006). B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *J Exp Med*, 203(4), 871-881.

Kubo, A., Nagao, K., Yokouchi, M., Sasaki, H., & Amagai, M. (2009). External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. *J Exp Med*, 206(13), 2937-2946.

Kumar, V., Abbas, A. K., & Aster, J. C. (2021). Inflammation and Repair in Robbins & Cotran Pathologic Basis of Disease (pp. 71-113). Elsevier, Philadelphia.

Kumar, V., Donthireddy, L., Marvel, D., Condamine, T., Wang, F., Lavilla-Alonso, S., et al. (2017). Cancer-Associated Fibroblasts Neutralize the Anti-tumor Effect of CSF1 Receptor Blockade by Inducing PMN-MDSC Infiltration of Tumors. *Cancer Cell*, 32(5), 654-668.e655.

Kwon, M., Yeo, S. C., Lee, J. S., & Park, J. J. (2019). Not CD68 but stabilin-1 expression is associated with the risk of recurrence in patients with oral cavity squamous cell carcinoma. *Head Neck*, 41(7), 2058-2064.

Kzhyshkowska, J., Gratchev, A., Martens, J. H., Pervushina, O., Mamidi, S., Johansson, S., et al. (2004). Stabilin-1 localizes to endosomes and the trans-Golgi network in human macrophages and interacts with GGA adaptors. *J Leukoc Biol*, 76(6), 1151-1161.

Kzhyshkowska, J., Gratchev, A., Schmuttermaier, C., Brundiers, H., Krusell, L., Mamidi, S., et al. (2008). Alternatively activated macrophages regulate extracellular levels of the hormone placental lactogen via receptor-mediated uptake and transcytosis. *J Immunol*, 180(5), 3028-3037.

Kzhyshkowska, J., Mamidi, S., Gratchev, A., Kremmer, E., Schmuttermaier, C., Krusell, L., et al. (2006). Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway. *Blood*, 107(8), 3221-3228.

Kzhyshkowska, J., Workman, G., Cardó-Vila, M., Arap, W., Pasqualini, R., Gratchev, A., et al. (2006). Novel function of alternatively activated macrophages: stabilin-1-mediated clearance of SPARC. *J Immunol*, 176(10), 5825-5832.

La Fleur, L., Botling, J., He, F., Pelicano, C., Zhou, C., He, C., et al. (2021). Targeting MARCO and IL37R on Immunosuppressive Macrophages in Lung Cancer Blocks Regulatory T Cells and Supports Cytotoxic Lymphocyte Function. *Cancer Res*, 81(4), 956-967.

Laffitte, B. A., Joseph, S. B., Walczak, R., Pei, L., Wilpitz, D. C., Collins, J. L., et al. (2001). Autoregulation of the human liver X receptor alpha promoter. *Mol Cell Biol*, 21(22), 7558-7568.

Larkin, J., Minor, D., D'Angelo, S., Neyns, B., Smylie, M., Miller, W. H., et al. (2018). Overall Survival in Patients With Advanced Melanoma Who Received Nivolumab Versus Investigator's Choice Chemotherapy in CheckMate 037: A Randomized, Controlled, Open-Label Phase III Trial. *J Clin Oncol*, 36(4), 383-390.

Latz, E., Schoenemeyer, A., Visintin, A., Fitzgerald, K. A., Monks, B. G., Knetter, C. F., et al. (2004). TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol*, 5(2), 190-198.

Lauber, K., Bohn, E., Kröber, S. M., Xiao, Y. J., Blumenthal, S. G., Lindemann, R. K., et al. (2003). Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell*, 113(6), 717-730.

Lauterbach, M. A., Hanke, J. E., Serefidou, M., Mangan, M. S. J., Kolbe, C. C., Hess, T., et al. (2019). Toll-like Receptor Signaling Rewires Macrophage Metabolism and Promotes Histone Acetylation via ATP-Citrate Lyase. *Immunity*, 51(6), 997-1011.e1017.

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., et al. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell*, 159(6), 1312-1326.

Laviron, M., Petit, M., Weber-Delacroix, E., Combes, A. J., Arkal, A. R., Barthélémy, S., et al. (2022). Tumor-associated macrophage heterogeneity is driven by tissue territories in breast cancer. *Cell Rep*, 39(8), 110865.

Leach, D. R., Krummel, M. F., & Allison, J. P. (1996). Enhancement of antitumor immunity by CTLA-4 blockade. *Science*, 271(5256), 1734-1736.

Lee, A., Whyte, M. K., & Haslett, C. (1993). Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. *J Leukoc Biol*, 54(4), 283-288.

Lee, S. J., Park, S. Y., Jung, M. Y., Bae, S. M., & Kim, I. S. (2011). Mechanism for phosphatidylserine-dependent erythrophagocytosis in mouse liver. *Blood*, 117(19), 5215-5223.

Lee-Rueckert, M., Lappalainen, J., Leinonen, H., Plihtari, R., Nordström, T., Åkerman, K., et al. (2020). Acidic extracellular pH promotes accumulation of free cholesterol in human monocyte-derived macrophages via inhibition of ACAT1 activity. *Atherosclerosis*, 312, 1-7.

Leibing, T., Riedel, A., Xi, Y., Adrian, M., Krzistetzko, J., Kirkamm, C., et al. (2023). Deficiency for scavenger receptors Stabilin-1 and Stabilin-2 leads to age-dependent renal and hepatic depositions of fasciclin domain proteins TGFBI and Periostin in mice. *Aging Cell*, 22(9), e13914.

Lesbats, J., Brillac, A., Reisz, J. A., Mukherjee, P., Lhuissier, C., Fernández-Monreal, M., et al. (2025). Macrophages recycle phagocytosed bacteria to fuel immunometabolic responses. *Nature*, 640(8058), 524-533.

Li, J., Lee, Y., Li, Y., Jiang, Y., Lu, H., Zang, W., et al. (2018). Co-inhibitory Molecule B7 Superfamily Member 1 Expressed by Tumor-Infiltrating Myeloid Cells Induces Dysfunction of Anti-tumor $CD8^+$ T cells. *Immunity*, 48(4), 773-786.e775.

Li, Q., Harden, J. L., Anderson, C. D., & Egilmez, N. K. (2016). Tolerogenic Phenotype of IFN-γ-Induced IDO+ Dendritic Cells Is Maintained via an Autocrine IDO-Kynurenine/AhR-IDO Loop. *J Immunol*, 197(3), 962-970.

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7), 923-930.

Lin, E. Y., Li, J. F., Bricard, G., Wang, W., Deng, Y., Sellers, R., et al. (2007). Vascular endothelial growth factor restores delayed tumor progression in tumors depleted of macrophages. *Mol Oncol*, 1(3), 288-302.

Lin, E. Y., Nguyen, A. V., Russell, R. G., & Pollard, J. W. (2001). Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med*, 193(6), 727-740.

Lin, H., Wei, S., Hurt, E. M., Green, M. D., Zhao, L., Vatan, L., et al. (2018). Host expression of PD-L1 determines efficacy of PD-L1 pathway blockade-mediated tumor regression. *J Clin Invest*, 128(2), 805-815.

Lin, L., Chen, Y. S., Yao, Y. D., Chen, J. Q., Chen, J. N., Huang, S. Y., et al. (2015). CCL18 from tumor-associated macrophages promotes angiogenesis in breast cancer. *Oncotarge*t, 6(33), 34758-34773.

Lin, S. Y., Hu, F. F., Miao, Y. R., Hu, H., Lei, Q., Zhang, Q., et al. (2019). Identification of STAB1 in Multiple Datasets as a Prognostic Factor for Cytogenetically Normal AML: Mechanism and Drug Indications. *Mol Ther Nucleic Acids*, 18, 476-484.

Link, V. M., Duttke, S. H., Chun, H. B., Holtman, I. R., Westin, E., Hoeksema, M. A., et al. (2018). Analysis of Genetically Diverse Macrophages Reveals Local and Domain-wide Mechanisms that Control Transcription Factor Binding and Function. *Cell*, 173(7), 1796-1809.e1717.

Liu, J., Zhang, N., Li, Q., Zhang, W., Ke, F., Leng, Q., et al. (2011). Tumor-associated macrophages recruit CCR6+ regulatory T cells and promote the development of colorectal cancer via enhancing CCL20 production in mice. *PLoS One*, 6(4), e19495.

Liu, Z., Gao, Z., Li, B., Li, J., Ou, Y., Yu, X., et al. (2022). Lipid-associated macrophages in the tumor-adipose microenvironment facilitate breast cancer progression. *Oncoimmunology*, 11(1), 2085432.

Liu, Z., Gu, Y., Chakarov, S., Bleriot, C., Kwok, I., Chen, X., et al. (2019). Fate Mapping via Ms4a3-Expression History Traces Monocyte-Derived Cells. *Cell*, 178(6), 1509-1525.e1519.

Loberg, R. D., Ying, C., Craig, M., Yan, L., Snyder, L. A., & Pienta, K. J. (2007). CCL2 as an important mediator of prostate cancer growth in vivo through the regulation of macrophage infiltration. *Neoplasia*, 9(7), 556-562.

Long, G. V., Dummer, R., Hamid, O., Gajewski, T. F., Caglevic, C., Dalle, S., et al. (2019). Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. *Lancet Oncol*, 20(8), 1083-1097.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*, 15(12), 550.

Loyher, P. L., Hamon, P., Laviron, M., Meghraoui-Kheddar, A., Goncalves, E., Deng, Z., et al. (2018). Macrophages of distinct origins contribute to tumor development in the lung. *J Exp Med*, 215(10), 2536-2553.

Lu, B., Rutledge, B. J., Gu, L., Fiorillo, J., Lukacs, N. W., Kunkel, S. L., et al. (1998). Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J Exp Med*, 187(4), 601-608.

Luecke, S., Sheu, K. M., & Hoffmann, A. (2021). Stimulus-specific responses in innate immunity: Multilayered regulatory circuits. *Immunity*, 54(9), 1915-1932.

Lövgren, T., Eloranta, M. L., Båve, U., Alm, G. V., & Rönnblom, L. (2004). Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis Rheum*, 50(6), 1861-1872.

Machnik, A., Neuhofer, W., Jantsch, J., Dahlmann, A., Tammela, T., Machura, K., et al. (2009). Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med*, 15(5), 545-552.

Manegold, C., van Zandwijk, N., Szczesna, A., Zatloukal, P., Au, J. S. K., Blasinska-Morawiec, M., et al. (2012). A phase III randomized study of gemcitabine and cisplatin with or without PF-3512676 (TLR9 agonist) as first-line treatment of advanced non-small-cell lung cancer. *Ann Oncol*, 23(1), 72-77.

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., & Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*, 25(12), 677-686.

Marliot, F., Lafontaine, L., & Galon, J. (2020). Immunoscore assay for the immune classification of solid tumors: Technical aspects, improvements and clinical perspectives. *Methods Enzymol*, 636, 109-128.

Marquet, J., Lasoudris, F., Cousin, C., Puiffe, M. L., Martin-Garcia, N., Baud, V., et al. (2010). Dichotomy between factors inducing the immunosuppressive enzyme IL-4-induced gene 1 (IL4I1) in B lymphocytes and mononuclear phagocytes. *Eur J Immunol*, 40(9), 2557-2568.

Martinez, F. O., & Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep*, 6, 13.

Martinez, F. O., Gordon, S., Locati, M., & Mantovani, A. (2006). Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol*, 177(10), 7303-7311.

Martinon, F., Burns, K., & Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell*, 10(2), 417-426.

Mass, E., Ballesteros, I., Farlik, M., Halbritter, F., Günther, P., Crozet, L., et al. (2016). Specification of tissue-resident macrophages during organogenesis. *Science*, 353(6304).

Massard, C., Michiels, S., Ferté, C., Le Deley, M. C., Lacroix, L., Hollebecque, A., et al. (2017). High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: Results of the MOSCATO 01 Trial. *Cancer Discov*, 7(6), 586-595.

Mathios, D., Kim, J. E., Mangraviti, A., Phallen, J., Park, C. K., Jackson, C. M., et al. (2016). Anti-PD-1 antitumor immunity is enhanced by local and abrogated by systemic chemotherapy in GBM. *Sci Transl Med*, 8(370), 370ra180.

Matusiak, M., Hickey, J. W., van IJzendoorn, D. G. P., Lu, G., Kidzinski, L., Zhu, S., et al. (2024). Spatially Segregated Macrophage Populations Predict Distinct Outcomes In Colon Cancer. *Cancer Discov*, 14(8), 1418-1439.

McDermott, D. F., Sosman, J. A., Sznol, M., Massard, C., Gordon, M. S., Hamid, O., et al. (2016). Atezolizumab, an Anti-Programmed Death-Ligand 1 Antibody, in Metastatic Renal Cell Carcinoma: Long-Term Safety, Clinical Activity, and Immune Correlates From a Phase Ia Study. *J Clin Oncol*, 34(8), 833-842.

McDonald, B., Zucoloto, A. Z., Yu, I. L., Burkhard, R., Brown, K., Geuking, M. B., et al. (2020). Programing of an Intravascular Immune Firewall by the Gut Microbiota Protects against Pathogen Dissemination during Infection. *Cell Host Microbe*, 28(5), 660-668.e664.

McDonald, P. P., Fadok, V. A., Bratton, D., & Henson, P. M. (1999). Transcriptional and translational regulation of inflammatory mediator production by endogenous TGF-beta in macrophages that have ingested apoptotic cells. *J Immunol*, 163(11), 6164-6172.

McGinnis, C. S., Murrow, L. M., & Gartner, Z. J. (2019). DoubletFinder: Doublet Detection in Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. *Cell Syst*, 8(4), 329-337.e324.

McGovern, K., Castro, A. C., Cavanaugh, J., Coma, S., Walsh, M., Tchaicha, J., et al. (2022). Discovery and Characterization of a Novel Aryl Hydrocarbon Receptor Inhibitor, IK-175, and Its Inhibitory Activity on Tumor Immune Suppression. *Mol Cancer Ther*, 21(8), 1261-1272.

McGrath, K. E., Frame, J. M., Fegan, K. H., Bowen, J. R., Conway, S. J., Catherman, S. C., et al. (2015). Distinct Sources of Hematopoietic Progenitors Emerge before HSCs and Provide Functional Blood Cells in the Mammalian Embryo. *Cell Rep*, 11(12), 1892-1904.

McWhorter, F. Y., Wang, T., Nguyen, P., Chung, T., & Liu, W. F. (2013). Modulation of macrophage phenotype by cell shape. *Proc Natl Acad Sci U S A*, 110(43), 17253-17258.

Medeiros, A. I., Serezani, C. H., Lee, S. P., & Peters-Golden, M. (2009). Efferocytosis impairs pulmonary macrophage and lung antibacterial function via PGE2/EP2 signaling. *J Exp Med*, 206(1), 61-68.

Medzhitov, R., Preston-Hurlburt, P., & Janeway, C. A. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*, 388(6640), 394-397.

Mellman, I., Chen, D. S., Powles, T., & Turley, S. J. (2023). The cancer-immunity cycle: Indication, genotype, and immunotype. *Immunity*, 56(10), 2188-2205.

Meric-Bernstam, F., Sweis, R. F., Hodi, F. S., Messersmith, W. A., Andtbacka, R. H. I., Ingham, M., et al. (2022). Phase I Dose-Escalation Trial of MIW815 (ADU-S100), an Intratumoral STING Agonist, in Patients with Advanced/Metastatic Solid Tumors or Lymphomas. *Clin Cancer Res*, 28(4), 677-688.

Merico, D., Isserlin, R., Stueker, O., Emili, A., & Bader, G. D. (2010). Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. *PLoS One*, 5(11), e13984.

Merika, M., Williams, A. J., Chen, G., Collins, T., & Thanos, D. (1998). Recruitment of CBP/p300 by the IFN beta enhanceosome is required for synergistic activation of transcription. *Mol Cell*, 1(2), 277-287.

Metchnikoff, É. (1893). Lectures on the comparative pathology of inflammation, delivered at the Pasteur Instutute in 1891 (Translated by F. A. Starling and E. H. Starling). Kegan Paul, Trench, Trübner & Co., London.

Miller, C. M., Donner, A. J., Blank, E. E., Egger, A. W., Kellar, B. M., Østergaard, M. E., et al. (2016). Stabilin-1 and Stabilin-2 are specific receptors for the cellular internalization of phosphorothioate-modified antisense oligonucleotides (ASOs) in the liver. *Nucleic Acids Res*, 44(6), 2782-2794.

Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J., & Hill, A. M. (2000). M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol*, 164(12), 6166-6173.

Misharin, A. V., Morales-Nebreda, L., Reyfman, P. A., Cuda, C. M., Walter, J. M., McQuattie-Pimentel, A. C., et al. (2017). Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. *J Exp Med*, 214(8), 2387-2404.

Miyanishi, M., Tada, K., Koike, M., Uchiyama, Y., Kitamura, T., & Nagata, S. (2007). Identification of Tim4 as a phosphatidylserine receptor. *Nature*, 450(7168), 435-439.

Molawi, K., Wolf, Y., Kandalla, P. K., Favret, J., Hagemeyer, N., Frenzel, K., et al. (2014). Progressive replacement of embryo-derived cardiac macrophages with age. *J Exp Med*, 211(11), 2151-2158.

Molgora, M., Esaulova, E., Vermi, W., Hou, J., Chen, Y., Luo, J., et al. (2020). TREM2 Modulation Remodels the Tumor Myeloid Landscape Enhancing Anti-PD-1 Immunotherapy. *Cell,* 182(4), 886-900.e817.

Mondor, I., Baratin, M., Lagueyrie, M., Saro, L., Henri, S., Gentek, R., et al. (2019). Lymphatic Endothelial Cells Are Essential Components of the Subcapsular Sinus Macrophage Niche. *Immunity*, 50(6), 1453-1466.e1454.

Moore, M. A., & Metcalf, D. (1970). Ontogeny of the haemopoietic system: yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. *Br J Haematol*, 18(3), 279-296.

Mosig, S., Rennert, K., Krause, S., Kzhyshkowska, J., Neunübel, K., Heller, R., et al. (2009). Different functions of monocyte subsets in familial hypercholesterolemia: potential function of CD14+ CD16+ monocytes in detoxification of oxidized LDL. *FASEB J*, 23(3), 866-874.

Mossadegh-Keller, N., Sarrazin, S., Kandalla, P. K., Espinosa, L., Stanley, E. R., Nutt, S. L., et al. (2013). M-CSF instructs myeloid lineage fate in single haematopoietic stem cells. *Nature*, 497(7448), 239-243.

Mounier, R., Théret, M., Arnold, L., Cuvellier, S., Bultot, L., Göransson, O., et al. (2013). AMPKα1 regulates macrophage skewing at the time of resolution of inflammation during skeletal muscle regeneration. *Cell Metab*, 18(2), 251-264.

Mulder, K., Patel, A. A., Kong, W. T., Piot, C., Halitzki, E., Dunsmore, G., et al. (2021). Cross-tissue single-cell landscape of human monocytes and macrophages in health and disease. *Immunity*, 54(8), 1883-1900.e1885.

Muller P. A., Koscsó B., Rajani G. M., Stevanovic K., Berres M., Hashimoto D., et al. (2014). Crosstalk between muscularis macrophage and enteric neurons regulates gastrointestinal motility. *Cell*, 158(2):300-13.

Mutka, M., Virtakoivu, R., Joensuu, K., Hollmén, M., & Heikkilä, P. (2022). Clever-1 positive macrophages in breast cancer. *Breast Cancer Res Treat*, 195(3), 237-248.

Nagata, S. (2018). Apoptosis and Clearance of Apoptotic Cells. Annu Rev Immunol, 36, 489-517.

Nagy L., Tontonoz P., Alvarez J. G., Chen H., Evans R. M. (1998). Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. *Cell*, 93(2), 229-40.

Nairz, M., Theurl, I., Ludwiczek, S., Theurl, M., Mair, S. M., Fritsche, G., et al. (2007). The co-ordinated regulation of iron homeostasis in murine macrophages limits the availability of iron for intracellular Salmonella typhimurium. *Cell Microbiol*, 9(9), 2126-2140.

Nakamura, A., Ebina-Shibuya, R., Itoh-Nakadai, A., Muto, A., Shima, H., Saigusa, D., et al. (2013). Transcription repressor Bach2 is required for pulmonary surfactant homeostasis and alveolar macrophage function. *J Exp Med*, 210(11), 2191-2204.

Nalio Ramos, R., Missolo-Koussou, Y., Gerber-Ferder, Y., Bromley, C. P., Bugatti, M., Núñez, N. G., et al. (2022). Tissue-resident FOLR2+ macrophages associate with CD8+ T cell infiltration in human breast cancer. *Cell*, 185(7), 1189-1207.e1125.

Nasir, I., McGuinness, C., Poh, A. R., Ernst, M., Darcy, P. K., & Britt, K. L. (2023). Tumor macrophage functional heterogeneity can inform the development of novel cancer therapies. *Trends Immunol*, 44(12), 971-985.

Nelson, J. K., Koenis, D. S., Scheij, S., Cook, E. C., Moeton, M., Santos, A., et al. (2017). EEPD1 Is a Novel LXR Target Gene in Macrophages Which Regulates ABCA1 Abundance and Cholesterol Efflux. *Arterioscler Thromb Vasc Biol*, 37(3), 423-432.

Netea, M. G., Domínguez-Andrés, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., et al. (2020). Defining trained immunity and its role in health and disease. *Nat Rev Immunol*, 20(6), 375-388.

Netea, M. G., Quintin, J., & van der Meer, J. W. (2011). Trained immunity: a memory for innate host defense. *Cell Host Microbe*, 9(5), 355-361.

Neupane, A. S., Willson, M., Chojnacki, A. K., Vargas, E. S. C. F., Morehouse, C., Carestia, A., et al. (2020). Patrolling Alveolar Macrophages Conceal Bacteria from the Immune System to Maintain Homeostasis. *Cell*, 183(1), 110-125.e111.

Newman, A. M., Steen, C. B., Liu, C. L., Gentles, A. J., Chaudhuri, A. A., Scherer, F., et al. (2019). Determining cell type abundance and expression from bulk tissues with digital cytometry. *Nat Biotechnol*, 37(7), 773-782.

Nguyen, K. D., Qiu, Y., Cui, X., Goh, Y. P., Mwangi, J., David, T., et al. (2011). Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature*, 480(7375), 104-108.

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science*, 308(5726), 1314-1318.

Niño-Castro, A., Abdullah, Z., Popov, A., Thabet, Y., Beyer, M., Knolle, P., et al. (2014). The IDO1-induced kynurenines play a major role in the antimicrobial effect of human myeloid cells against Listeria monocytogenes. *Innate Immun*, 20(4), 401-411.

Noel, M., O'Reilly, E. M., Wolpin, B. M., Ryan, D. P., Bullock, A. J., Britten, C. D., et al. (2020). Phase 1b study of a small molecule antagonist of human chemokine (C-C motif) receptor 2 (PF-04136309) in combination with nab-paclitaxel/gemcitabine in first-line treatment of metastatic pancreatic ductal adenocarcinoma. *Invest New Drugs*, 38(3), 800-811.

Novakovic, B., Habibi, E., Wang, S. Y., Arts, R. J. W., Davar, R., Megchelenbrink, W., et al. (2016). β-Glucan Reverses the Epigenetic State of LPS-Induced Immunological Tolerance. *Cell*, 167(5), 1354-1368.e1314.

Nywening, T. M., Belt, B. A., Cullinan, D. R., Panni, R. Z., Han, B. J., Sanford, D. E., et al. (2018). Targeting both tumour-associated CXCR2. *Gut*, 67(6), 1112-1123.

O'Donnell, J. S., Teng, M. W. L., & Smyth, M. J. (2019). Cancer immunoediting and resistance to T cell-based immunotherapy. *Nat Rev Clin Oncol*, 16(3), 151-167.

O'Leary, M. C., Lu, X., Huang, Y., Lin, X., Mahmood, I., Przepiorka, D., et al. (2019). FDA Approval Summary: Tisagenlecleucel for Treatment of Patients with Relapsed or Refractory B-cell Precursor Acute Lymphoblastic Leukemia. *Clin Cancer Res*, 25(4), 1142-1146.

O'Sullivan, C., Lewis, C. E., Harris, A. L., & McGee, J. O. (1993). Secretion of epidermal growth factor by macrophages associated with breast carcinoma. *Lancet*, 342(8864), 148-149.

Obeid, M., Tesniere, A., Ghiringhelli, F., Fimia, G. M., Apetoh, L., Perfettini, J. L., et al. (2007). Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med*, 13(1), 54-61.

Ohmori, Y., & Hamilton, T. A. (1998). STAT6 is required for the anti-inflammatory activity of interleukin-4 in mouse peritoneal macrophages. *J Biol Chem*, 273(44), 29202-29209.

Okabe, Y., & Medzhitov, R. (2014). Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell*, 157(4), 832-844.

Okabe, Y., & Medzhitov, R. (2016). Tissue biology perspective on macrophages. *Nat Immunol*, 17(1), 9-17.

Okada, N., Kawazoe, H., Takechi, K., Matsudate, Y., Utsunomiya, R., Zamami, Y., et al. (2019). Association Between Immune-Related Adverse Events and Clinical Efficacy in Patients with Melanoma Treated With Nivolumab: A Multicenter Retrospective Study. *Clin Ther*, 41(1), 59-67.

Olson, M. C., Scott, E. W., Hack, A. A., Su, G. H., Tenen, D. G., Singh, H., et al. (1995). PU. 1 is not essential for early myeloid gene expression but is required for terminal myeloid differentiation. *Immunity*, 3(6), 703-714.

Ong, L. L., Li, W., Oldigs, J. K., Kaminski, A., Gerstmayer, B., Piechaczek, C., et al. (2010). Hypoxic/normoxic preconditioning increases endothelial differentiation potential of human bone marrow CD133+ cells. *Tissue Eng Part C Methods*, 16(5), 1069-1081.

Oosterhof, N., Chang, I. J., Karimiani, E. G., Kuil, L. E., Jensen, D. M., Daza, R., et al. (2019). Homozygous Mutations in CSF1R Cause a Pediatric-Onset Leukoencephalopathy and Can Result in Congenital Absence of Microglia. *Am J Hum Genet*, 104(5), 936-947.

Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., et al. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature*, 478(7368), 197-203.

Oren, R., Farnham, A. E., Saito, K., Milofsky, E., & Karnovsky, M. L. (1963). Metabolic patterns in three types of phagocytizing cells. *J Cell Biol*, 17(3), 487-501.

Ostuni, R., Piccolo, V., Barozzi, I., Polletti, S., Termanini, A., Bonifacio, S., et al. (2013). Latent enhancers activated by stimulation in differentiated cells. *Cell*, 152(1-2), 157-171.

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan-a web and mobile app for systematic reviews. *Syst Rev*, 5(1), 210.

Pagès, F., Mlecnik, B., Marliot, F., Bindea, G., Ou, F. S., Bifulco, C., et al. (2018). International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet*, 391(10135), 2128-2139.

Palani, S., Elima, K., Ekholm, E., Jalkanen, S., & Salmi, M. (2016). Monocyte Stabilin-1 Suppresses the Activation of Th1 Lymphocytes. *J Immunol*, 196(1), 115-123.

Palis, J., Robertson, S., Kennedy, M., Wall, C., & Keller, G. (1999). Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. *Development*, 126(22), 5073-5084.

Palsson-McDermott, E. M., Curtis, A. M., Goel, G., Lauterbach, M. A., Sheedy, F. J., Gleeson, L. E., et al. (2015). Pyruvate kinase M2 regulates Hif-1α activity and IL-1β induction and is a critical determinant of the warburg effect in LPS-activated macrophages. *Cell Metab*, 21(1), 65-80.

Park, S. Y., Bae, D. J., Kim, M. J., Piao, M. L., & Kim, I. S. (2012). Extracellular low pH modulates phosphatidylserine-dependent phagocytosis in macrophages by increasing stabilin-1 expression. *J Biol Chem*, 287(14), 11261-11271.

Park, S. Y., Jung, M. Y., Lee, S. J., Kang, K. B., Gratchev, A., Riabov, V., et al. (2009). Stabilin-1 mediates phosphatidylserine-dependent clearance of cell corpses in alternatively activated macrophages. *J Cell Sci*, 122(Pt 18), 3365-3373.

Park, S. Y., & Kim, J. S. (2020). A short guide to histone deacetylases including recent progress on class II enzymes. *Exp Mol Med*, 52(2), 204-212.

Park, S. Y., Kim, S. Y., Kang, K. B., & Kim, I. S. (2010). Adaptor protein GULP is involved in stabilin-1-mediated phagocytosis. *Biochem Biophys Res Commun*, 398(3), 467-472.

Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R., Lafaille, J. J., et al. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell*, 155(7), 1596-1609.

Parnaik, R., Raff, M. C., & Scholes, J. (2000). Differences between the clearance of apoptotic cells by professional and non-professional phagocytes. *Curr Biol*, 10(14), 857-860.

Passlick, B., Flieger, D., & Ziegler-Heitbrock, H. W. (1989). Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood*, 74(7), 2527-2534.

Patel, A. A., Zhang, Y., Fullerton, J. N., Boelen, L., Rongvaux, A., Maini, A. A., et al. (2017). The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. *J Exp Med*, 214(7), 1913-1923.

Peranzoni, E., Lemoine, J., Vimeux, L., Feuillet, V., Barrin, S., Kantari-Mimoun, C., et al. (2018). Macrophages impede CD8 T cells from reaching tumor cells and limit the efficacy of anti-PD-1 treatment. *Proc Natl Acad Sci U S A*, 115(17), E4041-E4050.

Pfeifer, J. D., Wick, M. J., Roberts, R. L., Findlay, K., Normark, S. J., & Harding, C. V. (1993). Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature*, 361(6410), 359-362.

Pirzgalska, R. M., Seixas, E., Seidman, J. S., Link, V. M., Sánchez, N. M., Mahú, I., et al. (2017). Sympathetic neuron-associated macrophages contribute to obesity by importing and metabolizing norepinephrine. *Nat Med*, 23(11), 1309-1318.

Ploeger, D. T., Hosper, N. A., Schipper, M., Koerts, J. A., de Rond, S., & Bank, R. A. (2013). Cell plasticity in wound healing: paracrine factors of M1/M2 polarized macrophages influence the phenotypical state of dermal fibroblasts. *Cell Commun Signal*, 11(1), 29.

Podgrabinska, S., Braun, P., Velasco, P., Kloos, B., Pepper, M. S., & Skobe, M. (2002). Molecular characterization of lymphatic endothelial cells. *Proc Natl Acad Sci U S A*, 99(25), 16069-16074.

Politz, O., Gratchev, A., McCourt, P. A., Schledzewski, K., Guillot, P., Johansson, S., et al. (2002). Stabilin-1 and -2 constitute a novel family of fasciclin-like hyaluronan receptor homologues. *Biochem J*, 362(Pt 1), 155-164.

Popescu, D. M., Botting, R. A., Stephenson, E., Green, K., Webb, S., Jardine, L., et al. (2019). Decoding human fetal liver haematopoiesis. *Nature*, 574(7778), 365-371.

Pozzi, L. A., Maciaszek, J. W., & Rock, K. L. (2005). Both dendritic cells and macrophages can stimulate naive CD8 T cells in vivo to proliferate, develop effector function, and differentiate into memory cells. *J Immunol*, 175(4), 2071-2081.

PrabhuDas, M. R., Baldwin, C. L., Bollyky, P. L., Bowdish, D. M. E., Drickamer, K., Febbraio, M., et al. (2017). A Consensus Definitive Classification of Scavenger Receptors and Their Roles in Health and Disease. *J Immunol*, 198(10), 3775-3789.

Prieto, P. A., Yang, J. C., Sherry, R. M., Hughes, M. S., Kammula, U. S., White, D. E., et al. (2012). CTLA-4 blockade with ipilimumab: long-term follow-up of 177 patients with metastatic melanoma. *Clin Cancer Res*, 18(7), 2039-2047.

Prince, S., Viitala, M., Sjöroos, R., Bendes, Á. Á., Rannikko, J. H., Patten, D. A., et al. (2025). Secreted Clever-1 modulates T cell responses and impacts cancer immunotherapy efficacy. *Theranostics*, 15(15), 7501-7527.

Proia, T. A., Singh, M., Woessner, R., Carnevalli, L., Bommakanti, G., Magiera, L., et al. (2020). Antisense Oligonucleotide Remodels the Suppressive Tumor Microenvironment to Enhance Immune Activation in Combination with Anti-PD-L1. *Clin Cancer Res*, 26(23), 6335-6349.

Punt, J., Stranford, S., Jones, P., & Owen, J. A. (2019a). Cells, organs and microenvironments of the immune system in Kuby Immunology 8th (pp. 31-65). W. H. Freeman and Company, New York.

Punt, J., Stranford, S., Jones, P., & Owen, J. A. (2019b). Effector responses: antibody- and cell-mediated immunity in Kuby Immunology 8th. (pp. 433-468). W. H. Freeman and Company, New York.

Punt, J., Stranford, S., Jones, P., & Owen, J. A. (2019c). Innate Immunity in Kuby Immunology 8th. (pp. 113-160). W. H. Freeman and Company, New York.

Punt, J., Stranford, S., Jones, P., & Owen, J. A. (2019d). Recognition and response in Kuby Immunology 8th (pp. 69-109). W. H. Freeman and Company, New York.

Punt, J., Stranford, S., Jones, P., & Owen, J. A. (2019e). T-cell Activation, helper subset differentiation and memory in Kuby Immunology 8th (pp. 359-375). W. H. Freeman and Company, New York.

Qian, B., Deng, Y., Im, J. H., Muschel, R. J., Zou, Y., Li, J., et al. (2009). A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. *PLoS One*, 4(8), e6562.

Qian, B. Z., Li, J., Zhang, H., Kitamura, T., Zhang, J., Campion, L. R., et al. (2011). CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature*, 475(7355), 222-225.

Qiao, Y., Giannopoulou, E. G., Chan, C. H., Park, S. H., Gong, S., Chen, J., et al. (2013). Synergistic activation of inflammatory cytokine genes by interferon-γ-induced chromatin remodeling and toll-like receptor signaling. *Immunity*, 39(3), 454-469.

Quaranta, V., Rainer, C., Nielsen, S. R., Raymant, M. L., Ahmed, M. S., Engle, D. D., et al. (2018). Macrophage-Derived Granulin Drives Resistance to Immune Checkpoint Inhibition in Metastatic Pancreatic Cancer. *Cancer Res*, 78(15), 4253-4269.

Quintin, J., Saeed, S., Martens, J. H. A., Giamarellos-Bourboulis, E. J., Ifrim, D. C., Logie, C., et al. (2012). Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe*, 12(2), 223-232.

Ramachandran, P., Pellicoro, A., Vernon, M. A., Boulter, L., Aucott, R. L., Ali, A., et al. (2012). Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. *Proc Natl Acad Sci U S A*, 109(46), E3186-3195.

Ramspott, J. P., Bekkat, F., Bod, L., Favier, M., Terris, B., Salomon, A., et al. (2018). Emerging Role of IL-4-Induced Gene 1 as a Prognostic Biomarker Affecting the Local T-Cell Response in Human Cutaneous Melanoma. *J Invest Dermatol*, 138(12), 2625-2634.

Rantakari, P., Patten, D. A., Valtonen, J., Karikoski, M., Gerke, H., Dawes, H., et al. (2016). Stabilin-1 expression defines a subset of macrophages that mediate tissue homeostasis and prevent fibrosis in chronic liver injury. *Proc Natl Acad Sci U S A*, 113(33), 9298-9303.

Rappolee, D. A., Mark, D., Banda, M. J., & Werb, Z. (1988). Wound macrophages express TGF-alpha and other growth factors in vivo: analysis by mRNA phenotyping. *Science*, 241(4866), 708-712.

Reilley, M. J., McCoon, P., Cook, C., Lyne, P., Kurzrock, R., Kim, Y., et al. (2018). STAT3 antisense oligonucleotide AZD9150 in a subset of patients with heavily pretreated lymphoma: results of a phase 1b trial. *J Immunother Cancer*, 6(1):119.

Reiss, K. A., Angelos, M. G., Dees, E. C., Yuan, Y., Ueno, N. T., Pohlmann, P. R., et al. (2025). CAR-macrophage therapy for HER2-overexpressing advanced solid tumors: a phase 1 trial. *Nat Med*, 31(4), 1171-1182.

Restifo, N. P., Smyth, M. J., & Snyder, A. (2016). Acquired resistance to immunotherapy and future challenges. *Nat Rev Cancer*, 16(2), 121-126.

Richards, J. O., Karki, S., Lazar, G. A., Chen, H., Dang, W., & Desjarlais, J. R. (2008). Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. *Mol Cancer Ther*, 7(8), 2517-2527.

Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., & Glass, C. K. (1998). The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature*, 391(6662), 79-82.

Ries, C. H., Cannarile, M. A., Hoves, S., Benz, J., Wartha, K., Runza, V., et al. (2014). Targeting Tumor-Associated Macrophages with Anti-CSF-1R Antibody Reveals a Strategy for Cancer Therapy. *Cancer Cell*, 25(6):846-859.

Robert, C., Schachter, J., Long, G. V., Arance, A., Grob, J. J., Mortier, L., et al. (2015). Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med*, 372(26), 2521-2532.

Roberts, A. B., Sporn, M. B., Assoian, R. K., Smith, J. M., Roche, N. S., Wakefield, L. M., et al. (1986). Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci U S A*, 83(12), 4167-4171.

Roberts, A. W., Lee, B. L., Deguine, J., John, S., Shlomchik, M. J., & Barton, G. M. (2017). Tissue-Resident Macrophages Are Locally Programmed for Silent Clearance of Apoptotic Cells. *Immunity*, 47(5), 913-927.e916.

Robinson, B. D., Sica, G. L., Liu, Y. F., Rohan, T. E., Gertler, F. B., Condeelis, J. S., et al. (2009). Tumor microenvironment of metastasis in human breast carcinoma: a potential prognostic marker linked to hematogenous dissemination. *Clin Cancer Res*, 15(7), 2433-2441.

Robinson, S. C., Scott, K. A., Wilson, J. L., Thompson, R. G., Proudfoot, A. E., & Balkwill, F. R. (2003). A chemokine receptor antagonist inhibits experimental breast tumor growth. *Cancer Res*, 63(23), 8360-8365.

Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A., & Bazan, J. F. (1998). A family of human receptors structurally related to Drosophila Toll. *Proc Natl Acad Sci U S A*, 95(2), 588-593.

Rodríguez-Prados, J. C., Través, P. G., Cuenca, J., Rico, D., Aragonés, J., Martín-Sanz, P., et al. (2010). Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J Immunol*, 185(1), 605-614.

Rogado, J., Sánchez-Torres, J. M., Romero-Laorden, N., Ballesteros, A. I., Pacheco-Barcia, V., Ramos-Leví, A., et al. (2019). Immune-related adverse events predict the therapeutic efficacy of anti-PD-1 antibodies in cancer patients. *Eur J Cancer*, 109, 21-27.

Rogers, C., Fernandes-Alnemri, T., Mayes, L., Alnemri, D., Cingolani, G., & Alnemri, E. S. (2017). Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. *Nat Commun*, 8, 14128.

Roh-Johnson, M., Bravo-Cordero, J. J., Patsialou, A., Sharma, V. P., Guo, P., Liu, H., et al. (2014). Macrophage contact induces RhoA GTPase signaling to trigger tumor cell intravasation. *Oncogene*, 33(33), 4203-4212.

Roquilly, A., Jacqueline, C., Davieau, M., Mollé, A., Sadek, A., Fourgeux, C., et al. (2020). Alveolar macrophages are epigenetically altered after inflammation, leading to long-term lung immunoparalysis. *Nat Immunol*, 21(6), 636-648.

Ruffell, B., Chang-Strachan, D., Chan, V., Rosenbusch, A., Ho, C. M., Pryer, N., et al. (2014). Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. *Cancer Cell*, 26(5), 623-637.

Saeed, S., Quintin, J., Kerstens, H. H., Rao, N. A., Aghajanirefah, A., Matarese, F., et al. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science*, 345(6204), 1251086.

Saio, M., Radoja, S., Marino, M., & Frey, A. B. (2001). Tumor-infiltrating macrophages induce apoptosis in activated CD8(+) T cells by a mechanism requiring cell contact and mediated by both the cell-associated form of TNF and nitric oxide. *J Immunol*, 167(10), 5583-5593.

Sakai, M., Troutman, T. D., Seidman, J. S., Ouyang, Z., Spann, N. J., Abe, Y., et al. (2019). Liver-Derived Signals Sequentially Reprogram Myeloid Enhancers to Initiate and Maintain Kupffer Cell Identity. *Immunity*, 51(4), 655-670.e658.

Salmi, M., Koskinen, K., Henttinen, T., Elima, K., & Jalkanen, S. (2004). CLEVER-1 mediates lymphocyte transmigration through vascular and lymphatic endothelium. *Blood*, 104(13), 3849-3857.

Salomon, R., & Dahan, R. (2022). Next Generation CD40 Agonistic Antibodies for Cancer Immunotherapy. *Front Immunol*, 13, 940674.

Sandhu, S. K., Papadopoulos, K., Fong, P. C., Patnaik, A., Messiou, C., Olmos, D., et al. (2013). A first-in-human, first-in-class, phase I study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 in patients with solid tumors. *Cancer Chemother Pharmacol*, 71(4), 1041-1050.

Sanjuan, M. A., Dillon, C. P., Tait, S. W., Moshiach, S., Dorsey, F., Connell, S., et al. (2007). Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature*, 450(7173), 1253-1257.

Sato, K., Akamatsu, H., Murakami, E., Sasaki, S., Kanai, K., Hayata, A., et al. (2018). Correlation between immune-related adverse events and efficacy in non-small cell lung cancer treated with nivolumab. *Lung Cancer*, 115, 71-74.

Sato, S., Nomura, F., Kawai, T., Takeuchi, O., Mühlradt, P. F., Takeda, K., et al. (2000). Synergy and cross-tolerance between toll-like receptor (TLR) 2- and TLR4-mediated signaling pathways. *J Immunol*, 165(12), 7096-7101.

Scaffidi, P., Misteli, T., & Bianchi, M. E. (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*, 418(6894), 191-195.

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*, 74(4), 691-705.

Schledzewski, K., Falkowski, M., Moldenhauer, G., Metharom, P., Kzhyshkowska, J., Ganss, R., et al. (2006). Lymphatic endothelium-specific hyaluronan receptor LYVE-1 is expressed by stabilin-1+, F4/80+, CD11b+ macrophages in malignant tumours and wound healing tissue in vivo and in bone marrow cultures in vitro: implications for the assessment of lymphangiogenesis. *J Pathol*, 209(1), 67-77.

Schledzewski, K., Géraud, C., Arnold, B., Wang, S., Gröne, H. J., Kempf, T., et al. (2011). Deficiency of liver sinusoidal scavenger receptors stabilin-1 and -2 in mice causes glomerulofibrotic nephropathy via impaired hepatic clearance of noxious blood factors. *J Clin Invest*, 121(2), 703-714.

Schneider, C., Nobs, S. P., Kurrer, M., Rehrauer, H., Thiele, C., & Kopf, M. (2014). Induction of the nuclear receptor PPAR-γ by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages. *Nat Immunol*, 15(11), 1026-1037.

Schreiber, R. D., Old, L. J., & Smyth, M. J. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*, 331(6024), 1565-1570.

Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science*, 336(6077), 86-90.

Scott, C. L., Zheng, F., De Baetselier, P., Martens, L., Saeys, Y., De Prijck, S., et al. (2016). Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nat Commun*, 7, 10321.

Serbina, N. V., & Pamer, E. G. (2006). Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol*, 7(3), 311-317.

Shankaran, V., Ikeda, H., Bruce, A. T., White, J. M., Swanson, P. E., Old, L. J., et al. (2001). IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature*, 410(6832), 1107-1111.

Sharma, P., Hu-Lieskovan, S., Wargo, J. A., & Ribas, A. (2017). Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell*, 168(4), 707-723.

Sharma, P., Siddiqui, B. A., Anandhan, S., Yadav, S. S., Subudhi, S. K., Gao, J., et al. (2021). The Next Decade of Immune Checkpoint Therapy. *Cancer Discov*, 11(4), 838-857.

Sharma, S. K., Chintala, N. K., Vadrevu, S. K., Patel, J., Karbowniczek, M., & Markiewski, M. M. (2015). Pulmonary alveolar macrophages contribute to the premetastatic niche by suppressing antitumor T cell responses in the lungs. *J Immunol*, 194(11), 5529-5538.

Shaw, T. N., Houston, S. A., Wemyss, K., Bridgeman, H. M., Barbera, T. A., Zangerle-Murray, T., et al. (2018). Tissue-resident macrophages in the intestine are long lived and defined by Tim-4 and CD4 expression. *J Exp Med*, 215(6):1507-18.

Sheban, F., Phan, T. S., Xie, K., Ingelfinger, F., Gur, C., Shapir Itai, Y., et al. (2025). ZEB2 is a master switch controlling the tumor-associated macrophage program. *Cancer Cell*, 43(7), 1227-1241.e1211.

Shemer, A., Grozovski, J., Tay, T. L., Tao, J., Volaski, A., Süß, P., et al. (2018). Engrafted parenchymal brain macrophages differ from microglia in transcriptome, chromatin landscape and response to challenge. *Nat Commun*, 9(1), 5206.

Shetty, S., Weston, C. J., Oo, Y. H., Westerlund, N., Stamataki, Z., Youster, J., et al. (2011). Common lymphatic endothelial and vascular endothelial receptor-1 mediates the transmigration of regulatory T cells across human hepatic sinusoidal endothelium. *J Immunol*, 186(7), 4147-4155.

Sheu, K. M., Guru, A. A., & Hoffmann, A. (2023). Quantifying stimulus-response specificity to probe the functional state of macrophages. *Cell Syst*, 14(3), 180-195.e185.

Shih, V. F., Kearns, J. D., Basak, S., Savinova, O. V., Ghosh, G., & Hoffmann, A. (2009). Kinetic control of negative feedback regulators of NF-kappaB/RelA determines their pathogen- and cytokine-receptor signaling specificity. *Proc Natl Acad Sci U S A*, 106(24), 9619-9624.

Shimokado, K., Raines, E. W., Madtes, D. K., Barrett, T. B., Benditt, E. P., & Ross, R. (1985). A significant part of macrophage-derived growth factor consists of at least two forms of PDGF. *Cell*, 43(1), 277-286.

Sica, A., Saccani, A., Bottazzi, B., Polentarutti, N., Vecchi, A., van Damme, J., et al. (2000). Autocrine production of IL-10 mediates defective IL-12 production and NF-kappa B activation in tumor-associated macrophages. *J Immunol*, 164(2), 762-767.

Sikic, B. I., Lakhani, N., Patnaik, A., Shah, S. A., Chandana, S. R., Rasco, D., et al. (2019). First-in-Human, First-in-Class Phase I Trial of the Anti-CD47 Antibody Hu5F9-G4 in Patients With Advanced Cancers. *J Clin Oncol*, 37(12), 946-953.

Siu, L. L., Wang, D., Hilton, J., Geva, R., Rasco, D., Perets, R., et al. (2022). First-in-Class Anti-immunoglobulin-like Transcript 4 Myeloid-Specific Antibody MK-4830 Abrogates a PD-1 Resistance Mechanism in Patients with Advanced Solid Tumors. *Clin Cancer Res*, 28(1), 57-70.

Smythies, L. E., Sellers, M., Clements, R. H., Mosteller-Barnum, M., Meng, G., Benjamin, W. H., et al. (2005). Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. *J Clin Invest*, 115(1):66-75.

Snelgrove, R. J., Goulding, J., Didierlaurent, A. M., Lyonga, D., Vekaria, S., Edwards, L., et al. (2008). A critical function for CD200 in lung immune homeostasis and the severity of influenza infection. *Nat Immunol*, 9(9), 1074-1083.

Son, M., Frank, T., Holst-Hansen, T., Wang, A. G., Junkin, M., Kashaf, S. S., et al. (2022). Spatiotemporal NF-κB dynamics encodes the position, amplitude, and duration of local immune inputs. *Sci Adv*, 8(35), eabn6240.

Soria, G., Yaal-Hahoshen, N., Azenshtein, E., Shina, S., Leider-Trejo, L., Ryvo, L., et al. (2008). Concomitant expression of the chemokines RANTES and MCP-1 in human breast cancer: a basis for tumor-promoting interactions. *Cytokine*, 44(1), 191-200.

Spira, A., Gordon, M., Henry, J., Patel, S., Sehgai, K., Sen, S., et al. (2023). Abstract 756 First-in-human, open-label, multicenter, phase 1 clinical study to evaluate safety, tolerability, pharmacokinetics and pharmacodynamics of anti Siglec-15 PYX-106 in subjects with advanced solid tumors. *J Immunother Cancer*, 11(Suppl 1), A852.

Spranger, S., Spaapen, R. M., Zha, Y., Williams, J., Meng, Y., Ha, T. T., et al. (2013). Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. *Sci Transl Med*, 5(200), 200ra116.

Starr, R., Willson, T. A., Viney, E. M., Murray, L. J., Rayner, J. R., Jenkins, B. J., et al. (1997). A family of cytokine-inducible inhibitors of signalling. *Nature*, 387(6636), 917-921.

Stein, M., Keshav, S., Harris, N., & Gordon, S. (1992). Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med*, 176(1), 287-292.

Stout, R. D., Jiang, C., Matta, B., Tietzel, I., Watkins, S. K., & Suttles, J. (2005). Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol*, 175(1), 342-349.

Strieter, R. M., Chensue, S. W., Basha, M. A., Standiford, T. J., Lynch, J. P., Baggiolini, M., et al. (1990). Human alveolar macrophage gene expression of interleukin-8 by tumor necrosis factor-alpha, lipopolysaccharide, and interleukin-1 beta. *Am J Respir Cell Mol Biol*, 2(4), 321-326.

Su, P., Wang, Q., Bi, E., Ma, X., Liu, L., Yang, M., et al. (2020). Enhanced Lipid Accumulation and Metabolism Are Required for the Differentiation and Activation of Tumor-Associated Macrophages. *Cancer Res*, 80(7), 1438-1450.

Su, X., Yu, Y., Zhong, Y., Giannopoulou, E. G., Hu, X., Liu, H., et al. (2015). Interferon-γ regulates cellular metabolism and mRNA translation to potentiate macrophage activation. *Nat Immunol*, 16(8), 838-849.

Sul, J., Blumenthal, G. M., Jiang, X., He, K., Keegan, P., & Pazdur, R. (2016). FDA Approval Summary: Pembrolizumab for the Treatment of Patients With Metastatic Non-Small Cell Lung Cancer Whose Tumors Express Programmed Death-Ligand 1. *Oncologist*, 21(5), 643-650.

Sun, L., Wu, J., Du, F., Chen, X., & Chen, Z. J. (2013). Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*, 339(6121), 786-791.

Suzuki, T., Maranda, B., Sakagami, T., Catellier, P., Couture, C. Y., Carey, B. C., et al. (2011). Hereditary pulmonary alveolar proteinosis caused by recessive CSF2RB mutations. *Eur Respir J*, 37(1), 201-204.

Suzuki, T., Sakagami, T., Rubin, B. K., Nogee, L. M., Wood, R. E., Zimmerman, S. L., et al. (2008). Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. *J Exp Med*, 205(12), 2703-2710.

Svedberg, F. R., Brown, S. L., Krauss, M. Z., Campbell, L., Sharpe, C., Clausen, M., et al. (2019). The lung environment controls alveolar macrophage metabolism and responsiveness in type 2 inflammation. *Nat Immunol*, 20(5), 571-580.

Swann, J. B., & Smyth, M. J. (2007). Immune surveillance of tumors. *J Clin Invest*, 117(5), 1137-1146.

Swirski, F. K., Nahrendorf, M., Etzrodt, M., Wildgruber, M., Cortez-Retamozo, V., Panizzi, P., et al. (2009). Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science*, 325(5940), 612-616.

Szekanecz, Z., Haines, G. K., Lin, T. R., Harlow, L. A., Goerdt, S., Rayan, G., et al. (1994). Differential distribution of intercellular adhesion molecules (ICAM-1, ICAM-2, and ICAM-3) and the MS-1 antigen in normal and diseased human synovia. Their possible pathogenetic and clinical significance in rheumatoid arthritis. *Arthritis Rheum*, 37(2), 221-231.

Szulzewsky, F., Pelz, A., Feng, X., Synowitz, M., Markovic, D., Langmann, T., et al. (2015). Glioma-associated microglia/macrophages display an expression profile different from M1 and M2 polarization and highly express Gpnmb and Spp1. *PLoS One*, 10(2), e0116644.

Tadayon, S., Dunkel, J., Takeda, A., Eichin, D., Virtakoivu, R., Elima, K., et al. (2021). Lymphatic Endothelial Cell Activation and Dendritic Cell Transmigration Is Modified by Genetic Deletion of Clever-1. *Front Immunol*, 12, 602122.

Takahashi, K., & Naito, M. (1993). Development, differentiation, and proliferation of macrophages in the rat yolk sac. *Tissue Cell*, 25(3), 351-362.

Takahashi, K., Yamamura, F., & Naito, M. (1989). Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: a light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. *J Leukoc Biol*, 45(2), 87-96.

Takamiya, R., Ohtsubo, K., Takamatsu, S., Taniguchi, N., & Angata, T. (2013). The interaction between Siglec-15 and tumor-associated sialyl-Tn antigen enhances TGF-β secretion from monocytes/macrophages through the DAP12-Syk pathway. *Glycobiology*, 23(2), 178-187.

Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T., et al. (1999). Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity*, 11(4), 443-451.

Tamoutounour, S., Guilliams, M., Montanana Sanchis, F., Liu, H., Terhorst, D., Malosse, C., et al. (2013). Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity*, 39(5), 925-938.

Tamura, T., Nagamura-Inoue, T., Shmeltzer, Z., Kuwata, T., & Ozato, K. (2000). ICSBP directs bipotential myeloid progenitor cells to differentiate into mature macrophages. *Immunity*, 13(2), 155-165.

Tamura, Y., Adachi, H., Osuga, J., Ohashi, K., Yahagi, N., Sekiya, M., et al. (2003). FEEL-1 and FEEL-2 are endocytic receptors for advanced glycation end products. *J Biol Chem*, 278(15), 12613-12617.

Tang-Huau, T. L., Gueguen, P., Goudot, C., Durand, M., Bohec, M., Baulande, S., et al. (2018). Human in vivo-generated monocyte-derived dendritic cells and macrophages cross-present antigens through a vacuolar pathway. *Nat Commun*, 9(1), 2570.

Tashireva, L. A., Denisov, E. V., Gerashchenko, T. S., Pautova, D. N., Buldakov, M. A., Zavyalova, M. V., et al. (2017). Intratumoral heterogeneity of macrophages and fibroblasts in breast cancer is associated with the morphological diversity of tumor cells and contributes to lymph node metastasis. *Immunobiology*, 222(4), 631-640.

Tau, G. Z., Cowan, S. N., Weisburg, J., Braunstein, N. S., & Rothman, P. B. (2001). Regulation of IFN-gamma signaling is essential for the cytotoxic activity of CD8(+) T cells. *J Immunol*, 167(10), 5574-5582.

Tervahartiala, M., Taimen, P., Mirtti, T., Koskinen, I., Ecke, T., Jalkanen, S., et al. (2017). Immunological tumor status may predict response to neoadjuvant chemotherapy and outcome after radical cystectomy in bladder cancer. *Sci Rep*, 7(1), 12682.

Terpstra, V. & van Berkel, T. J. (2000). Scavenger receptors on liver Kupffer cells mediate the in vivo uptake of oxidatively damaged red blood cells in mice. Blood. 95(6):2157-63.

Thakral, K. K., Goodson, W. H., & Hunt, T. K. (1979). Stimulation of wound blood vessel growth by wound macrophages. *J Surg Res*, 26(4), 430-436.

Thisted, T., Smith, F. D., Mukherjee, A., Kleschenko, Y., Feng, F., Jiang, Z. G., et al. (2024). VISTA checkpoint inhibition by pH-selective antibody SNS-101 with optimized safety and pharmacokinetic profiles enhances PD-1 response. *Nat Commun*, 15(1), 2917.

Timperi, E., Gueguen, P., Molgora, M., Magagna, I., Kieffer, Y., Lopez-Lastra, S., et al. (2022). Lipid-Associated Macrophages Are Induced by Cancer-Associated Fibroblasts and Mediate Immune Suppression in Breast Cancer. *Cancer Res*, 82(18), 3291-3306.

Tolcher, A., Lavon Vandross, A., Moser, J., Gordon, M., Harrop, K., & Lechleider, R. (2022). Abstract: A phase 1/2 dose escalation/expansion study of OR2805 alone or in combination in subjects with advanced solid tumors. *J Clin Oncol*, 40(16_suppl), TPS2693.

Tsai, R. K., & Discher, D. E. (2008). Inhibition of "self" engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. *J Cell Biol*, 180(5), 989-1003.

Tsou, C. L., Peters, W., Si, Y., Slaymaker, S., Aslanian, A. M., Weisberg, S. P., et al. (2007). Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest*, 117(4), 902-909.

Tumeh, P. C., Harview, C. L., Yearley, J. H., Shintaku, I. P., Taylor, E. J., Robert, L., et al. (2014). PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*, 515(7528), 568-571.

Uderhardt, S., Martins, A. J., Tsang, J. S., Lämmermann, T., & Germain, R. N. (2019). Resident Macrophages Cloak Tissue Microlesions to Prevent Neutrophil-Driven Inflammatory Damage. *Cell*, 177(3), 541-555.e517.

Vacchelli, E., Galluzzi, L., Eggermont, A., Fridman, W. H., Galon, J., Sautès-Fridman, C., et al. (2012). Trial watch: FDA-approved Toll-like receptor agonists for cancer therapy. *Oncoimmunology*, 1(6), 894-907.

van de Laar, L., Saelens, W., De Prijck, S., Martens, L., Scott, C. L., Van Isterdael, G., et al. (2016). Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity*, 44(4), 755-768.

van Elsas, M. J., Middelburg, J., Labrie, C., Roelands, J., Schaap, G., Sluijter, M., et al. (2024). Immunotherapy-activated T cells recruit and skew late-stage activated M1-like macrophages that are critical for therapeutic efficacy. *Cancer Cell*, 42(6), 1032-1050.e10.

van Furth, R., Cohn, Z. A., Hirsch, J. G., Humphrey, J. H., Spector, W. G., & Langevoort, H. L. (1972). The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ*, 46(6), 845-852.

Vanden Berghe, T., Vanlangenakker, N., Parthoens, E., Deckers, W., Devos, M., Festjens, N., et al. (2010). Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features. *Cell Death Differ*, 17(6), 922-930.

Varga, T., Mounier, R., Patsalos, A., Gogolák, P., Peloquin, M., Horvath, A., et al. (2016). Macrophage PPARγ, a Lipid Activated Transcription Factor Controls the Growth Factor GDF3 and Skeletal Muscle Regeneration. *Immunity*, 45(5), 1038-1051.

Varol, C., Landsman, L., Fogg, D. K., Greenshtein, L., Gildor, B., Margalit, R., et al. (2007). Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med*, 204(1), 171-180.

Vats, D., Mukundan, L., Odegaard, J. I., Zhang, L., Smith, K. L., Morel, C. R., et al. (2006). Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab*, 4(1), 13-24.

Vermare, A., Guérin, M. V., Peranzoni, E., & Bercovici, N. (2022). Dynamic $CD8^+$ T cell Cooperation with Macrophages and Monocytes for Successful Cancer Immunotherapy. *Cancers (Basel)*, 14(14), 3546.

Vestweber, D. (2015). How leukocytes cross the vascular endothelium. *Nat Rev Immunol*, 15(11), 692-704.

Viitala, M., Virtakoivu, R., Tadayon, S., Rannikko, J., Jalkanen, S., & Hollmén, M. (2019). Immunotherapeutic Blockade of Macrophage Clever-1 Reactivates the CD8+ T-cell Response against Immunosuppressive Tumors. *Clin Cancer Res*, 25(11), 3289-3303.

Voabil, P., de Bruijn, M., Roelofsen, L. M., Hendriks, S. H., Brokamp, S., van den Braber, M., et al. (2021). An ex vivo tumor fragment platform to dissect response to PD-1 blockade in cancer. *Nat Med*, 27(7), 1250-1261.

Von Hoff, D. D. (1998). There are no bad anticancer agents, only bad clinical trial designs--twenty-first Richard and Hinda Rosenthal Foundation Award Lecture. *Clin Cancer Res*, 4(5), 1079-1086.

Walsh, L. J., Goerdt, S., Pober, J. S., Sueki, H., & Murphy, G. F. (1991). MS-1 sinusoidal endothelial antigen is expressed by factor XIIIa+, HLA-DR+ dermal perivascular dendritic cells. *Lab Invest*, 65(6), 732-741.

Wang, B., Huang, H., Yang, M., Yang, W., Liu, Z., Hou, W., et al. (2019). Microlocalization and clinical significance of stabilin-1. *World J Urol*, 38(3), 709-716.

Wang, C., Chen, T., Zhang, J., Yang, M., Li, N., Xu, X., et al. (2009). The E3 ubiquitin ligase Nrdp1 'preferentially' promotes TLR-mediated production of type I interferon. *Nat Immunol*, 10(7), 744-752.

Wang, X., Sathe, A. A., Smith, G. R., Ruf-Zamojski, F., Nair, V., Lavine, K. J., et al. (2020). Heterogeneous origins and functions of mouse skeletal muscle-resident macrophages. *Proc Natl Acad Sci U S A*, 117(34), 20729-20740.

Wang, Y., Chen, T., Han, C., He, D., Liu, H., An, H., et al. (2007). Lysosome-associated small Rab GTPase Rab7b negatively regulates TLR4 signaling in macrophages by promoting lysosomal degradation of TLR4. *Blood*, 110(3), 962-971.

Warheit, D. B., & Hartsky, M. A. (1993). Role of alveolar macrophage chemotaxis and phagocytosis in pulmonary clearance responses to inhaled particles: comparisons among rodent species. *Microsc Res Tech*, 26(5), 412-422.

Wei, S., Nandi, S., Chitu, V., Yeung, Y. G., Yu, W., Huang, M., et al. (2010). Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. *J Leukoc Biol*, 88(3), 495-505.

Weinstock, C., Khozin, S., Suzman, D., Zhang, L., Tang, S., Wahby, S., et al. (2017). U.S. Food and Drug Administration Approval Summary: Atezolizumab for Metastatic Non-Small Cell Lung Cancer. *Clin Cancer Res*, 23(16), 4534-4539.

West, A. P., Brodsky, I. E., Rahner, C., Woo, D. K., Erdjument-Bromage, H., Tempst, P., et al. (2011). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature*, 472(7344), 476-480.

Willenborg, S., Lucas, T., van Loo, G., Knipper, J. A., Krieg, T., Haase, I., et al. (2012). CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair. *Blood*, 120(3), 613-625.

Wolchok, J. D., Chiarion-Sileni, V., Rutkowski, P., Cowey, C. L., Schadendorf, D., Wagstaff, J., et al. (2025). Final, 10-Year Outcomes with Nivolumab plus Ipilimumab in Advanced Melanoma. *N Engl J Med*, 392(1), 11-22.

Wu, J., Sun, L., Chen, X., Du, F., Shi, H., Chen, C., et al. (2013). Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science*, 339(6121), 826-830.

Wu, K., Kryczek, I., Chen, L., Zou, W., & Welling, T. H. (2009). Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res*, 69(20), 8067-8075.

Wu, P., Wu, D., Zhao, L., Huang, L., Chen, G., Shen, G., et al. (2016). Inverse role of distinct subsets and distribution of macrophage in lung cancer prognosis: a meta-analysis. *Oncotarget*, 7(26), 40451-40460.

Wu, S. Z., Al-Eryani, G., Roden, D. L., Junankar, S., Harvey, K., Andersson, A., et al. (2021). A single-cell and spatially resolved atlas of human breast cancers. *Nat Genet*, 53(9), 1334-1347.

Wu, W. C., Sun, H. W., Chen, H. T., Liang, J., Yu, X. J., Wu, C., et al. (2014). Circulating hematopoietic stem and progenitor cells are myeloid-biased in cancer patients. *Proc Natl Acad Sci U S A*, 111(11), 4221-4226.

Wyckoff, J., Wang, W., Lin, E. Y., Wang, Y., Pixley, F., Stanley, E. R., et al. (2004). A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res*, 64(19), 7022-7029.

Wyckoff, J. B., Wang, Y., Lin, E. Y., Li, J. F., Goswami, S., Stanley, E. R., et al. (2007). Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. *Cancer Res*, 67(6), 2649-2656.

Xiong, D., Wang, Y., & You, M. (2020). A gene expression signature of $TREM2^{hi}$ macrophages and γδ T cells predicts immunotherapy response. *Nat Commun*, 11(1), 5084.

Yan, D., Kowal, J., Akkari, L., Schuhmacher, A. J., Huse, J. T., West, B. L., et al. (2017). Inhibition of colony stimulating factor-1 receptor abrogates microenvironment-mediated therapeutic resistance in gliomas. *Oncogene*, 36(43), 6049-6058.

Yao, J., Mackman, N., Edgington, T. S., & Fan, S. T. (1997). Lipopolysaccharide induction of the tumor necrosis factor-alpha promoter in human monocytic cells. Regulation by Egr-1, c-Jun, and NF-kappaB transcription factors. *J Biol Chem*, 272(28), 17795-17801.

Yao, Y., Jeyanathan, M., Haddadi, S., Barra, N. G., Vaseghi-Shanjani, M., Damjanovic, D., et al. (2018). Induction of Autonomous Memory Alveolar Macrophages Requires T Cell Help and Is Critical to Trained Immunity. *Cell*, 175(6), 1634-1650.e1617.

Ye, S., Cohen, D., Belmar, N. A., Choi, D., Tan, S. S., Sho, M., et al. (2019). A Bispecific Molecule Targeting CD40 and Tumor Antigen Mesothelin Enhances Tumor-Specific Immunity. *Cancer Immunol Res*, 7(11), 1864-1875.

Yeku, O. O., Barve, M., Tan, W. W., Wang, J., Patnaik, A., LoRusso, P., et al. (2025). Myeloid targeting antibodies PY159 and PY314 for platinum-resistant ovarian cancer. *J Immunother Cancer*, 13(3), e010959.

Yona, S., Kim, K. W., Wolf, Y., Mildner, A., Varol, D., Breker, M., et al. (2013). Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity*, 38(1), 79-91.

Yu, J., Green, M. D., Li, S., Sun, Y., Journey, S. N., Choi, J. E., et al. (2021). Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. *Nat Med*, 27(1), 152-164.

Yu, K., Cao, Y., Zhang, Z., Wang, L., Gu, Y., Xu, T., et al. (2025). Blockade of CLEVER-1 restrains immune evasion and enhances anti-PD-1 immunotherapy in gastric cancer. *J Immunother Cancer*, 13(5).

Yáñez, A., Coetzee, S. G., Olsson, A., Muench, D. E., Berman, B. P., Hazelett, D. J., et al. (2017). Granulocyte-Monocyte Progenitors and Monocyte-Dendritic Cell Progenitors Independently Produce Functionally Distinct Monocytes. *Immunity*, 47(5), 890-902.e894.

Zahalka, S., Starkl, P., Watzenboeck, M. L., Farhat, A., Radhouani, M., Deckert, F., et al. (2022). Trained immunity of alveolar macrophages requires metabolic rewiring and type 1 interferon signaling. *Mucosal Immunol*, 15(5), 896-907.

Zanoni, I., Ostuni, R., Marek, L. R., Barresi, S., Barbalat, R., Barton, G. M., et al. (2011). CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. *Cell*, 147(4), 868-880.

Zeitler, L., & Murray, P. J. (2023). IL4i1 and IDO1: Oxidases that control a tryptophan metabolic nexus in cancer. *J Biol Chem*, 299(6), 104827.

Zeng, Z., Surewaard, B. G. J., Wong, C. H. Y., Geoghegan, J. A., Jenne, C. N. & Kubes, P. (2016) CRIg functions as a macrophage pattern recognition receptor to directly bind and capture blood-borne gram-positive bacteria. *Cell Host Microbe*, 20(1):99-106.

Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., et al. (2019). Metabolic regulation of gene expression by histone lactylation. *Nature*, 574(7779), 575-580.

Zhang, J., Gratchev, A., Riabov, V., Mamidi, S., Schmuttermaier, C., Krusell, L., et al. (2009). A novel GGA-binding site is required for intracellular sorting mediated by stabilin-1. *Mol Cell Biol*, 29(22), 6097-6105.

Zhang, Q. W., Liu, L., Gong, C. Y., Shi, H. S., Zeng, Y. H., Wang, X. Z., et al. (2012). Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One*, 7(12), e50946.

Zhang, S., Weinberg, S., DeBerge, M., Gainullina, A., Schipma, M., Kinchen, J. M., et al. (2019). Efferocytosis Fuels Requirements of Fatty Acid Oxidation and the Electron Transport Chain to Polarize Macrophages for Tissue Repair. *Cell Metab*, 29(2), 443-456.e445.

Zhong, W., Rao, Z., Xu, J., Sun, Y., Hu, H., Wang, P., et al. (2022). Defective mitophagy in aged macrophages promotes mitochondrial DNA cytosolic leakage to activate STING signaling during liver sterile inflammation. *Aging Cell*, 21(6), e13622.

Zhou, X., Franklin, R. A., Adler, M., Carter, T. S., Condiff, E., Adams, T. S., et al. (2022). Microenvironmental sensing by fibroblasts controls macrophage population size. *Proc Natl Acad Sci U S A*, 119(32), e2205360119.

Zhu, Y., Herndon, J. M., Sojka, D. K., Kim, K. W., Knolhoff, B. L., Zuo, C., et al. (2017). Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression. *Immunity*, 47(2), 323-338.e326.

Ziegler-Heitbrock, L., Ancuta, P., Crowe, S., Dalod, M., Grau, V., Hart, D. N., et al. (2010). Nomenclature of monocytes and dendritic cells in blood. *Blood*, 116(16), e74-80.

Zigmond, E., Samia-Grinberg, S., Pasmanik-Chor, M., Brazowski, E., Shibolet, O., Halpern, Z., et al. (2014). Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. *J Immunol*, 193(1), 344-353.

Ziogas, A., Novakovic, B., Ventriglia, L., Galang, N., Tran, K. A., Li, W., et al. (2025). Long-term histone lactylation connects metabolic and epigenetic rewiring in innate immune memory. *Cell*, 188(11), 2992-3012.e2916.

Zordan, P., Rigamonti, E., Freudenberg, K., Conti, V., Azzoni, E., Rovere-Querini, P., et al. (2014). Macrophages commit postnatal endothelium-derived progenitors to angiogenesis and restrict endothelial to mesenchymal transition during muscle regeneration. *Cell Death Dis*, 5(1), e1031.

Ålgars, A., Irjala, H., Vaittinen, S., Huhtinen, H., Sundström, J., Salmi, M., et al. (2012). Type and location of tumor-infiltrating macrophages and lymphatic vessels predict survival of colorectal cancer patients. *Int J Cancer*, 131(4), 864-873.

Ålgars, A., Kemppinen, L., Fair-Mäkelä, R., Mustonen, H., Haglund, C., & Jalkanen, S. (2021). Stage I-IV Colorectal Cancer Prognosis Can Be Predicted by Type and Number of Intratumoral Macrophages and CLEVER-1. *Cancers (Basel)*, 13(23).

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