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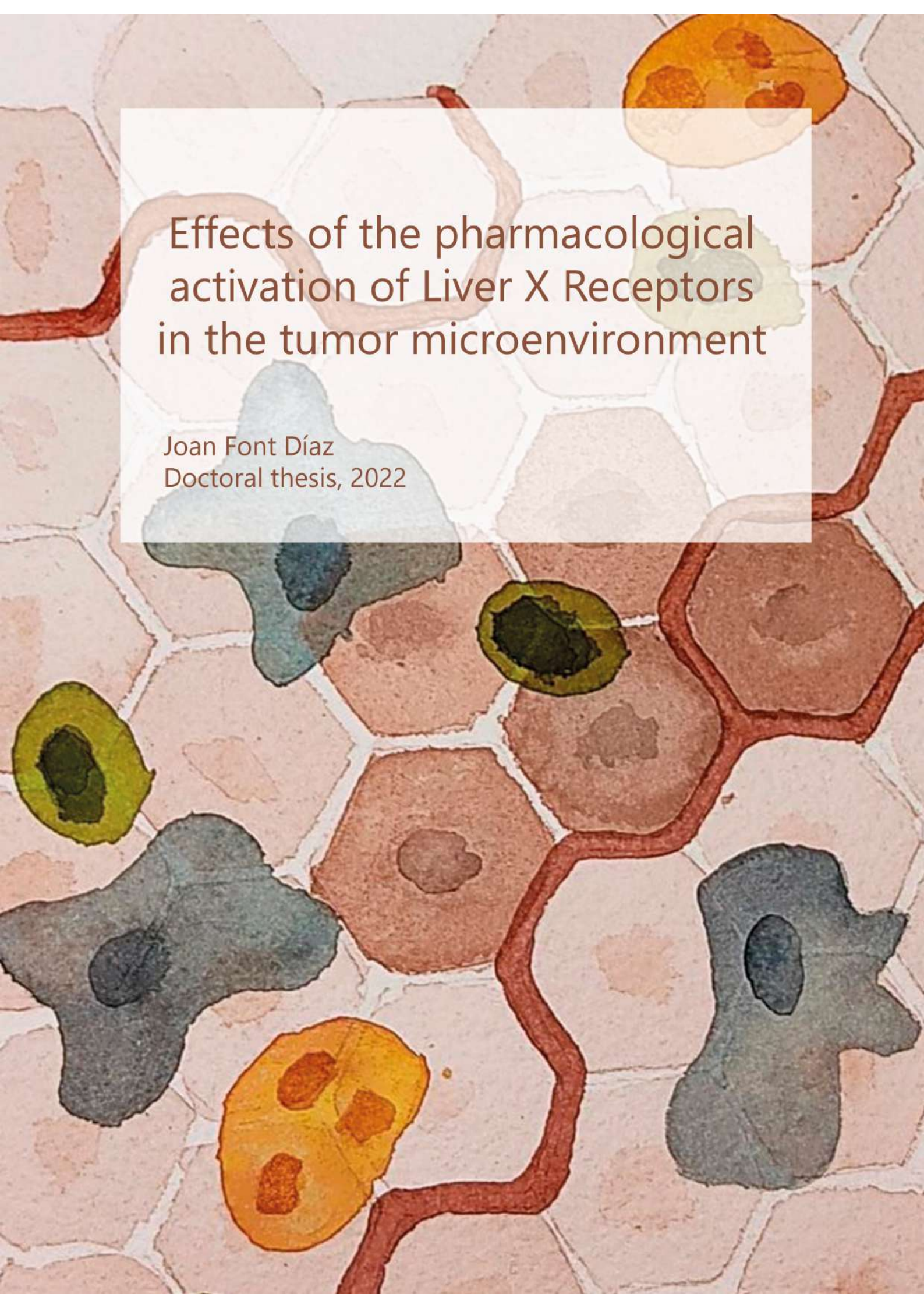

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Abstract

Nuclear receptors are a superfamily of ligand-dependent transcription factors that are involved in numerous biological processes in homeostasis and disease. Liver X receptors (LXRs) are members of the nuclear receptors family that are regulated by oxidized forms of cholesterol (oxysterols) and other byproducts of cholesterol metabolism and biosynthesis. In addition, there are synthetic agonists, such as T0901317 (T1317), which have higher affinity and stability. LXRs are key factors in the regulation of lipid homeostasis and in the modulation of inflammation. There are two LXRs isoforms, LXRα (NR1H3) and LXRβ (NR1H2), encoded by two different genes and with differential tissue distribution. In order to bind to the DNA and regulate the expression of target genes, LXRs form heterodimers with the retinoid X receptors (RXRs), another member of the nuclear receptors family. LXRs can also repress the expression of genes, for example interacting with co-repressor complexes through transrepression.

In the last decade, there has been a growing interest in LXRs as therapeutic targets against cancer. Activation of LXRs with high doses of synthetic agonists exerts direct antiproliferative, cytostatic and pro-apoptotic effects *in vitro* in many cancer cell lines. In addition, pharmacological activation of LXRs can inhibit tumor progression in pre-clinical models in mice. Interestingly, in our own previous studies using a syngeneic model of Lewis lung carcinoma, activation of the LXR pathway with T1317 suppressed tumor growth in wild type but not in LXR-deficient mice, underlining the importance of functional expression of LXRs in the host for the antitumoral effects of the agonist. In addition, the expression of the chemokines CCL17 and CCL22, key attractants for regulatory T (Treg) cells, and the transcription factor IRF4 was inhibited in T1317-treated TAMs *in vivo* and *ex vivo*.

Based on these observations, we further explored the actions of the LXR agonist in the tumor microenvironment. Tumor-associated macrophages (TAMs) are the most abundant immune cells in the tumor microenvironment, and they strongly contribute to the establishment of an immunosuppressive environment. We studied two TAM subpopulations with distinct phenotypic characteristics and intra-tumoral localization, named MHCII^{high} TAMs and MHCII^{low} TAMs after their differential expression of MHCII. Collectively, treatment with T1317 impacted the transcriptional profile of TAMs at multiple levels, suppressing several mechanisms used by these cells for the maintenance of the immunosuppressed environment. Among these effects, activation of LXRs caused a decrease in the abundance of Tregs in the tumor, without affecting their immunosuppressive or proliferative capabilities, nor their peripheral abundance. Concomitantly with the inhibition of CCL17 expression in TAMs, the results suggested that LXR activation reduced Treg intratumoral abundance through blocking their recruitment. In this sense, a functional systemic expression of IRF4 was found necessary for the T1317-mediated inhibition of tumor growth.

In addition, the inhibitory effect of LXR activation on CCL17, CCL22 and IRF4 expression was also observed in human macrophages derived from peripheral mononuclear cells

from healthy donors, suggesting that the crosstalk between LXRs and the IRF4-CCL17/CCL22 axis is evolutionary conserved and may be also relevant in humans.

Overall, these results shed new light on the mechanisms of LXR agonists as antitumoral drugs targeting the tumor microenvironment.

CONTENT

ABBREVIATIONS

- ABC – ATP binding cassette
- ADP – Adenosine diphosphate
- ADPR – Adenosine diphosphate ribose
- AIM – Apoptotic inhibitory factor secreted by macrophages
- APC – Antigen presenting cell
- ApoE – Apolipoprotein E
- Arg1 – Arginase 1
- ATP – Adenosine triphosphate
- BCR – B cell receptor
- BMDM - Bone marrow-derived macrophages
- cADPR – Cyclic adenosine diphosphate ribose
- CCL – Chemokine (C-C motif) ligand
- CCR – Chemokine (C-C motif) receptor
- CD – Cluster differentiation
- CDK – Cyclin-dependent kinases
- COX – Cyclooxygenases
- CSF-1 – Colony-stimulating factor 1, also known as macrophage colony-stimulating factor (M-CSF)
- CSF-1R – Colony-stimulating factor 1 receptor, also known as macrophage colony-stimulating factor receptor (M-CSFR)
- CTLA-4 – Cytotoxic T-lymphocyte-associated protein 4
- CXCL – Chemokine (C-X-C motif) Ligand
- CXCR – Chemokine (C-X-C motif) Receptor
- DBD – DNA binding domain
- DC – Dendritic cell
- DDA – Dendrogenin A
- DMEM – Dulbecco's modified eagle medium
- DMSO – Dimethyl sulfoxide
- ELISA – Enzyme-linked immunosorbent assay
- FASN – Fatty acid synthase
- FBS – Fetal bovine serum
- Fc – Fragment crystallizable
- FoxP3 – Forkhead box P3
- G-CSF – Granulocyte-colony stimulating factor
- G-MDSC – Granulocytic myeloid-derived suppressor cell
- GM-CSF – Granulocyte macrophage-colony stimulating factor
- GM-CSFR – Granulocyte macrophage-colony stimulating factor receptor
- HBSS – Hank’s balanced salt solution
- HC – Hydroxycholesterol

- HDL – High density lipoprotein
- HRP – Horseradish peroxidase
- HSC – Hematopoietic stem cell
- IDO1/IDO2 – Indoleamine-2,3-dioxygenase 1-2
- IDOL – Inducible degrader of LDL receptor
- IFN – Interferon
- IFNR – Interferon receptor
- IL – Interleukin
- IL-R – Interleukin receptor
- iNOS – Inducible nitric oxide synthase
- IRF – Interferon regulatory factor
- JAK – Janus kinase
- LBD – Ligand binding domain
- LCOR – Ligand-dependent nuclear receptor co-repressor
- LDL – Low density lipoprotein
- LDLR – Low density lipoprotein receptor
- LPL – Lipoprotein lipase
- LPS – Lipopolysaccharide
- LRP – LDL receptor related protein
- LT – Lymphotoxin
- LXR – Liver X receptor
- LXRE – LXR response element
- M-MDSC – Monocytic-myeloid-derived suppressor cells
- MDSC – Myeloid-derived suppressor cells
- MerTK – Mer tyrosine kinase
- MAPK – Mitogen-activated protein kinases
- Mgl2 – Macrophage galactose n-acetyl-galactosamine-specific lectin 2
- MHC – Major histocompatibility complex
- MMP – Metalloproteinase
- MoDCs – Monocyte-derived dendritic cells
- MyD – Myeloid differentiation primary response
- NAADP – Nicotinic acid adenine dinucleotide phosphate
- NAD – Nicotinamide adenine dinucleotide
- NADP – Nicotinamide adenine dinucleotide phosphate
- NCoR – Nuclear receptor co-repressor
- NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells
- NK – Natural killer
- NLRP3 – NOD-like receptor family pyrin domain-containing 3
- Nos2 – Nitric oxide synthase 2 (see iNOS)
- NR – Nuclear receptor
- PAMPs – Pathogen-associated molecular patterns
- PBS – Phosphate buffer saline
- PD-1 – Programmed cell death protein 1

- PD-L1/2 – Programmed cell death-ligand ½
- PI3K – Phosphatidylinositol 3-kinase
- PRR – Pattern recognition receptors
- *Ptgs* – Prostaglandin-endoperoxide synthase
- PUFA – Polyunsaturated fatty acid
- Retnla – Resistin-like alpha
- RNS – Reactive nitrogen species
- RORγT – Retinoic-acid-receptor-related orphan receptor γT
- ROS – Reactive oxygen species
- RPMI – Roswell Park memorial institute
- RXR – Retinoid X receptor
- SCD – Stearoyl-CoA desaturase
- SLAP/SLAP2 – Src-like adaptor proteins
- SOCS – Suppressor of cytokine signaling
- *Srebf1* – See SREBP1c
- SREBP1c – Sterol regulatory element binding protein 1c
- STAT – Signal transcription and activator of transcription
- TADC – Tumor-associated dendritic cell
- TAM – Tumor-associated macrophage
- TGFβ – Transforming growth factor β
- Th – Helper T cell
- TLR – Toll-like receptor
- TME – Tumor microenvironment
- TNFα – Tumor necrosis factor α
- Treg – T regulatory cell
- TTP – Tristetraprolin
- VEGF – Vascular endothelial growth factor
- VEGFR – Vascular endothelial growth factor receptor
- WT – Wild type

INTRODUCTION

1. The immune system: an overview

The immune system is a complex network of organs, tissues, cells, and molecules that work in an organized and synchronized manner to survey and protect the organism from both external and internal threats. Roughly, it can be divided into several levels of defense: physical and chemical barriers, and innate and adaptive immune systems.

The innate immune system is composed of molecules and cells that recognize and attack pathogens very shortly after the pathogen invades the organism. The cells that belong to the innate immune system are primarily the myeloid cells, including macrophages, neutrophils, and dendritic cells (DCs), and the innate lymphoid cells, like the natural killer (NK) cells. All these cells have an arsenal of pattern recognition receptors (PRR) that can bind and recognize a variety of pathogen-associated molecular patterns (PAMPs) (Punt *et al*., 2019). PAMP recognition by PRR activates local phagocytes, like macrophages that rapidly engulf and digest pathogens by phagocytosis. It can also lead to the production of molecules that have direct antimicrobial capabilities, or that causes the physiological changes that are known as inflammation (Punt *et al*., 2019).

The innate immune cells have the crucial role of activating the third level of defense: the adaptive immune system composed by T and B lymphocytes (Punt *et al*., 2019). The activation of the adaptive immune response is initiated usually by DCs. Immature DCs are specialized phagocytic cells that migrate from the bone marrow to reside and survey the peripheral tissues in search of pathogens. In the onset of an infection, they are attracted to the inflammation site. Upon phagocytosis and activation antigen-bearing DCs migrate to the lymph nodes, where they present antigens coupled to major histocompatibility complexes (MHC) to T lymphocytes (Punt *et al*., 2019).

T cells are key elements for the adaptive immune response. In the thymus, pre-T cells acquire a unique T cell receptor (TCR) that recognizes a specific antigen. After thymic selection, two types of T lymphocytes are present in the circulation, cluster differentiation (CD)4 positive (mainly helper T (Th) cells) or CD8 positive (cytotoxic T cells). Naïve T cells migrate through the bloodstream to peripheral lymphoid organs where they will be activated upon recognition of specific peptides presented by antigen

presenting cells (APC) (Punt *et al*., 2019). Activated T cells undergo clonal expansion. The resulting daughter cells, with the same antigen specificity, will differentiate into effector T cells and will migrate to the infection site. Effector cytotoxic T cells will destroy infected cells whereas Th will activate other immune cells and coordinate the immune response. Some effector T cells stay at peripheral lymphoid organs where they will interact with B cells (Punt *et al*., 2019).

B cells are the other key cellular component in adaptive immune responses. They originate in the bone marrow. Each mature B cell has a different antigen-specific receptor called B cell receptor (BCR). The secreted forms of the BCR are known as antibodies. Once bound to antigens, antibodies directly neutralize the pathogen or label it (opsonization) for a more efficient elimination by the innate immune system (Punt *et al*., 2019). B cells are activated in the secondary lymphoid organs upon recognition of specific antigens. Interaction with effector Th cells is also essential for B cell activation. Through this interaction B cells present antigens to Th cells, which then produce signals required for B cell activation. Upon the T cell-dependent activation of B cells, they undergo clonal expansion within specialized structures called germinal centers. Some activated B cells will then differentiate plasma cells that will produce antibodies. In addition, memory B cells will survey the body to generate a secondary response if the specific pathogen causes an infection again (Punt *et al*., 2019).

Aside from the crucial contribution in activating the adaptive immune response, innate immunity also synergizes with effector T cell and B cell responses in the elimination of pathogens. For example, effector T cells can activate macrophages through paracrine or endocrine cytokine production. Activated macrophages can phagocytose and eliminate pathogens more efficiently through the recognition of opsonizing antibodies (Punt *et al*., 2019).

A full-scale immune response is a powerful reaction that damages healthy cells and tissues in the process of eliminating the threat. Thus, the immune response must be regulated to prevent excessive damage to the body. Once the pathogen is eliminated, most of the activated immune cells die through apoptosis and are phagocytosed. Memory cells will survive and survey the organism prepared to rapidly activate and fight if the pathogen manages to infect again (Punt *et al*., 2019). In addition, regulatory cells,

like regulatory T cells (Treg) can control the response through the secretion of regulatory and inhibitory cytokines (Punt *et al.*, 2019). Besides limiting an excessive immune response, Treg are also responsible for establishing tolerance to autoantigens and to the antigens of the microbiota, induce tolerance to food antigens and regulate the immunity of the fetus and placenta. Loss of Treg homeostasis can result in effector T cell responses against self-antigens and lead to an autoimmune disorder (Shevyrev & Tereshchenko, 2020).

1.1 The immune system in the tumor microenvironment

A tumor is a heterogeneous entity that is not only composed by tumoral cells, but rather a collection of host and infiltrating cells, extracellular matrix, blood vessels, and secreted factors. This complex network is known as the tumor microenvironment (TME). Immune cells are a very important compartment in the TME, and while initially the immune system reacts against the tumor to defend the host, tumor cells can exploit inflammation and immune cells to support their growth and progression (reviewed in Upadhyay *et al.*, 2018).

Innate and adaptive immune responses crosstalk and display immune surveillance for tumoral cells. At initial stages, innate immune cells may infiltrate the tumor and display antitumoral actions, such as direct killing of tumor cells, destruction of tumor-associated blood vessels or extracellular matrix and inhibition of angiogenesis (reviewed in Upadhyay *et al.*, 2018). Tumor-derived DNA has been found in the cytosol of infiltrated DCs. Through the activation of the stimulator of interferon genes (STING) pathway, DCs sense tumoral DNA, produce type I interferons (IFN), and prime T cells (reviewed in Woo *et al.*, 2015). NK cells kill tumoral cells by releasing granzymes and perforins, and secrete cytokines, such as IFNγ, which inhibits tumor cell proliferation, stimulates the production of anti-angiogenic factors, and promotes the recruitment of DCs and effector T cells (Langers *et al.*, 2012). Pro-inflammatory macrophages secrete a wide range of cytotoxic factors, such as reactive oxygen species (ROS), nitric oxide, reactive nitrogen species (RNS) and pro-inflammatory cytokines (Pan *et al.*, 2020). In addition, they stimulate Th1 responses and, along with NK cells, eliminate tumoral cells by antibody-

dependent cell mediated cytotoxicity (Bruns *et al*., 2015). Tumoral antigens are processed by APCs and presented to the adaptive immune system. Cytotoxic T cells may detect the abnormal tumor antigens and eliminate tumor cells. In addition, they also produce IFNγ. Th1 cells support cytotoxic T cells through the secretion of interleukin (IL)-2 and IFNγ (reviewed in Anderson & Simon, 2020).

Despite the capacity of the immune system to detect and eliminate tumors, the high mutation rate and genome plasticity of tumoral cells allows them to proliferate uncontrollably until an equilibrium phase with the host is established, enabling the tumor to eventually escape from the immune system. Over time, tumoral cells evade the immune system by either inducing immune tolerance to tumoral antigens or through suppression of the immune cells. In this sense, tumor cells, as well as stromal cells, modulate the activity of infiltrating immune cells through the secretion of cytokines and other factors. This results in the reprogramming of the infiltrated immune cells, which will afterwards play a determinant role in tumor progression, promoting angiogenesis, invasion, metastasis, and immune suppression of the antitumoral response (reviewed in Upadhyay *et al*., 2018).

Among the different mechanisms promoting immunosuppression, a key element is the expression of the immunomodulatory receptor programed death-ligand (PD-L)1 in tumor and stromal cells which interacts with programmed cell death protein (PD)-1 on T cells and inhibits effector T cell responses through the induction of T cell anergy, exhaustion and apoptosis (Iwai *et al*., 2002).

Broadly, the cells that most contribute in the TME to tumor promotion are tumor-associated macrophages (TAMs) (which will be further discussed in the sections “Macrophages in the tumor microenvironment” and “Pro-tumoral effects of tumor-associated macrophages”), regulatory DCs, Tregs and myeloid-derived suppressor cells (MDSCs).

DCs have inherently antitumorigenic functions in the body, but factors secreted in the TME can reprogram DCs to support tumor progression (Fu & Jiang, 2018). Such regulatory DCs either present antigens inefficiently, which disarms T cell antitumoral activity (Stoitzner *et al*., 2008; Engelhardt *et al*., 2012), or dampen pro-inflammatory

responses in the tumor by stimulating Th2 cells, which enhance tumor progression by secreting IL-4 and IL-13 (Wu *et al*., 2014 A). In addition, regulatory DCs express the enzyme indoleamine-2,3-dioxygenases (IDO), which participates in tryptophan metabolism and limits T cell function, and PD-L1, which contributes to their immunosuppressive actions. Regulatory DCs also display reduced secretion of type I IFN (Harden *et al*., 2012).

Tregs also display a potent immunosuppressive activity in the TME. Tregs can be recruited to the tumor through several chemokines, such as C-C motif chemokine ligand (CCL)5, CCL17 and CCL22, secreted in the TME (Curiel *et al*., 2004; Schlecker *et al*., 2012). They may also be differentiated or reprogrammed from other infiltrating T cell populations. In Tregs, PD-1/PD-L1 or PD-L2 interactions promote their development and maintenance through the stabilization of forkhead box P3 (FoxP3) expression (Francisco *et al*., 2009). It has been observed that PD-L1 synergizes with transforming growth factor (TGF)β to promote differentiation of naïve T cells into Tregs (Francisco *et al*., 2009). In addition, TGFβ, IL-2 and prostaglandin E2 (PGE2) secreted in the TME are able to reprogram Th17 cells to acquire a Treg phenotype. These reprogrammed T cells have an overlapping phenotype with Th17 and Tregs and express CD25 and C-C chemokine receptor (CCR)4 (Downs-Canner *et al*., 2017).

Tregs exert their immunomodulatory functions in the TME through several mechanisms, both contact-dependent and by secretion of immunomodulatory mediators. Tregs constitutively express the checkpoint molecule cytotoxic T-lymphocyte-associated protein (CTLA)-4, which competes with the costimulatory molecule CD28 for the binding with CD80/86 on APCs. Thus, CTLA-4 competitive binding blocks CD28-mediated costimulatory signals and inhibits effector T cell proliferation, cytokine production and survival pathways (Curran *et al*., 2010; Grosso & Jure-Kunkel, 2013). Activated Tregs also upregulate lymphocyte activation gene-3, an immunomodulatory protein analog for CD4. Although its specific mechanism of immunosuppression is still not clear, it is likely displayed through the interaction with MHC II, thus impairing DC maturation and inducing anergy in tumor infiltrating T cells (Liang *et al*., 2008). Studies also report that Treg-derived perforin and granzyme induce immunosuppression by targeting NK and

cytotoxic T cells, incapacitating them to eliminate tumor cells (Zhao *et al*., 2006; Cullen *et al*., 2010).

Tregs also secrete anti-inflammatory mediators in the TME. IL-10 inhibits IFNγ-mediated APC activation (thus preventing T cell activation) and stabilizes the suppressive phenotype of recently activated Treg (Dennis *et al*., 2013; Mittal *et al*., 2015). TGFβ downregulates the expression of IL-2 in the TME and induces cell cycle arrest and inhibits T cell proliferation (Wrzesinski *et al*., 2007). IL-35 also inhibits T cell proliferation through G1-S cell cycle arrest (Pylayeva-Gupta, 2016).

Tregs express CD25, the high affinity receptor for IL-2. Expression of CD25 contributes to immunosuppression by out-competing infiltrating T cells for IL-2, thus depriving surrounding T cells from a key survival and expansion signal and resulting in the induction of T cell apoptosis (Pandiyan *et al*., 2007). Finally, Tregs produce adenosine, whose accumulation in the TME causes immunosuppression through the inhibition of pro-inflammatory pathways (Romio *et al*., 2011; Allard *et al*., 2016).

Another important cell type that promotes immunosuppression in the TME is the MDSC. In the TME, MDSCs can be subdivided in two subsets: monocytic MDSCs (M-MDSCs), with a $CD11b^{high}$, $LY6C^{high}$, and $LY6G^{low}$ phenotype, and granulocytic MDSCs (G-MDSCs), characterized by $CD11b^{high}$, $LY6C^{low}$, and $LY6G^{high}$ expression (Bronte *et al*., 2016). Due to the enhanced expression of immunosuppressive molecules, both subtypes present in the TME have an increased suppressive phenotype compared to MDSCs found in peripheral lymphoid organs (Kumar *et al*., 2016). MDSCs in peripheral lymphoid organs use several major pathways for immunosuppression. They produce ROS, nitric oxide and peroxynitrite, which can nitrate chemokines and block the access of cytotoxic T cells to the tumor (Nagaraj *et al*., 2007; Molon *et al*., 2011; Raber *at al*., 2014). They also express enzymes that catabolize and reduce the availability of amino acids that are essential for T cell function and proliferation. For example, the expression of arginase (Arg)1 reduces the availability of L-arginine (Raber *et al*., 2012), and the expression of IDO reduce the availability of L-tryptophan (Yu *et al.*, 2013). Finally, MDSCs also reduce the availability of L-cysteine though the expression of the xCT cysteine/glutamate transporter, which uptakes cysteine, and the lack of expression of the alanine-serine-cysteine transporter Asc-1, which exports cysteine, resulting in the consumption of L-cysteine from the

surrounding area (Srivastava *et al*., 2010). MDSCs also produce Il-10 and TGFβ (Kumar *et al*., 2016 B), activate Tregs (Pan *et al*., 2010), and affect NK function (Mao *et al*., 2014). The inflammatory and hypoxic conditions of the TME, causes alterations in the MDSC phenotype resulting in a more potent non-specific immunosuppressive activity (reviewed in Kumar *et al*., 2016 B). Intratumoral MDSCs increase their expression of Arg1 and inducible nitric oxide synthase (iNOS) and downregulate ROS production (Corzo *et al*., 2010; Schlecker *et al*., 2012), upregulate their expression of PD-L1 (Noman *et al*., 2014) and increase their secretion of CCL4 and CCL5, thus enhancing Treg recruitment to the TME (Schlecker *et al*., 2012). Lastly, MDSCs in the TME also enhance angiogenesis and promote epithelial-mesenchymal transition through IL-6 secretion (Condamine *et al*., 2015; Condamine *et al*., 2016). In addition, they can differentiate into TAMs contributing to the maintenance of the TAM intratumoral pool (Corzo *et al*., 2010; Strauss *et al*., 2015).

2. The macrophage: biology, polarization, and functions

Macrophages are mononuclear cells from the innate immune system. They have great importance, both in host defense and in the maintenance of homeostasis in several tissues. First, they phagocytose and eliminate pathogens, secrete a wide range of cytokines and other mediators that trigger the inflammatory response, and present antigens to T lymphocytes, thus stimulating the adaptive immune response. Second, they contribute to the negative regulation and resolution of the immune response by eliminating dead cells and promoting tissue repair. Third, they have critical roles in the developmental process of many organs and tissues, like mammary glands, bones, and brain. Therefore, macrophages are remarkably versatile cells, capable of responding to the tissue microenvironment and polarizing and switching their phenotype to adapt their functions. Their phenotypic plasticity is a hallmark of macrophages, which allows them to display completely different functions depending on the disease state or developmental stage.

In mice, tissue resident macrophages from many compartments start to colonize the tissue during early embryonic stages (Epelman *et al*., 2014). For example, microglia, specialized macrophages that reside in the central nervous system, originate exclusively from the yolk sack. Alveolar macrophages, in contrast, start to colonize the lungs from the fetal liver myelopoiesis (Epelman *et al*., 2014). If resident macrophages are not eliminated because of an inflammatory event, their maintenance in the adult mice is achieved through self-renewal and does not depend on the influx from macrophages derived from blood monocytes (Locati *et al*., 2020). In homeostatic conditions, macrophages originating from adult bone marrow precursors also contribute to the tissue resident populations, completely in some tissues, like the intestines, and partially in others, like the dermis, heart, and kidney. In humans, there is also evidence that supports the dual origin of tissue macrophages, either blood monocyte-derived or local, self-maintained from fetal origin (Bassler *et al*., 2019).

In general, evidence suggests that self-renewing tissue resident macrophages have a major role in the maintenance of tissue homeostasis whereas monocyte-derived macrophages respond to pathological stimuli (Locati *et al*., 2020). In pathological conditions, macrophages derived from blood monocytes infiltrate the tissue and promote inflammation. Is still a matter of debate for how long these inflammatory cells stay in the tissue after the resolution of inflammation and if they adopt or not the phenotype of tissue-resident macrophages.

2.1 Macrophage classification

The classification of macrophage subtypes, given the huge phenotypic diversity of subpopulations in homeostasis and disease and their capacity to shift phenotype depending on the stimuli, is even today a matter of discussion. Based on studies *in vitro*, macrophages are frequently subdivided in two paradigmatic phenotypes. Macrophages stimulated with the gram-negative bacterial compound lipopolysaccharide (LPS) and/or the cytokine IFNγ undergo “classical” activation, which many authors refer to as M1 phenotype. In contrast, macrophages stimulated with IL-4, undergo an “alternative” activation which is also known as the M2 phenotype (Murray *et al*., 2014).

The initial M1/M2 dichotomy has been further adapted to denominate different activation scenarios (M2 was expanded into subcategories, M2a, M2b, M2c). In this classification, M1 and M2 are paradigmatic poles of a spectrum of phenotypes, which reinforces the idea that it is very difficult to easily distribute macrophages in defined groups (Figure 1) (Mantovani, 2016).

Canonical M1 macrophages, or classically activated, are specialized in the development of pro-inflammatory responses (Figure 1). Typically, macrophages polarize to an M1 phenotype to assist the host against infections. They secrete high levels of pro-inflammatory cytokines (IL-6, IL-12, IL-23, tumor necrosis factor (TNF)α), have anti-microbial activities, produce ROS, nitric oxide and RNS, and stimulate the shift of Th cells towards the Th1 phenotype. They also have increased expression of the high affinity Fcγ receptor (CD16/CD32), and the costimulatory molecules CD80 and CD86, thus displaying an enhanced capacity to phagocytose antibody-opsonized materials and activate Th cells, respectively.

In the opposite part of the spectrum, M2 macrophages, or alternatively activated macrophages, are involved in anti-inflammatory and immunomodulatory responses (Figure 1). They also play a crucial role in damaged tissue repair and angiogenesis. They highly express the anti-inflammatory cytokine IL-10, Arg1, mannose receptor CD206, the vascular endothelial growth factor (VEGF) and the chemokines CCL17 and CCL22, which are important attractants of Th2 and Tregs.

Researchers use to target lineage-specific markers to analyze immune cell populations. With macrophages, the difficulty dwells in the fact that different macrophage activation states are related to shifts in the expression of a large number of genes, but none of them completely defines an activation state. While many authors use the M1/M2 simplified vision of macrophage polarization, the real situation in the tissue, in which a variety of cytokines and growth factors participate in defining the final phenotype, is more complex and multiple phenotypes coexist in the same pathogenic scenario (Wynn *et al.*, 2013). Therefore, macrophage polarization goes far beyond the M1/M2 dichotomy, even though M1 and M2 macrophages can be found in pathological conditions and are important in the immune response. A particularly important example

is the tumor-associated macrophage (TAM), which will be extensively reviewed further in this introduction.

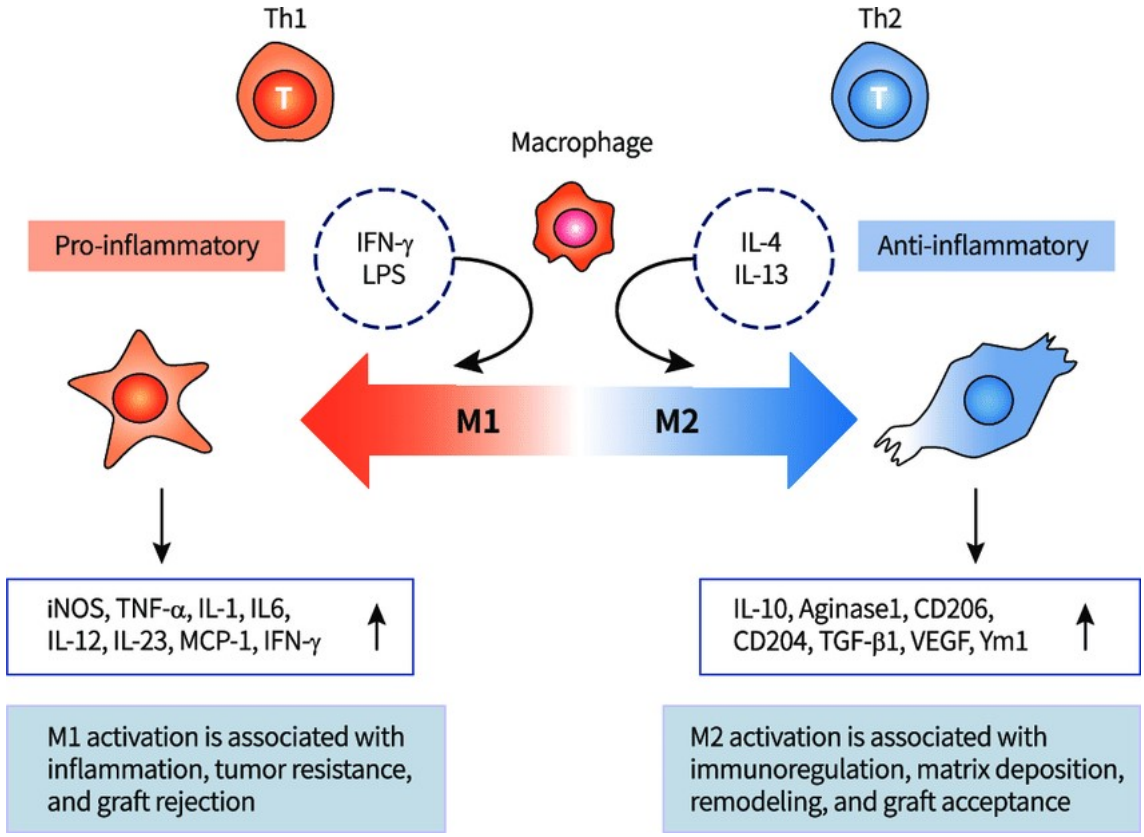


Figure 1. **Archetypical M1 and M2 polarization of macrophages**. IFNγ and LPS are the classical signals that promote M1 polarization, whereas IL-4 and IL-13 induce M2 polarization. The M1 phenotype in macrophages is involved in the induction of inflammation, tumor resistance, and graft rejection. The M2 phenotype in macrophages is associated with immune modulation, tissue repair and remodeling, and graft acceptance, MCP, monocyte chemoattractant protein, Ym1, chitinase-like 3. Figure obtained from Lee, 2019.

2.2 Macrophages and the tumor microenvironment

Macrophages are present in the immune cell compartment infiltrated in all tumors. They are, in general, double edge swords, able to promote antitumoral and pro-tumoral responses, depending on the signals and stimuli of the microenvironment. TAMs play a role in all the phases of tumor development, from the initiation to metastasis. They are key drivers of carcinogenic inflammation, promote genetic instability, contribute to sabotage antitumoral immune responses, and promote tumor cell invasion and metastasis (reviewed in Mantovani *et al*., 2017) (Figure 2).

For a long time, the literature held that TAMs arise from circulating monocytes from the blood compartment recruited by the tumor cells or non-malignant cells in the tumor environment. In that direction, it was shown that in murine mammary cancer, tissue-resident macrophages are eliminated, and the TAM compartment is refilled with monocyte-derived macrophages (Franklin *et al*., 2014). However, later studies in mice suggested that tissue-resident macrophages from embryonic progenitors can also become part of the TAM compartment in some cancers such as glioma (Hambardzumyan *et al*., 2016) and pancreatic ductal adenocarcinoma (Zhu *et al*., 2017). In humans, the absence of reliable TAM markers and the lack of in-depth extensive studies makes it more difficult to draw conclusions about the ontogeny of TAMs. The current assumption is that, in at least some human cancers, there is a coexistence of TAMs from different origins and that the TME is the driver of their education. In addition, although TAM proliferation has been observed in some cancer models, this mechanism does not always explain the maintenance of TAM numbers within the tumor. This observation suggests that the recruitment of circulating cells, including monocytes and M-MDSCs highly contributes to the maintenance of TAM populations, (Figure 2) (Franklin *et al*., 2014; Strauss *et al*., 2015; Kumar *et al*., 2016 A). Nevertheless, the origin of TAMs does not seem to affect their phenotype and functions in the TME.

2.3 Pro-tumoral effects of tumor-associated macrophages

TAMs exert multiple pro-tumoral actions in different stages of tumor development (Figure 2). Chronic inflammation is one of the factors that highly contribute to the emergence of cancer, and macrophages are key for the establishment of a non-resolved inflammatory microenvironment. Recent studies have proposed that TAMs may enhance genetic instability by the production of ROS, thus promoting carcinogenesis (Bonavita *et al*., 2015). TAMs also participate in tumor growth and metastasis. They produce growth factors, *e.g.* the epidermal growth factor (EGF), that stimulate the proliferation of several cancer cells (O'Sullivan *et al*., 1993; Haque *et al*., 2019). In addition, TAMs promote tissue remodeling through the deposition of fibrotic tissue, or the release of proteolytic enzymes, such as metalloproteinases (MMP), which digest and modify the extracellular matrix, thus facilitating tumoral cell dissemination (Vinnakota *et al*., 2017; Fu *et al*., 2020). Also, TAMs can stimulate angiogenesis and lymphangiogenesis (Murdoch *et al*., 2008; Fu *et al*., 2020), and, in metastatic sites, they generate a supportive niche for metastatic cells (Mantovani *et al*., 2017).

Despite the diversity in TAM phenotypes in most cancers, TAMs ultimately polarize into immunosuppressive cells. On one hand, TAMs can recruit Tregs through the secretion of chemokines (*e.g*. CCL17 and CCL22) and stimulate their activation through the production of IL-10 and TGFβ (Mantovani *et al*., 2017). On the other hand, TAMs can suppress T cell activity through additional indirect mechanisms, including the consumption of arginine upon Arg1 expression, the production of immunosuppressive metabolites and tryptophan depletion through the IDO1 and 2 pathways (Murray *et al*., 2014), or the secretion of prostaglandins as a product of cyclooxygenase (COX1 and COX2)-mediated arachidonic acid metabolism (Biswas, 2015). TAMs can also mediate T cell suppression directly through the expression of PD-L1 and PD-L2, B7-H4 (Kryczek *et al.,* 2006) and the V-domain Ig suppressor of T cell activation (VISTA), which may display similar functions (Wang *et al*., 2011).

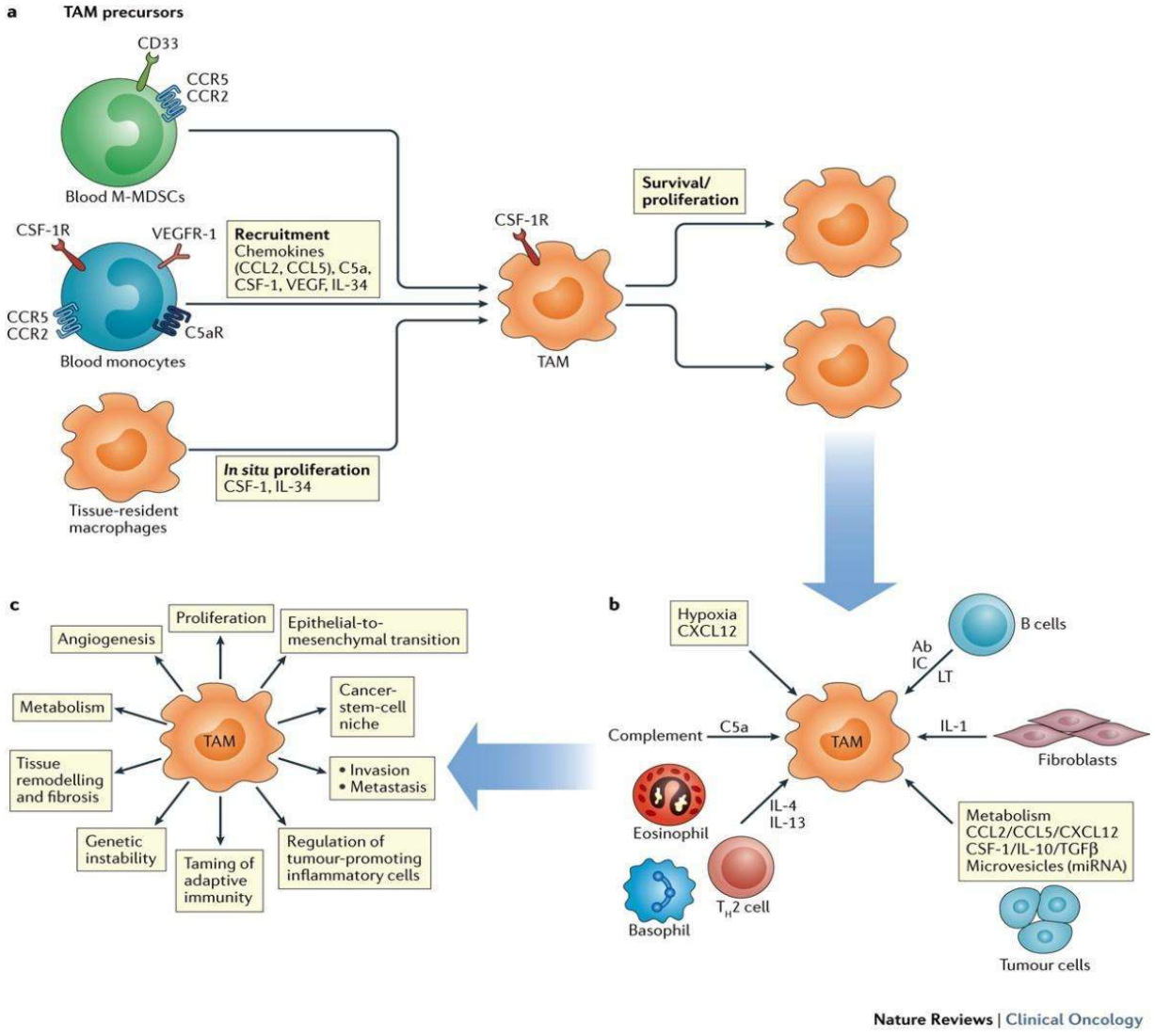


Figure 2. **A schematic representation of the roles of TAMs in tumor progression**. a) Both blood monocytes and M-MDSCs are recruited to the TME through a diverse range of chemoattractants and differentiate into TAMs. In some cancer types, tissue resident macrophages can also contribute to the TAM pool. Within the tumor, TAMs survive and further proliferate in response to growth factors. b) Many different signals can contribute to modulate TAM activation and polarization. Those signals are different within each tumor and can be delivered from cancer cells, local non-malignant cells (such as fibroblasts), or other cells from the TME (*e.g.* other immune cells). c) TAMs participate in every step of tumor development and deeply affect tumor biology through a wide range of pro-tumoral functions. CSF, colony-stimulating factor 1, CSF-R, colony-stimulating factor receptor 1, CXCL, C-X-C motif chemokine ligand, Ab Ic, antibody

immune complexes, LT, lymphotoxin, miRNA, micro-RNA. Figure obtained from Mantovani *et al*, 2017.

High numbers of TAMs correlate with a worse prognosis in many types of solid tumors, strengthening the view that TAMs promote tumor development (Kawahara *et al*., 2010; Zhang *et al*., 2012). In this sense, the abundance and density of TAMs correlated with the tumor grade and a worsened outcome in patients with breast carcinoma (Bingle *et al.,* 2002), and with a more advanced-stage disease in patients of breast or bladder cancer (Leek *et al.*, 1996; Hanada *et al*., 2000). In contrast, in patients with specific tumor types (prostate, colorectal and non-small-cell lung carcinomas), a high macrophage infiltration correlated with a favorable prognosis, which may probably be related to the positive effects of TAMs on the response to chemotherapy treatment (Zhang *et al*., 2012).

2.4 TAM subpopulations: MHCII^{high} TAMs and MHCII^{low} TAMs

One of the most important characteristics of solid tumors is that, due to their abnormal growth, angiogenesis and metabolic alterations, different regions are created within the tumor, with an uneven distribution of metabolites and oxygen. The result is a gradient of metabolites and oxygen that depends on the intratumoral blood vessels architecture. Going further away from the vessels, oxygen pressure decreases and there is also a decrease in glucose, while lactate concentration rises. Thus, macrophages in intervessel regions must adapt to moderate to severe hypoxia conditions, and to acidosis caused by the increased lactate (Arneth, 2020). It has been shown that hypoxia and lactate induce the production of angiogenesis factors by TAMs, as well as Arg1 expression (Carmona-Fontaine *et al*., 2017). Thus, TAMs may display different functions also as a response to specific conditions in different regions of the tumor.

In this doctoral thesis, we have used a model of experimental subcutaneous tumors described by Movahedi and collaborators, in which two subpopulations of TAMs have been defined. These subpopulations display distinctive intratumoral spatial distribution and differential expression of MHC class II molecules: MHCII^{high} TAMs and MHCII^{low} TAMs (Movahedi *et al*., 2010).

MHCII^{low} TAMs are primarily found in the hypoxic regions of the tumor, have an M2-like gene expression pattern and high angiogenic activity. In contrast, MHCII^{high} TAMs can be localized in the normoxic regions of the tumor, have a more pro-inflammatory phenotype and low angiogenic activity. Both subpopulations are highly immunosuppressive and have a low antigen-presenting capacity. Also, both MHCII^{low} TAMs and, especially MHCII^{high} TAMs secrete the chemokines CCL17 and CCL22 (Movahedi *et al*., 2010). Moreover, they show distinct responsiveness to key growth factors. On one hand, MHCII^{low} TAMs are affected by macrophage-colony stimulating factor (M-CSF) and are unresponsive to granulocyte macrophage-colony stimulating factor (GM-CSF). On the other hand, GM-CSF does influence the fine-tuning of the MHCII^{high} TAM phenotype (Van Overmeire *et al*., 2016).

3. The GM-CSF pathway

GM-CSF is a member of the colony stimulating factors superfamily, along with M-CSF and the granulocyte-colony stimulating factor (G-CSF). While M-CSF displays three active dimeric forms (secreted, anchored to the extracellular matrix and as a transmembrane protein), GM-CSF and G-CSF can only be detected as secreted monomers. Originally, CSFs were described as growth factors capable of stimulating the formation of colonies of myeloid cells from murine bone marrow hematopoietic precursors *in vitro* (reviewed in Becher *et al*., 2016). GM-CSF was first detected in conditioned medium from murine lung tissue stimulated with LPS and it was able to stimulate the proliferation of granulocytes and macrophages derived from bone marrow *in vitro* (Burgess *et al*., 1977). Nowadays, it is known that GM-CSF stimulates the proliferation of a diverse range of cells from hematopoietic origin, not only macrophages and granulocytes, but also erythroid cells and megakaryocytes, in a dose-dependent manner (reviewed in Hong *et al*., 2016).

The biological sources of GM-CSF are very diverse, including macrophages, mast cells, B cells, activated T cells, fibroblasts, vascular endothelial cells, epithelial cells (Shi *et al*., 2006; Ponomarev *et al*., 2007; Codarri *et al*., 2011; Greter *et al*., 2012; Li *et al*., 2015;

Shiomi & Usui, 2015), and an important number of cancer cell types (Perez *et al*., 2009; Lammel *et al*., 2012; Revoltella *et al*., 2012; Urdinguio *et al*., 2013). Production of GM-CSF is stimulated by pro-inflammatory cytokines like IL-6, IL-1, and TNFα. In contrast, its expression can be suppressed by the cytokines IL-10, IL-4, and IFNγ (reviewed in Hong *et al*., 2016). The homeostatic sera levels of GM-CSF are often extremely low or even indetectable, only expressed in non-sterile tissues, like gut or lungs; but the levels of circulating GM-CSF rise dramatically in response to pro-inflammatory stimuli (Petrina *et al*., 2021).

The GM-CSF receptor (GM-CSFR), also known as CD116 is a heterodimeric complex with two subunits: the alpha chain and the beta chain. The alpha chain possesses an extracellular ligand-binding domain specific for GM-CSF, whereas the beta chain, shared with the receptors for IL-3 and IL-5, promotes signal transduction (Onetto-Pothier *et al*., 1990; Kitamura *et al*., 1991) (Figure 3).

GM-CSF signaling can be controlled negatively by downregulation of the subunits of the GM-CSFR or the dephosphorylation of downstream mediators. Src-like adaptor proteins (SLAP and SLAP2) can interact with the GM-CSFR alpha chain resulting in the downregulation of both GM-CSFR subunits (Liontos *et al*., 2011). Suppressor of cytokine signaling 1 (SOCS1), can facilitate ubiquitination of the beta chain of GM-CSFR or JAK2, tagging them for degradation in the proteasome (Bunda *et al*., 2013).

The role of GM-CSF in homeostatic myelopoiesis is not as wide as that of the other CSF family members. Contrarily to mice deficient in M-CSF, with severe developmental defects due to their deficiencies in osteoclasts and tissue macrophages (Wiktor-Jedrzejczak *et al*., 1990; Dai *et al*., 2002), or mice deficient in G-CSF, which have severe neutropenia (Lieschke *et al*., 1994), GM-CSF deficiency impacts selectively development of alveolar macrophages from fetal monocytes (Guilliams *et al*., 2013; Gschwend *et al*., 2021) and the maintenance of tissue resident DCs (Bugunovic *et al*., 2009; Hirata *et al*., 2010; Greter *et al*., 2012). Deficiency in GM-CSF/GM-CSFR in mice causes a pathology named pulmonary alveolar proteinosis, characterized by an accumulation of surfactants in the alveoli (Becher *et al*., 2016). The defect in alveolar macrophages, which are responsible for surfactant clearance, is the basis for this disease.

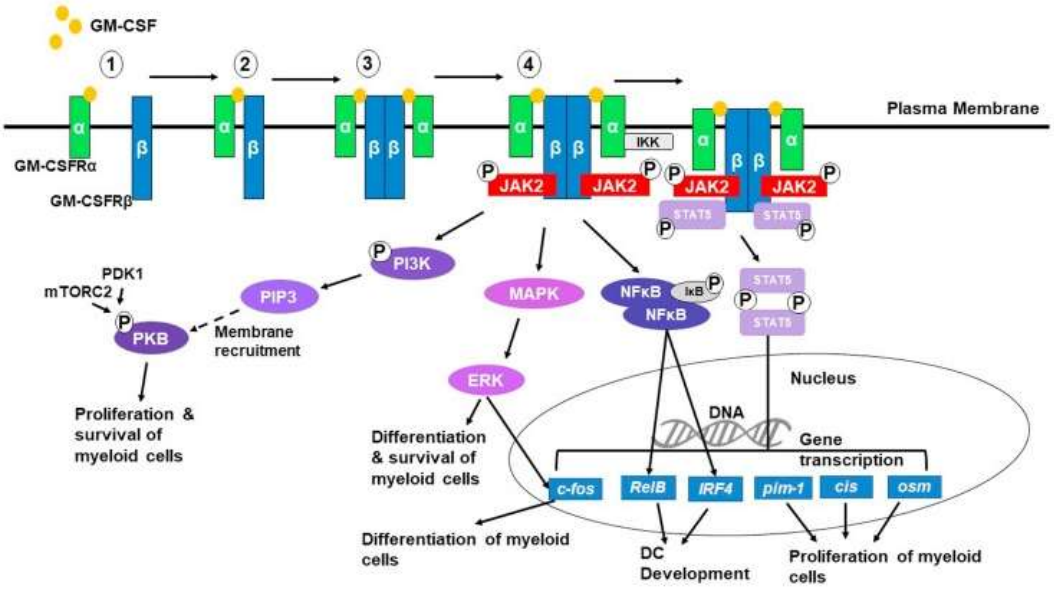


Figure 3. **GM-CSF signaling pathways.** Sequential events take place in response to GM-CSF. Fig. 1) GM-CSF binds to the alpha subunit of the GM-CSFR; 2) Dimerization of the two subunits of the GM-CSFR, alpha and beta, which enhances the binding affinity to GM-CSF; 3) Formation of the GM-CSFR hexameric complex followed by the lateral association of two hexameric complexes; 4) Activation of different signaling pathways. First, several Janus kinase 2 (JAK2) is recruited to the cytoplasmic tails of the beta subunits where they trans-phosphorylate each other. Phosphorylated JAK2 activates several signaling pathways, including signal transducer and activator of transcription 5 (STAT5), mitogen-activated protein kinases (MAPK), nuclear factor kappa B (NFκB) and the phosphatidylinositol 3-kinase (PI3K) pathways. The PI3K-protein kinase B (PKB)/Akt pathway replicates many downstream effectors that are important for the proliferation and survival of myeloid cells. The MAPK pathway, in particular extracellular signal-regulated kinases (ERK) phosphorylate transcription factors that contribute to myeloid cell differentiation and survival. Phosphorylated STAT5 homodimerize and translocate to the nucleus where they stimulate the transcription of genes that promote differentiation, survival, and proliferation of myeloid cells (such as c-fos, interferon regulatory factor (IRF)4, RelB, proto-oncogene serine/threonine-protein kinase (pim-1), cytokine Inducible SH2 containing protein (cis) and oncostatin M (osm)). Activated NFκB contributes to upregulate RelB and IRF4. Figure obtained from Petrina *et al.*, 2021.

Many important functions of GM-CSF have been studied in the context of inflammation. As mentioned above, basal production of GM-CSF is extremely low, but it rapidly rises after the onset of an inflammatory reaction. Myeloid cells (specially monocytes, macrophages, neutrophils, and mast cells) are major producers of GM-CSF (reviewed in Wicks *et al*., 2016). At the same time, GM-CSF stimulates differentiation of myeloid cells from hematopoietic progenitors in the bone marrow. For example, GM-CSF stimulates the differentiation, activation and survival of neutrophils and eosinophils. In neutrophils, it upregulates the expression of integrins (CD11b), increasing their capacity to enter in the inflamed tissues, boosts the generation of ROS, and enhances phagocytosis and generation of neutrophil extracellular traps (Yong *et al*., 1992; Yousefi *et al*., 2009; Futosi *et al*., 2013). On eosinophils, GM-CSF also has activating and pro-survival effects (Curran & Bertics, 2012; Wong *et al*., 2013; Liu *et al*., 2015). GM-CSF also induces the differentiation and survival of monocytes and macrophages and regulates several of their functions. It extends cell survival, promotes macrophage proliferation, induces the expression of MHC II molecules and PRRs, enhances phagocytosis and antigen processing and presentation, induces the generation of ROS and RNS, and upregulates the expression of pro-inflammatory cytokines and chemokines (reviewed in Hamilton, 2019).

In macrophages, stimulation *in vitro* with GM-CSF induces an “M1-like” phenotype, with the production of IL-6, IL-12, TNFα and IL-23 (Akagawa *et al*., 2006; Fleetwood *et al*., 2007), although *in vivo* the effects of the GM-CSF pathway in monocytes and macrophages may depend on other stimuli (or act in synergy with other pathways), like IFNγ, LPS, the IRF5 pathway or activin A, a growth factor from the TGFβ superfamily (Krausgruber *et al*., 2011; Sierra-Filardi *et al*., 2011; Borriello *et al*., 2017). In some circumstances, however, GM-CSF stimulated monocytes display an M2-like phenotype *in vivo* (Däbritz *et al*., 2015).

Monocyte-derived DCs (MoDC), which are at very low numbers in steady state, accumulate as a consequence of GM-CSF production during inflammation (Segura *et al*., 2013). Traditionally, GM-CSF has been used to differentiate DCs *in vitro* from monocytes or bone marrow progenitors, and these cells have similarities with MoDCs (Xu *et al*., 2007).

GM-CSF has also an important role as a mediator of inflammation during the Th17 response. Th17 cells are a pro-inflammatory subpopulation of Th cells that are defined by their expression of IL-17. They polarize in response to cytokines like IL-1β, IL-23, IL-6, or TGFβ (Ko *et al*., 2014). They are important in anti-bacterial and anti-fungal responses but are also strongly related to autoinflammatory and autoimmune disorders, such as multiple sclerosis, psoriasis, or rheumatoid arthritis (Ko *et al*., 2014). Th17 cells produce GM-CSF through activation of the transcription factor retinoic-acid-receptor-related orphan nuclear receptor gamma (RORγT), although other signaling pathways (*e.g.* IRF4 and NFκB) may also contribute depending on external stimuli (Codarri *et al*., 2011; Ruan *et al*., 2011; Man *et al*., 2013). In fact, many T cell-driven models of autoimmunity have a strong GM-CSF dependence, like the experimental allergic encephalomyelitis (EAE), collagen induced polyarthritis, myocarditis, and streptococcal cell wall arthritis among others (Campbell *et al*., 1998; Plater-Zyberk *et al*., 2007; Codarri *et al*., 2011; Wu *et al*., 2016). In addition, a specific subset of Th cells producing GM-CSF has been proposed, ThGM cells (reviewed in Herndler-Brandstetter and Flavell, 2014). Natural Killer T (NKT) cells can also produce GM-CSF and are important in the regulation of the neutrophil response against fungal infections (Bär *et al*., 2014).

These observations raise the hypothesis that GM-CSF expression by effector T cells, can affect the phenotype and numbers of APC at the inflammation site, specifically inducing the polarization toward pro-inflammatory macrophages and the generation of MoDCs. At the same time, myeloid cells may produce IL-23 and other pro-inflammatory chemokines that stimulate Th17 polarization (Sonderegger *et al*., 2008; Codarri *et al*., 2011; El-Behi *et al*., 2011).

3.1 GM-CSF in cancer

In cancer, the use of GM-CSF for antitumoral therapies has been an interesting field of study for a long time. Initial studies using secreting tumor vaccines and comparing the effectiveness of different pro-inflammatory molecules in B16 melanoma cells concluded that GM-CSF promoted the most effective and specific anti-tumor response (Dranoff *et al*., 1993). The idea behind the modification of cancer cells to secrete GM-CSF was to

stimulate a strong pro-inflammatory reaction, promoting the recruitment and activation of myeloid cells that would later present tumor-associated antigens from the tumor vaccine cells. A first clinical trial was performed in patients with metastatic renal cell carcinoma. Histology from intradermal biopsies showed that there was indeed an infiltration of immune cells in the injection sites (Simons *et al*., 1997). However, the investigation of the GM-CSF tumor vaccine as an antitumoral therapy came to an end when two large phase III clinical trials showed the lack of efficacy of the vaccines (Lawson *et al*., 2015). More recently, GM-CSF tumor vaccines have gained attention again thanks to their use in clinical trials in combination with the checkpoint blocker Ipilimumab (anti-CTLA4), were they demonstrated to be safe and more effective that ipilimumab alone (Hodi *et al*., 2014).

Another approach is based on the delivery of GM-CSF to the tumor site using viral-based carriers. T-VEC, a modified herpes virus that encodes GM-CSF, can replicate preferentially in cancer cells, produce GM-CSF and, through its oncolytic activity, promote the availability of tumor-associated antigens. It has shown therapeutic benefit in a phase III clinical trial with stage III-IV melanoma patients (Larocca *et al*., 2020). Such promising results have been studied also in combination with checkpoint inhibitors, like the anti-PD-1 antibody Pembrolizumab (Ribas *et al*., 2018).

Another possible antitumoral application of GM-CSF is the *in vitro* generation of DCs from patient monocytes, which are activated and loaded with tumor-associated antigens and used as vaccines. However, these cells do not migrate well to the lymph nodes and do not elicit a proper T cell response, which suggests that better optimization of the protocols are required (reviewed in Wimmers *et al*., 2014).

It is important to indicate, however, that GM-CSF can also have important pro-tumoral roles. It is common that tumor cells secrete GM-CSF or induce its secretion by other cells in the TME, reprogramming local macrophages into a immunosuppressive state and enhancing systemic myelopoiesis (Bayne *et al*., 2012; Kohanbash *et al*., 2013; Su *et al*., 2014; Waghray *et al*., 2016). Although tumor-associated myeloid cells are a very heterogeneous group of cells, they are primarily immunosuppressive. GM-CSF indeed stimulates the recruitment and differentiation of MDSCs in the TME (Wu *et al*., 2014 B). In addition, the production of GM-CSF by immature myeloid cells in the spleen also helps

to maintain splenic hematopoiesis (in mice), facilitating the constant supply of MDSC precursors (Wu *et al*., 2018).

GM-CSF secretion in the TME may also exert pro-tumoral roles through the induction of milk fat globule epidermal growth factor 8 (MFG-E8). This glycoprotein is induced in phagocytes to eliminate apoptotic bodies, secrete TGFβ and CCL22 and prime Tregs. In melanoma, MFG-E8 has been shown to promote resistance to apoptosis, angiogenesis, tumor cell invasion and metastasis, as well as to recruit Tregs to the tumor microenvironment (Junishi *et al*., 2009). Thus, the pro-tumoral roles of GM-CSF are an important downside for its use as an antitumoral therapy and they must be taken carefully into account in future studies.

4. Nuclear receptors

Nuclear receptors (NRs) conform a superfamily of ligand-activated transcription factors that are important for many biological processes, either in homeostasis (development, metabolism, reproduction, senescence) or in disease (metabolic diseases, cancer, etc.), and include several receptors for lipophilic vitamins, steroid hormones, lipid metabolites, and bile acids (Font-Díaz *et al*., 2021). NRs regulate gene expression at different levels, thus modulating a myriad of cell fate decisions. For this reason, NRs serve as targets for many drugs in a great range of disorders (NRs represent indeed the targets for nearly 15% of pharmacological drugs) (reviewed in Burris *et al*., 2013).

The superfamily of NRs is composed of 48 receptors in humans and 49 in mice. They share a common structure, with four different structural and functional domains: a long N-terminal domain, a DNA-binding domain (DBD), a hinge region, and a C-terminal ligand-binding domain (LBD) (reviewed in Fuentes-Prior *et al*., 2019). The N-terminal domain is highly variable in length and contains several regulatory regions. The DBD is highly conserved among all the NRs and is composed by two zinc fingers that interact with specific response elements in the genome. The flexible hinge region mediates receptor dimerization and DNA binding simultaneously and is equipped with the nuclear

localization signal. The large LBD recognizes the specific ligands and modulates the functional state of the NR. The LBD also participates in protein-protein interactions within the NRs subsets allowing the formation of heterodimers (reviewed in Font-Díaz *et al*., 2021).

The classification of the NR superfamily is based on their subcellular location and the ligand-induced effects on gene expression. Some NRs, including high affinity receptors for steroid hormones, such as the estrogen receptor, the androgen receptor, and the glucocorticoid receptor, are normally found in the cytoplasm. Upon ligand recognition, they translocate to the nucleus, undergo dimerization, and modulate transcription. Other NRs are normally found in the nucleus and bound to their target DNA sequences even in the absence of specific ligands, forming complexes with co-repressor proteins and blocking transcription. Ligand recognition causes conformational changes in the NRs that allow dissociation of the co-repressor proteins and promote interaction with coactivators. This is the case of many NRs that bind diet-derived ligands or molecules that are metabolic intermediates or byproducts, such as the peroxisome-proliferator activated receptors (PPARs), the vitamin D receptor, the retinoic acid receptor, or the liver X receptors (LXRs). These receptors form heterodimers with another NR, the retinoid X receptor (RXR). In addition, there are some NRs considered orphan, without a known ligand (reviewed in Font-Díaz *et al*., 2021).

NRs exerts their functions as transcriptional regulators through different mechanisms. Upon ligand recognition, they can activate the transcription directly by the interaction of their DBD to specific response elements, either as homodimers or heterodimers and interacting with coactivator proteins. Several NRs can also repress gene expression, in the presence or absence of ligand. In this sense, many NRs can interfere with other transcription factors in a ligand dependent manner, blocking their transcriptional activity (a phenomenon known as transrepression). Lastly, NRs can be functionally modulated through post-transcriptional modifications, such as phosphorylation, SUMOylation and ubiquitination (Sever *et al*., 2013).

4.1 Liver X Receptors

LXRs are transcription factors from the NR superfamily. Two isoforms have been described, NR1H3 (LXRα), and NR1H2 (LXRβ), with 78% homology in their amino acid sequence. The expression of LXRβ is widely distributed in the body, whereas LXRα is mainly expressed in tissues with a high metabolic activity, such as liver, intestine, kidneys, and adipose tissue. In the immune compartment, both LXR isoforms are expressed in macrophages, DCs, and neutrophils. B cells express LXRβ, whereas T cells express either LXRβ or both isoforms. The natural ligands of LXRs are oxysterols (such as 22(R)-hydroxycholesterol (HC), 24(S)-HC, 27-HC, and 24(S),25-epoxycholesterol) or intermediate molecules from the cholesterol biosynthesis pathway such as the cholesterol precursor desmosterol. LXRs can also be activated with synthetic high-affinity agonists (such as T0901317 and GW3965) which are normally used for investigating the effects of the pharmacological activation of LXRs *in vivo* and *in vitro* (reviewed in Glaría *et al*., 2020; Dixon *et al*., 2021).

LXRs bind to DNA as heterodimers with RXRs through the recognition of specific regions in the genome named LXR response elements (LXREs). In the steady state, LXRs are bound to co-repressor proteins such as nuclear receptor co-repressor 1 (NCoR1). In the presence of the agonist, the LXR-RXR heterodimer undergoes a conformational change that promotes the dissociation of the co-repressor complex and allows the recruitment of coactivators and the positive transcriptional regulation of their target genes (Wagner *et al*., 2003; Liang *et al*., 2019). A recent study by Ramón-Vázquez and collaborators suggests three different modes of action by which LXRs control gene transcription (Ramón-Vázquez *et al*., 2019). Mode I is also called the de-repression mode. In this case, the LXR-RXR heterodimer represses several target genes in the absence of agonists. Indeed, genes regulated this way have a higher expression in an LXR-deficient context due to de-repression. Once the LXR-RXR heterodimer is activated through ligand-binding, the expression of these genes is potently upregulated. Mode II describes classical ligand-dependent activation by which the LXR-RXR heterodimer upregulates transcription in the presence of the agonist, and target gene expression is inhibited in an LXR-deficient context. In mode III, the expression of several genes is not upregulated upon pharmacological activation of LXRs, but it is indeed reduced in an LXR-deficient

context, indicating that functional expression of LXRs is needed indirectly for the regulation of those genes (Ramón-Vázquez *et al*., 2019).

As it will be further developed in this introduction, LXRs induce the expression of many genes involved in lipid and glucose metabolism (reviewed in Glaría *et al*., 2020). Additionally, LXRα selectively regulates genes involved in the control of apoptosis and leukocyte migration, whereas LXRβ regulates genes that control lymphocyte differentiation and selection (Bensinger *et al*., 2008; Beceiro *et al*., 2018; Ramón-Vázquez *et al*., 2019). LXRs can also inhibit the transcription of several genes, including key mediators of inflammation. For example, through transrepression, SUMOylated LXRs can bind to repressor complexes in the promoter of pro-inflammatory genes and block the release of co-repressors in response to inflammatory signals, thus inhibiting the recruitment of the transcriptional machinery (Ghisletti *et al*., 2007; Huang *et al*., 2011). This topic is further developed in section "Role of Liver X Receptors in inflammation".

4.2 Roles of Liver X Receptors in metabolism

When cholesterol concentration in the blood is high, there is an increase in the concentration of oxidized forms of cholesterol (oxysterols), that are endogenous LXR ligands. To compensate for the increased cholesterol levels, LXRs upregulate the expression of gene networks involved in cholesterol transport and excretion. LXRs are therefore known as sterol sensors that facilitate cholesterol efflux and reverse cholesterol transport from peripheral cells such as macrophages to the liver, where it will be excreted as bile acids (Repa *et al*., 2000 B). To define in detail the biological roles of LXRs in lipid homeostasis, most of the studies have been performed with synthetic agonists.

In more detail, ligand-mediated activation of LXRs in peripheral cells, such as macrophages, promotes the upregulation of sterol transporter proteins from the ATP-binding cassette family (ABC), namely ABCA1 and ABCG1. Both transporters facilitate the transfer of the intracellular cholesterol out from the cells towards high density lipoprotein (HDL) particles. In addition, LXRs upregulate the expression of

apolipoprotein E (ApoE) in macrophages, which also participates in cholesterol efflux (Repa *et al*., 2000 B; Laffitte *et al*., 2001; Wang *et al*., 2007; Ouvrier *et al*., 2009).

Simultaneously, LXR activation upregulates the ubiquitin ligase inducible degrader of LDL receptor (IDOL), which promotes the degradation of the receptors for low density lipoproteins (LDL), thus reducing the uptake of extracellular cholesterol in peripheral cells. The neat result is a reduction in cholesterol accumulation in peripheral cells (Figure 4) (Zelcer *et al*., 2009).

In the liver, the activation of LXRs upregulates two more members of the ABC family of transporters, ABCG5 and ABCG8, that mediate the excretion of hepatic cholesterol to the intestine through the bile ducts. In mice, moreover, LXRα induces the expression of cholesterol 7-hydroxylase, which participates in the metabolization of cholesterol into bile acids. It has been also shown that LXRs activation may stimulate the transcription of hepatic lipoprotein lipase (LPL), which increases the uptake of HDL in the liver. Both ABCG5 and ABCG8 transporters are also upregulated by LXRs in the intestinal enterocytes, limiting the absorption of cholesterol and facilitating its excretion through the feces (Figure 4) (Peet *et al*., 1998; Repa *et al*., 2002; Yu *et al*., 2002; Wilund *et al*., 2004)

LXRs also exert transcriptional control over many genes involved in the synthesis of fatty acids. Important mentions are the master transcriptional regulator of fatty acid synthesis, sterol regulatory element binding protein 1c (SREBP1c), and several enzymes such as the fatty acid synthase (FASN), the acetyl-CoA carboxylase or the stearoyl-CoA desaturase (SCD) (Repa *et al*., 2000 A; Chu *et al*., 2006; Li *et al*., 2013). The activation of the LXR pathway leads to the increased production of triglycerides by the liver (Figure 4). In fact, treatment with synthetic LXRs ligands in both mice and humans causes abnormal accumulation of triglycerides and hepatic steatosis (Groot *et al*., 2005; Kirchgessner *et al*., 2016). Interestingly, in homeostatic conditions, a significant increase in cholesterol activates the LXR pathway, but also inactivates SREBP1c-mediated lipid synthesis pathway. Thus, it is possible that the LXR-mediated increase in fatty acid synthesis occurs only as a response to pharmacological LXR activation, and not as a physiological mechanism induced by an increase in blood cholesterol levels (Ignatova *et al*., 2013). On the other hand, beyond the quantitative accumulation of fatty acids,

pharmacological LXR activation leads to qualitative changes in the fatty acid profile and in the distribution of fatty acids within cellular lipid species. In this sense, LXRs promote the synthesis of polyunsaturated fatty acids (PUFAs) and their distribution in cellular lipids through the induction of several enzymes involved in long-chain fatty acid activation, desaturation, and elongation (Varin *et al*., 2015; reviewed in Jalil *et al*., 2019). Therefore, LXRs control dynamically the PUFA composition and physicochemical properties of cell membranes, which affects several biological processes (reviewed in Jalil *et al*., 2019).

LXR agonists also exert important anti-diabetic effects in mice. Pharmacological activation of LXRs leads to a decrease in hyperglycemia and an improvement in insulin sensitivity. The LXR pathway increases brown fat activity, up-regulates the glucose transporter 4 in adipose tissue and inhibits hepatic glucose production through the downregulation of many gluconeogenic enzymes in the liver (such as the phosphoenolpyruvate carboxykinase or the glucose-6-phosphatase) (Commendford *et al*., 2007, Korach-André *et al*., 2011).

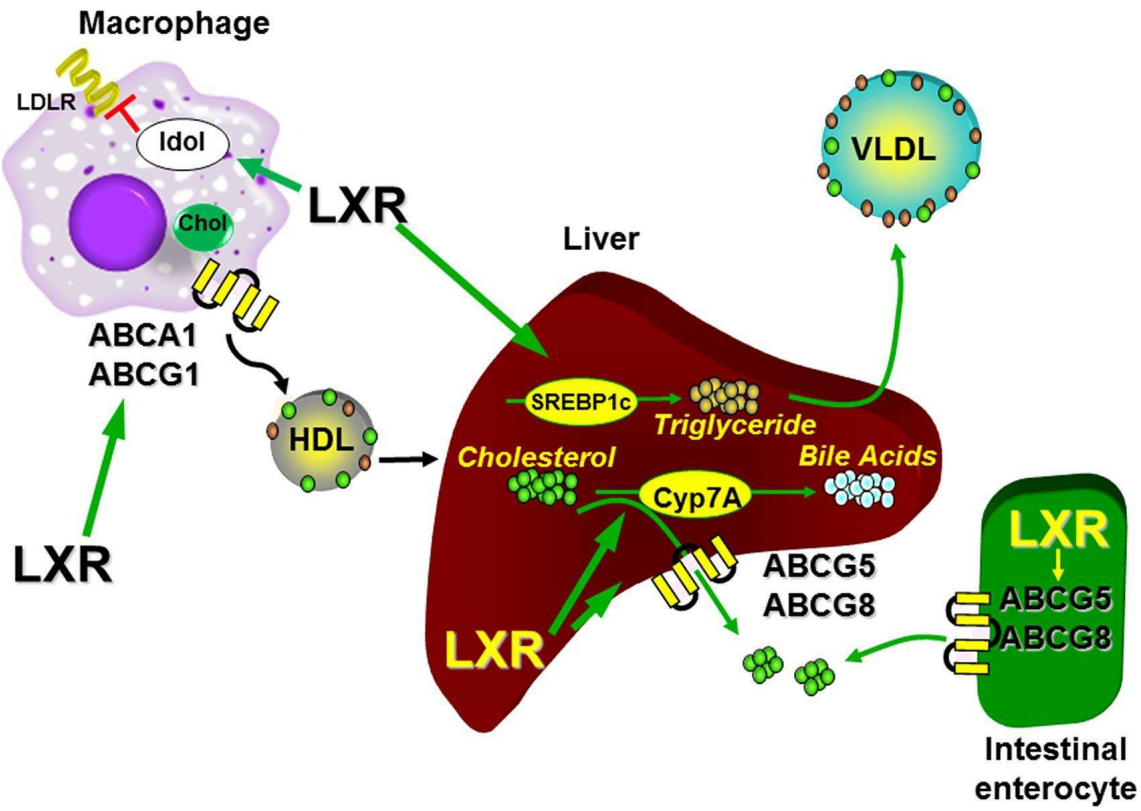


Figure 4. **Regulation of cholesterol homeostasis by LXR**. The accumulation of cholesterol increases the availability of endogenous LXR agonists. Activation of the LXR pathways in peripheral cells (*e.g.* macrophages) leads to the upregulation of the cholesterol transporters ABCA1 and ABCG1, which promote cholesterol efflux to HDL particles. The ubiquitin ligase IDOL is also induced, driving LDL receptor degradation to reduce cholesterol uptake. LXR-mediated upregulation of the transporters ABCG5 and ABCG8 in the liver allow the excretion of cholesterol to the bile while induction of the cytochrome CYP7A (in mice) increases the metabolization of cholesterol into bile acids. Upregulation of SREBP1c and other enzymes involved in lipogenesis lead to the synthesis of triglycerides and the secretion of very low-density lipoproteins. In the gut, induction of ABCG5 and ABCG8 reduces cholesterol absorption by intestinal enterocytes, thus favoring its elimination through the feces. Figure obtained from Schulman *et al*., 2017.

4.3 Roles of Liver X Receptors in inflammation

LXRs modulate inflammation through the negative regulation of the expression of several pro-inflammatory mediators. In this sense, pharmacological activation of the LXR pathway ameliorates to a certain extent the outcome of many disease models in mice, in part through the negative control of inflammation (reviewed in Glaría *et al*., 2020 and Zhao *et al*., 2021). Such diseases include atherosclerosis (Bischoff *et al*., 2010; Zeng *et al*., 2018), arthritis (Park *et al*., 2010), Alzheimer's disease (Rangaraju *et al*., 2018), neuroinflammatory diseases (Hindinger *et al*., 2006; Zhang-Gandhi & Drew, 2007), or lupus (Han *et al*., 2018).

One of the best characterized mechanisms through which LXRs exert their anti-inflammatory actions is transrepression. Through this action LXRs interfere with the activity of transcription factors generally involved in the positive control of a pro-inflammatory transcription program. As an example, SUMOylated LXRs block the activation of NF-κB by interacting with co-repressor complexes associated with NF-κB and preventing co-repressor release in response to TLR signaling (Ghisletti *et al*., 2007). SUMOylated LXRs also block the response to IFNγ in murine macrophages and astrocytes by interfering with STAT1 recruitment to target gene promoters (Figure 5) (Lee *et al*., 2009; Pascual-García *et al*., 2013).

In addition to transrepression, LXRs can inhibit the development of inflammation through positive regulation of several target genes (Figure 5). In this line, the upregulation of the cholesterol transporter ABCA1 in macrophages alters the cholesterol composition in the cell membrane, affecting the recruitment of mediators to lipid rafts and interfering with TLR signaling (Ito *et al*., 2015). Moreover, ABCA1 upregulation in murine macrophages has been also linked to an increased production of the anti-inflammatory cytokine IL-10 (Ma *et al*., 2012). On the other hand, LXRs induce the expression of the Mer tyrosine kinase (MerTK) in murine macrophages which enhances the capacity of these cells to phagocytose and eliminate apoptotic bodies. These effects are associated with suppression of the inflammatory response (Noelia *et al*., 2009). Other studies showed that, through the upregulation of IRF8, LXRs inhibit the transcription of the pro-inflammatory cytokine IL-18. Moreover, synthetic LXRs ligands downregulate the expression of both NOD-like receptor family pyrin domain- containing

3 (NLRP3), a central component of the inflammasome, and pro-IL-1β, thus blocking the maturation of IL-18 and IL-1β (Pourcet *et al*., 2016; Lei *et al*., 2017). LXR activation in murine macrophages can also upregulate the expression of the zinc-finger protein tristetraprolin (TTP), which mediates TNF-α mRNA instability. Through TTP activity, LXR activation promotes IL-1β, IL-6 and TNF-α mRNA decay (Xiao *et al*., 2017). The scavenger receptor apoptotic inhibitory factor secreted by macrophages (AIM)/CD5L, which expression is under the control of the LXR pathway (Joseph *et al*., 2004; Valledor *et al*., 2004), may also contribute to the anti-inflammatory actions of LXRs. In human monocytes, expression of AIM/CD5L leads to enhanced autophagy and the induction of an anti-inflammatory profile, similar to an IL-10-stimulated monocyte (Sanjurjo *et al*., 2018). Another mechanism that contributes to the negative control of inflammation is the synthesis of long chained PUFAs with anti-inflammatory properties. In this line, LXR activation induces SCD2 expression, either directly or through the intermediate upregulation of SREBP1c expression. SCD2 activity then favors the synthesis of oleic acid and palmitoleic acid, which display anti-inflammatory effects (Spann *et al*., 2012).

In DCs, in contrast to the observations in macrophages, LXR may have both anti-inflammatory and pro-inflammatory actions. On one hand, in human DCs, LXR activation has been shown to interfere with T cell stimulation, disrupting immune synapse formation through the downregulation of the actin-bundling protein fascin (Geyeregger *et al*., 2007). On the other hand, treatment with LXR ligands in human monocyte-derived DCs *in vitro* stimulated the production of pro-inflammatory cytokines and enhanced T $CD4^+$ cell activation, due to prolonged NF-κB activity (Töröcsik *et al*., 2010). Also, treatment with LXR agonists results in increased DCs chemotaxis to inflammatory sites in association with an enhanced response to the chemokines CCL19 and CCL21 (Beceiro *et al*., 2018). These contrasting observations indicate that more research needs to be done to uncover the factors that cause such opposed effects of LXR activation in DCs.

Outside the myeloid compartment, the LXR pathway has been shown to inhibit T cell proliferation. It has been shown that LXR activation inhibits T cell proliferation through the induction of ABCG1 expression and the subsequent alteration of the cellular cholesterol content. In that sense, LXRβ deficiency specifically promoted lymphocyte proliferation (Wang *et al*., 2018). Moreover, LXR agonists inhibited Th17 cell

proliferation potentially through SREBP1c-mediated interference with the transcription factor aryl hydrocarbon receptor (Cui *et al*., 2011). On the other hand, LXR induced an increase in the abundance of Tregs in murine gut, although the underlying molecular mechanism was not described (Herold *et al*., 2017).

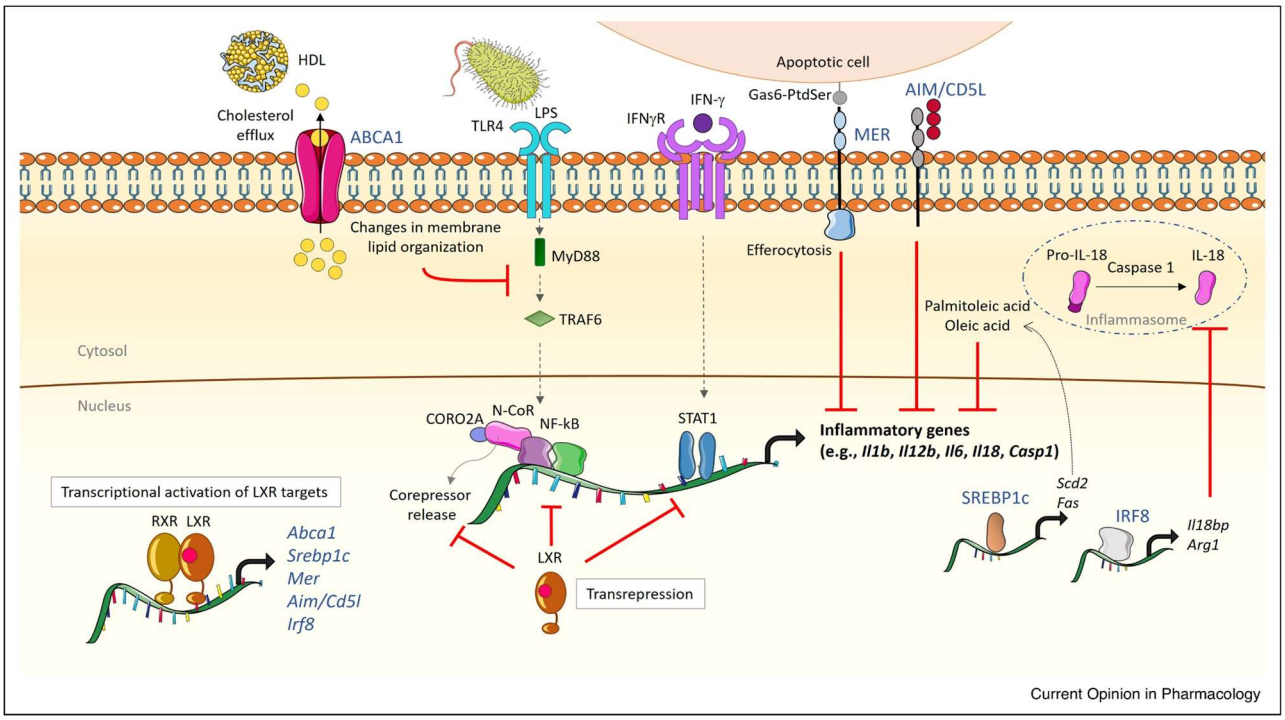


Figure 5. LXRs induce several inhibitory mechanisms of the inflammatory response in macrophages. Upon interaction with an agonist, LXRs mediate mechanism of transrepression, which interfere with the release of co-repressors or with the activity/recruitment of transcription factors (NF-κB, STAT1) required for TLR- or IFNγ-dependent inflammatory gene expression. In addition, LXRs indirectly inhibit inflammation through the transcriptional upregulation of LXR targets (in blue), involved in the modulation of metabolic and/or immune responses. ABCA1-mediated cholesterol efflux interferes with TLR signaling through the induction of changes in the lipid composition of the membrane. SREBP1c induces the expression of enzymes involved in the generation of lipids with anti-inflammatory properties. MER enhances efferocytosis, reducing the inflammatory potential of apoptotic bodies. AIM/CD5L induces the expression of molecules involved in the resolution of inflammation and promotes

an anti-inflammatory profile. IRF8 inhibits IL-18 activity through the expression of IL18 binding protein (IL18BP). Casp1, caspase 1; CORO2A, Coronin 2A; IFNγR, IFNγ receptor; MyD88, myeloid differentiation primary response 88; TRAF6, TNF receptor associated factor 6. Figure obtained from Glaría *et al*., 2020.

4.4 CD38, a transcriptional target of LXRs with roles in homeostasis and inflammation

CD38 is a multifunctional transmembrane enzyme that is expressed predominantly in immune cells (Reviewed in Malavasi *et al*., 2008). The CD38 promoter region contains binding sites for NF-kB, RXR, LXR, and STAT, and its expression is upregulated by pro-inflammatory cytokines, endotoxins, and IFNs (Musso *et al*., 2001; Kang *et al*., 2006; Matalonga *et al*., 2017). CD38 can function either as a receptor or as an enzyme. The primary enzymatic function of CD38 is the synthesis of adenosine diphosphate (ADP) ribose from nicotinamide adenine dinucleotide (NAD^+). It also catalyzes the synthesis of cyclic ADP ribose (cADPR) from NAD+ but due to CD38 inefficient cyclase activity, it must consume almost 100 molecules of NAD+ to generate one molecule of cADPR (Chini, 2009). In addition, in the presence of nicotinic acid and at acidic pH, CD38 can hydrolyze NAD phosphate (NADP) to generate nicotinic acid adenine dinucleotide phosphate (NAADP) (Gelman *et al*., 1993). Several products of CD38 catalytic activity are second messengers in calcium mobilization with relevant signaling consequences in diverse cellular contexts (Kwong *et al*., 2012; Lin *et al*., 2017). CD38 also metabolizes extracellular NAD+ precursors nicotinamide mononucleotide (NMN) and nicotinamide riboside prior to their intracellular transport for NAD+ biosynthesis (Camacho-Pereira *et al*., 2016). While the majority of CD38 molecules display their NADase functions as a transmembrane ecto-enzyme with their catalytic domain facing out from the cell membrane (Shrimp *et al*., 2014), CD38 can also be found with its catalytic domain facing inwards (Zhao *et al*., 2012), in intracellular membranes (Yamada *et al*., 1997), or even in a soluble form (Funaro *et al*., 1996).

One important role of CD38 in activated immune cells may be the reduction of NAD+ availability for pathogens, thus reducing their infective capacity. Indeed, in macrophages

upregulation of CD38 by the LXR pathway contributes to limit the infection of murine macrophages by *Salmonella* Typhimurium (Matalonga *et al*., 2017). It is possible that CD38 may also help immune cells to induce a metabolic collapse in extracellular pathogens that rely heavily on the uptake of external NAD+ and their precursors for survival, such as several *Haemophilus* members (Cynamon *et al*., 1998; Herbert *et al.*, 2003).

In addition, CD38 displays important roles in the maintenance of a pro-inflammatory phenotype in myeloid cells (Frasca *et al*., 2006; Matalonga *et al*., 2017; Schiavoni *et al*., 2018; Shu *et al.*, 2018), and in neutrophil and monocyte migration to infection sites (Partida-Sanchez *et al*., 2001; Estrada-Figueroa *et al*., 2011; Lischke *et al.*, 2013). Due to its many physiological and pathological roles, CD38 has a great potential as a therapeutic target for several human diseases (reviewed in Hogan *et al*., 2019 and in Glaría & Valledor, 2020).

4.5 Liver X receptors in cancer

Pharmacological activation of LXRs as an antitumoral therapy has been studied with rising interest in recent years. Synthetic LXR ligands exert potent cytostatic, pro-apoptotic and anti-proliferative effects *in vitro* in several cell lines from different types of cancers. Anti-proliferative effects have been also seen in primary cells (Pascual-García *et al*., 2011). In line with these effects, administration of LXRs ligands leads to a reduction in tumor progression in many murine cancer models (reviewed in Font-Díaz *et al*., 2021).

Several pathways have been proposed as mediators of the anti-proliferative actions of LXRs, including key proteins involved in cell cycle control. In this sense, LXR ligands repress the expression of positive regulators of the cell cycle (such as cyclins and/or cyclin-dependent kinases (CDKs)) (Vedin *et al*., 2009; Kim *et al*., 2010; Sasso *et al*., 2013; Vedin *et al*., 2013; Hu *et al*., 2014) promote the upregulation of cell cycle inhibitors (such as p21 or p27) (Fukuchi *et al*., 2004, Sasso *et al*., 2013; Vedin *et al*., 2013; Candelaria *et al*., 2014; Xiong *et al*., 2017), disrupt β-catenin/WNT signaling pathway (Youlin *et al*., 2017), and induce the expression of the suppressor of cytokine signaling 3 (SOCS3) (Fu *et al*., 2014). Moreover, LXR ligands have been shown to promote apoptosis in several

cancer cell lines, through the inhibition of the AKT pathway or the induction of caspase-dependent cell death (Pommier *et al*., 2010; Sasso *et al*., 2013; Derangere *et al*., 2014; Zhang *et al*., 2014).

Other studies suggest an important link between the regulation of lipid metabolism and the anti-proliferative or pro-apoptotic actions of LXRs in cancer cells. As described above in this introduction, the upregulation of the lipid transporter ABCG1 inhibits proliferation of T cells, but also of cancer cells, through the alteration of intracellular cholesterol (El Roz *et al*., 2012). In a study with pancreatic cancer cells stimulated with LXR ligands, the ABCG1-derived alterations in the cell membrane changed the structure of lipid rafts, interfering with Akt phosphorylation (Pommier *et al*., 2010). In glioblastoma cells, the LXR ligand LXR-623 (a partial agonist for LXRα and full agonist for LXRβ) promotes cell death due to cholesterol depletion. Upregulation of several mediators of cholesterol homeostasis, namely ABCA1, ABCG1, and IDOL, contributed to these effects (Villa *et al*., 2016). In pancreatic cancer cells, the activation of the LXR pathway, through the upregulation of FAS, causes an accumulation of triglycerides that leads to cell cycle arrest (Kim *et al*., 2010).

Intriguingly, despite the previous considerations, the LXR inverse agonist SR9243, which induced the interaction of LXRs with co-repressors, downregulated the expression of genes involved in gluconeogenesis and lipogenesis and inhibited the Warburg effect, thus promoting cell death in many cell lines (Flaveny *et al*., 2015). Thus, more investigation is needed to clarify the exact role of the LXR pathway and lipid metabolism in cancer cells.

Another interesting finding was the discovery of dendrogenin A (DDA), a partial LXR agonist, acting as a promoter of lethal autophagy in human acute myeloid leukemia and melanoma cells. DDA is a steroidal alkaloid that is formed by the enzymatic conjugation of histamine and a cholesterol derivative (De Medina *et al*., 2013). DDA, through the activation of LXRβ, upregulated the expression of the pro-autophagic mediators nuclear receptor 77 (also known as Nur77), neuron-derived orphan receptor 1 (also known as NOR1), and the microtubule-associated protein 1A/1B light chain 3 (also known as LC3), involved in autophagosome formation. Moreover, DDA blocked the LXRβ-mediated

repression of the transcription factor EB, which further contributed to stimulating autophagy and lysosome biogenesis (Segala *et al*., 2017).

In addition to the direct effects in cancer cells, LXR activation can affect tumor progression by acting on the TME. Through the alteration of cholesterol homeostasis in endothelial cells and the subsequent change in lipid raft organization, LXR agonists can interfere with VEGF receptor signaling and reduce angiogenesis in the tumor (Noghero *et al*., 2012). Furthermore, treatment with the LXR ligands GW3965, or the newly developed RGX-104, induces apoptosis in MDSC through the upregulation of *ApoE* transcription (Tavazoie *et al*., 2018). The decrease in the numbers of MDSCs in the tumor was accompanied by an increase in cytotoxic T cells and pro-inflammatory Th cells and correlated with a reduction in tumor progression and metastasis (Tavazoie *et al*., 2018). The authors also showed that the combined treatment with RGX-104 and anti-PD-1 immunotherapy have synergistic effects on the inhibition of tumor progression in mice. RGX-104 is currently in a phase 1, first-in-human, dose escalation and expansion clinical trial in patients with advanced solid tumors or lymphoma under standard treatments, with some very promising preliminary results ("A Study of RGX-104 in Patients with Advanced Solid Malignancies and Lymphoma", in ClinicalTrials.gov. Link available in bibliography).

Despite the well documented antitumoral activities of the LXR pathway, it must be noted that, in some studies LXR activation has been linked to potential pro-tumoral effects. In this line, activation of LXRα by potential endogenous agonists secreted in the TME downregulated the expression of CCR7 in DCs. The downregulation of CCR7 reduced the migratory capacity of DCs to the lymph nodes, impairing tumor antigen presentation to T cells (Villablanca *et al*., 2010). In contrast with these results, it has been observed that LXR activation is required for CCR7-dependent chemotaxis. In DCs, this effect depends on the upregulation of the expression of the multifunctional enzyme CD38, which is a recently identified LXR target (Matalonga *et al*., 2017; Beceiro *et al*., 2018). On the other hand, hyaluronic acid produced within the tumor induces cholesterol efflux in TAMs through increased expression of ABCA1 and ABCG1, promoting a pro-tumoral switch in these cells in response to IL-4 (Goossens *et al*., 2019). Although hyaluronic acid has not been shown to activate LXRs, the involvement of cholesterol transporters in the

acquisition of a pro-tumoral role in TAMs is intriguing. Therefore, more investigation needs to be carried out to clarify the roles of the different molecules secreted in the TME and the involvement of the LXR pathway in this compartment.

Investigating new, better, more specific, antitumoral therapies must be one of the challenges to achieve in the next decades. LXR agonists, through its plethora of antitumoral effects, have arisen as promising therapeutic targets. Exploiting their effects in the tumor microenvironment offers new possibilities for the development of strategies to tackle tumor drug resistance.

5. Previous results from the group

Before the initiation of this thesis, the group had performed studies evaluating the consequences of LXR activation on tumor progression in a model of syngenic tumor growth in C57BL/6J mice. Treatment with the LXR agonist T0901317 (T1317) reduced tumor growth in wild type (WT) mice but not in LXR-deficient mice, even though 3LL-R tumor cells expressed functional LXRs (Figure 6). These results suggested that the functional expression of LXRs in host cells was necessary for the inhibitory effects of T1317 on tumor growth.

In separate experiments in murine bone marrow-derived macrophages (BMDM) LXRs agonists inhibited the IL-4- and GM-CSF-induced expression of the chemokines CCL17 and CCL22 at the mRNA level. In addition, the mRNA expression levels of CCL17 were also reduced in $MHCII^{high}$ TAMs stimulated with T1317 *in vivo*, and in $MHCII^{low}$ TAMs stimulated with T1317 *ex vivo.* This effect was not observed in TAMs isolated from tumors developed in LXRs-deficient mice.

In addition, the use of macrophages deficient for IRF4 allowed us to propose that this transcription factor is necessary for the IL4- and GM-CSF-mediated induction of CCL17 and CCL22 expression. LXR agonists were capable of inhibiting the upregulation of IRF4 mRNA expression in murine BMDM stimulated with IL-4 or GM-CSSF and in TAMs *in vivo*. This effect was not observed in TAMs isolated from LXR-deficient mice.

The data mentioned in this section were included in two doctoral theses, "*Selective roles of the nuclear receptor LXR in the transcriptional control of classical and alternative macrophage activation"*, presented by Dr. Theresa León in 2013 and, especially, "*Papel del receptor nuclear LXR en la proliferación y perfil metastático de células tumorales y en la actividad de macrófagos asociados a tumor"*, presented by Dr. Josep María Carbó in 2017.

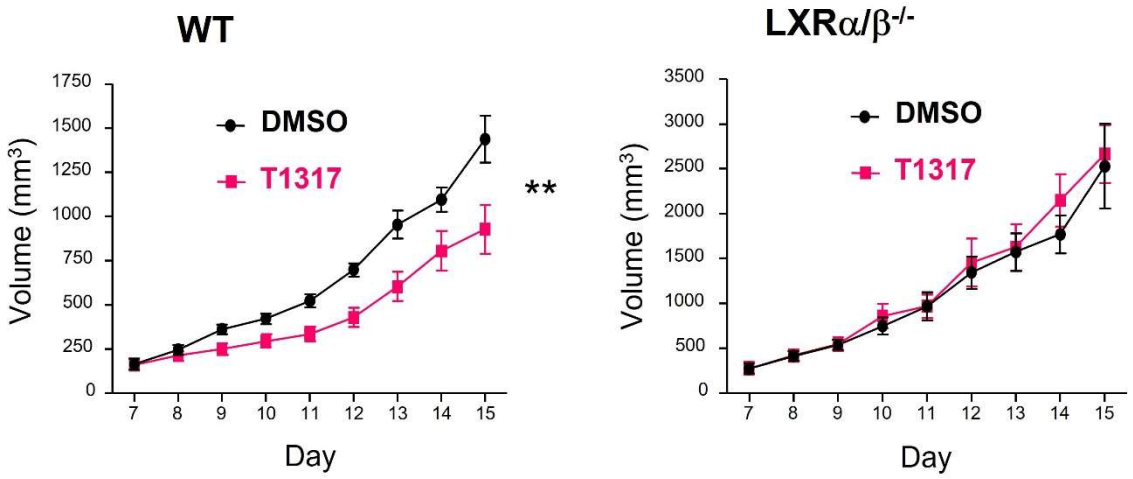


Figure 6. LXR activation inhibits tumor growth *in vivo*. C57BL/6 WT male mice (left) or LXR-deficient male mice (right) were subjected to subcutaneous injection of 3LL-R cells. The mice were treated daily with dimethyl sulfoxide (DMSO) or T1317 (15 mg/kg) from day 7 of tumor establishment. Graphic displays tumor volume progression curves. Mean ± SEM; n = 14 mice/group in WT mice and n = 8 (DMSO) and 9 (T1317) in LXR-deficient mice. Two-way ANOVA-repeated measures; **, $p < 0.01$. Data from Dr. Josep María Carbó doctoral thesis "*Papel del receptor nuclear LXR en la proliferación y perfil metastático de células tumorales y en la actividad de macrófagos asociados a tumor"*, 2017.

HYPOTHESIS

Based on the previous observations in our laboratory we hypothesized that LXR activation may exert important antitumoral actions in the TME. In this sense, through the inhibition of chemokine expression, LXR activation could affect the recruitment of Tregs to the tumor microenvironment, thus contributing to ameliorate tumor progression. In addition, given the key role of LXRs in the regulation of macrophage biology, we hypothesized that LXR activation could be influencing the expression profile of TAMs beyond the effects on chemokine expression.

OBJECTIVES

1 To study the effects of pharmacological LXR activation on tumor growth in different cancer settings.

2 To evaluate the effects of pharmacological LXR activation on several immune cell populations within the TME.

3 To explore the cellular and molecular mechanisms contributing to the antitumoral effects of pharmacological LXR activation.

MATERIALS AND METHODS

Reagents

The synthetic high affinity LXR agonists T0901317 (T1317) and GW3965 were purchased from Cayman Europe and Tocris, respectively. Recombinant murine GM-CSF and human M-CSF and IL-4 were purchased from PreproTech.

Animals

C57BL/6J mice were purchased from Envigo or Harlan and raised as a colony in our animal facility. LXR-deficient mice were initially donated by Dr. David Mangelsdorf (UT Southwestern Medical Center, Dallas, TX, USA) and backcrossed into C57BL/6 background for more than ten generations. FoxP3-EGFP mice were generated by crossing the NOD.FoxP3-EGFP strain (23) with C57BL/6J mice for five generations. PyMT mice with an FVB/N background were obtained from The Mouse Models of Human Cancers Consortium Repository (National Cancer Institute, Frederick, MD, USA) and backcrossed into the C57BL/6J background for nine generations. STAT1-deficient mice were donated by Dr. Anna Planas (IDIBAPS, Barcelona Spain) (Gorina *et al*., 2011) and raised as a colony under non-SPF conditions. Mice with specific p38α ablation in the myeloid cell lineages (p38αΔM) were donated by Dr. Ángel Nebreda (IRB, Barcelona, Spain) and were generated by crossing animals bearing the exon2 of p38α flanked by loxP sites (p38α loxP/loxP) (Heinrichsdorff *et al*., 2008; Ventura *et al*., 2007) with mice expressing the Cre recombinase under control of the LysozymeM gene promoter (LysM-Cre) (Clausen *et al*., 1999). All the mice were C57BL/6 background and Cre was always kept in heterozygosis. IRF4-deficient mice and control C57BL/6 mice were bred at the animal facility of the Biomedical Research Center at the University of Marburg, Germany. CD38-deficient mice were obtained from Jaime Sancho (Instituto de Parasitología y Biomedicina López Neyra, Granada, Spain) under an agreement with Jackson Laboratories (Cockayne *et al*., 1998). The mice were fed a regular chow diet. All the protocols requiring animal manipulation have been approved by the Institutional Animal Care and Use Committees from Parc Científic de Barcelona (#9672), Universitat de Barcelona (#7088), Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau (#7281) and the University of Marburg (RP Giessen, Germany).

Primary bone marrow-derived macrophages

Bone marrows were harvested from the femurs and tibias of six to ten-week-old mice. Bone marrow precursors were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 20% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich), and 30% conditioned media from the L929 cell line as a source of M-CSF.

Primary human macrophages

The mononuclear cell fractions from peripheral blood mononuclear cells samples from healthy donors were obtained through a ficoll density gradient centrifugation. Erythrocytes were removed with Red Blood Cell Lysis buffer (Invitrogen) following the manufacturer's instructions. The cells were seeded in culture plates and incubated in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% human serum for 30 min, allowing the cells to adhere to the culture plates. After discarding non-adherent cells, adhered cells, considered monocytes, were incubated in RPMI supplemented with 10% FBS and human recombinant M-CSF (50 ng/ml) to induce differentiation into macrophages. The protocol has been approved by the Bioethics Commission of the University of Barcelona and the blood samples were obtained from the Blood and Tissue Bank from *Generalitat de Catalunya*.

Cell lines culture conditions

The 3LL-R cell line (a murine lung Lewis carcinoma cell line) was kindly provided by Prof. Dr. Jo Van Ginderachter (Vrije Universiteit Brussel, Brussels, Belgium). 3LL-R cells were maintained in RPMI media containing L-glutamine (0.3 g/L) (BioWest) and supplemented with 10 % FBS. 3LL-R were used within 15 passages after thawing.

Generation of 3LL-R conditioned medium

3LL-R cells were cultured as indicated previously until passage five. To obtain the conditioned medium, cells were allowed to achieve 85% confluency. The medium was removed, the cells were washed once with PBS and then incubated with RPMI-2% FBS

for 24h at 37 ºC. The medium was recovered and centrifuged to eliminate cells and debris and the supernatant was aliquoted and stored at – 80 ºC.

Subcutaneous 3LL-R tumor model

3LL-R cells ($3x10^6$) were subcutaneously injected in the lower back of eight to ten-week-old WT, LXRα/β-deficient, IRF4-deficient, or CD38-deficient mice (C57BL/6J background). The tumors were allowed to grow for two weeks. At day 7, once the tumor was established and for the next 8 days, the animals received a daily dose of T1317 (15 mg/kg) through an intraperitoneal injection. Control mice received an equivalent dose of vehicle (DMSO) diluted in PBS. The tumors were measured using a digital caliper from day 7 to day 15 (length (D) and width (d) measures were taken), and tumor volume was calculated with the formula V = pi * (d2 * D)/6. At day 15, the mice were sacrificed, and the tumors were harvested and processed as described in the following sections.

PyMT mammary cancer model

Spontaneous tumor development was evaluated in PyMT transgenic mice expressing high levels of the transforming oncogene polyoma middle T antigen under the control of the mouse mammary tumor virus long terminal repeat promoter, which specifically directs expression in the mammary epithelium (Guy *et al*., 1992). After weaning, PyMT female mice were divided in two groups: one group was administered a regular chow diet (A04; Scientific Animal Food & Engineering) and a second group was administered the same diet supplemented with 50 mg/kg of T1317. The mice were monitored every three days for palpable tumors starting at six weeks of age. Tumor latency was defined as the time to the development of the first palpable tumor in each mouse. The mice were euthanized at 22 weeks of age. Total tumor burden was determined after all the mammary glands were carefully excised and weighed, and the mass of the tumor-bearing mammary glands was measured. Each mammary gland was labeled as in Cedó *et al*., 2016 (see Figure 7 for mammary gland distribution).

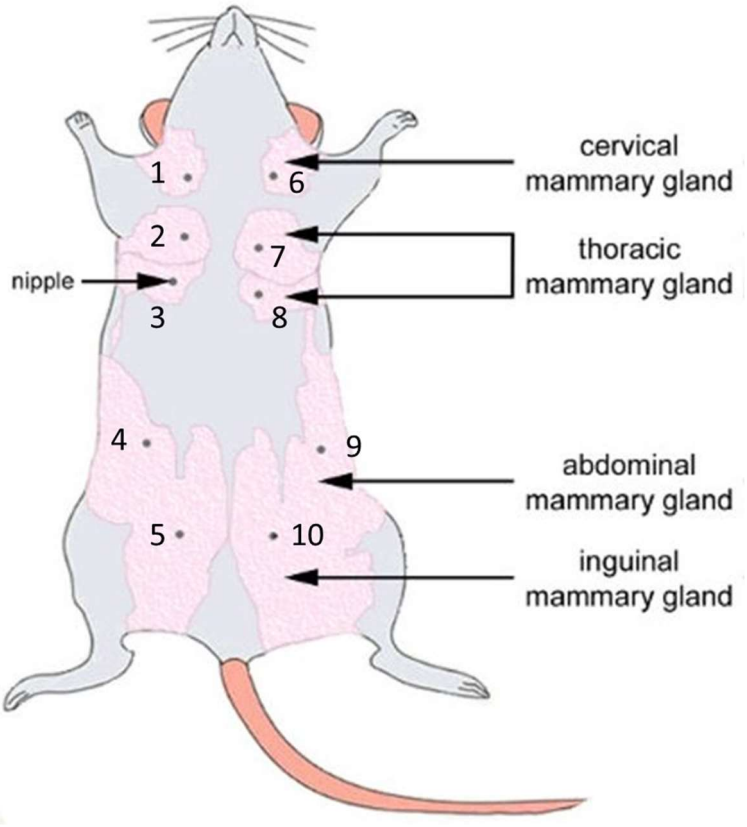


Figure 7. Schematic representation of the anatomical location of mammary glands in a female mouse. Figure adapted from Honvo-Houéto & Truchet, 2015.

Identification of intratumoral immune cell populations

Tumors were induced by the subcutaneous injection of 3LL-R cells ($3x10^6$) in host mice as described above. At day 15 post-injection, the tumors were harvested and mechanically disintegrated. Tumor homogenates were incubated with a cocktail of digestive enzymes (10 U/ml collagenase I, 400 U/ml collagenase IV and 30 U/ml DNAse I) (Worthington) for 25 minutes at 37ºC and subsequently filtered through a 70 µm cell strainer. Erythrocytes were eliminated through incubation with Red Blood Cell Lysis buffer (Invitrogen) following manufacturer's instructions. Mononuclear cells were isolated through a Lymphoprep density gradient (Stemcell Technologies) centrifugation. Final cell suspensions were diluted to a concentration of 10^7 cells/ml in PBS and incubated with Fc block (rat anti-mouse CD16/CD32, BD Biosciences) (1:50, 30 min, 4 ^oC). For myeloid cell analysis, the cells were incubated with specific fluorochrome-

labelled antibodies against CD11b, Ly6G, Ly6C and IA/IE (MHCII) (table 1) (1:100 in Hank's balanced salt solution (HBSS)-2 % FBS, 30 min, 4 ºC, dark conditions). Cell populations were analyzed through flow cytometry using a Gallios Flow Cytometer (Beckman-Coulter) (see gating strategy in Figure 8).

Antibody	Fluorochrome	Clone	Company
anti-Ly6C	AF647	ER-MP20	BioRad
anti-IA/IE (MHC II)	BV421	M5/114.15.2	BD Pharmingen
anti-CD11b	PE-Cy7	M1/70	eBioscience
anti-Ly6G	PerCP-Cy5.5	1A8	Biolegend
anti-CD4	Pacific blue	RM4-5	Biolegend
anti-CD8 (in FoxP3-EGFP tumors)	APC	53-6.7	Biolegend
anti-CD8	PE	53-6.7	Biolegend
anti-FoxP3- (for intracellular staining)	APC	FJK-16s	eBioscience
anti-CD16/CD32 (for Fcγ blocking)		2.4G2	BD Biosciences

Table 1. Antibodies used for intratumoral immune cell identification, quantification, isolation and purification through flow cytometry and cell sorting.

For lymphoid cell determination, the cells were incubated with specific antibodies against CD4 and CD8 (table 1) (1:100 in HBSS-2 % FBS, 40 min, 4 ºC, dark conditions). The

cells were then fixed and permeabilized using the FoxP3/Transcription factor staining buffer set (Invitrogen) following the manufacturers' specifications and incubated with anti-FoxP3 antibodies (table 1) (30 min, room temperature, dark conditions). The cells were analyzed by flow cytometry using a Gallios Flow Cytometer (Beckman-Coulter) (Figure 8).

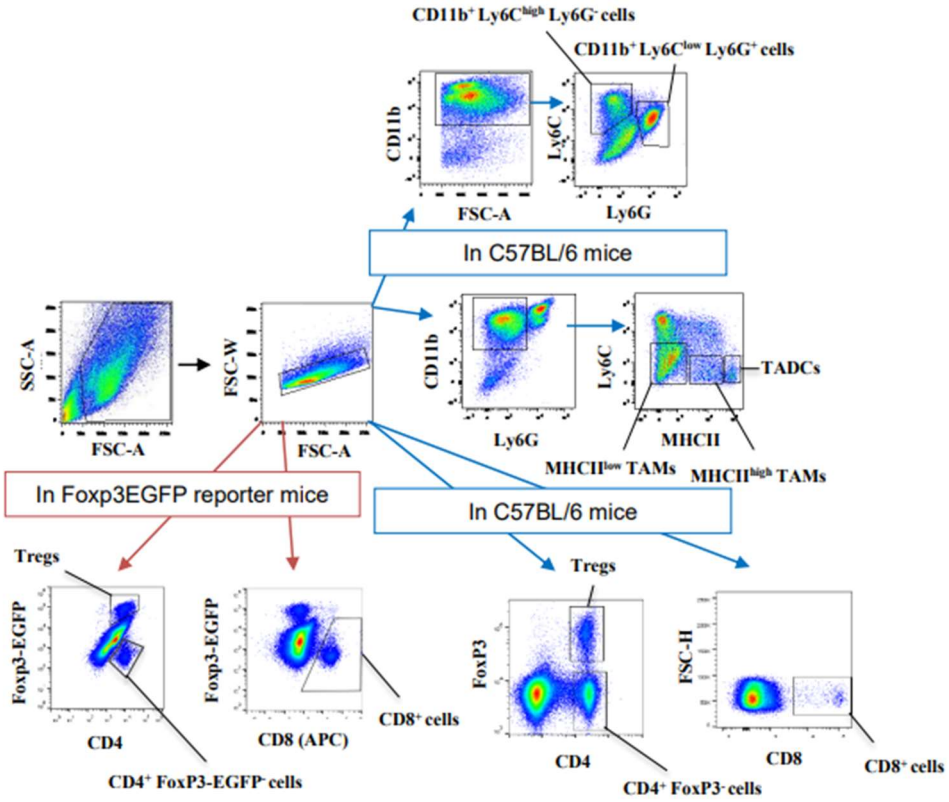


Figure 8. Gating strategy for the identification of immune cell populations within 3LL-R tumors. Cell suspensions were enriched for mononuclear cells using a Lymphoprep gradient density separation method. Arrows indicate the sequence of gating. Cells presenting a lower SSC-FSC profile were initially discriminated. Among the selected cells, singlets were gated and analyzed using the following selection criteria. In C57BL/6 mice and knock out models, myeloid cell determination was carried out in two steps. First, CD11b+ cells were gated, and two populations were analyzed based on the expression of Ly6C and Ly6G: CD11b+/Ly6C^{high}/Ly6G- cells and CD11b+/Ly6C^{low}/Ly6G+ cells. Second, CD11b+/Ly6G- cells were gated and three populations were identified based on the lack of expression of Ly6C and differential expression levels of MHC II, namely MHCII^{low} TAMs, MHCII^{high} TAMs and TADCs (MHCII^{bright}). For lymphoid cell determination, CD8+ cells (mostly cytotoxic T lymphocytes), CD4+/FoxP3- (predominantly Th cells) and Tregs (CD4+/FoxP3+) were analyzed. In FoxP3-EGFP transgenic mice, Tregs were considered CD4+/FoxP3-EGFP+.

Identification of intratumoral lymphoid cell populations in FoxP3-EGFP mice

For lymphocyte cell determination, 3LL-R cells were injected subcutaneously in FoxP3-EGFP reporter mice. The tumors were collected at day 10 post injection and cell suspensions were generated and blocked with Fc block as described above. The cells were then incubated with specific antibodies against CD4 and CD8 (table 1) as described above and analyzed using a Gallios Flow Cytometer (Beckman-Coulter). Tregs were identified via EGFP expression analysis (Figure 8).

Identification of murine spleen lymphoid cell populations

For some experiments, spleens were mechanically disaggregated and filtered through a 70 µm nylon strainer. After erythrocyte lysis and centrifugation, the cells were resuspended in PBS. Lymphoid cells populations were stained with specific fluorochrome-labelled antibodies (table 1) and analyzed by flow cytometry as described above (Figure 8).

In vivo Treg depletion

To reduce systemic Treg abundance, male FoxP3-EGFP reporter mice were treated with anti-CD25 antibodies (InVivoMab anti-mouse CD25 (IL-2Ra), clone PC-61.5.3, Bio X Cell (#BE0012)) (200 µg per animal diluted in PBS; intraperitoneal injection) at days 2, 5 and 8 post-3LL-R cell injection. Isotype control was administered to control mice (InVivoMAb rat IgG isotype control anti-horseradish peroxidase, clone HRPN, Bio X Cell (#BE0088)). At day 5, and until day 9, the mice received intraperitoneally a daily dose of T1317 (15 mg/kg), or vehicle (DMSO) diluted in PBS. Tumor volume was measured as described above. On day 10, the mice were sacrificed, and the spleens and tumors were harvested and processed as described in previous sections.

Isolation of TAMs by cell sorting

Tumors were induced, harvested, and processed as indicated in previous sections. A cell suspension from a pool of tumors was generated (five tumors in each experiment). The cells were incubated with the antibodies chosen for myeloid cell analysis (see table 1). $MHCII^{high}$ TAM and $MHCII^{low}$ TAM populations were isolated using a FACSaria Fusion cell sorter (BD Bioscience). For *ex vivo* experiments, TAMs were cultured in RPMI-10 % FBS, supplemented with L-glutamine, HEPES, 10 mM sodium pyruvate, non-essential amino acids (BioWest) and 3.7 nM 2-mercaptoethanol (Sigma-Aldrich).

Isolation of Tregs by cell sorting

Tumors were induced in FoxP3-EGFP transgenic mice as indicated in previous sections. Tumors were harvested at day 10 post-injection and processed in pools to get a cell suspension. Tregs were sorted as $EGFP^{+}$ cells using a FACSaria Fusion cell sorter. The cells were maintained in RPMI-10 % FBS for subsequent analysis. Alternatively, Tregs were isolated from the spleens of FoxP3-EGFP mice.

Treg proliferation assays

Tregs isolated by cell sorting were stained with CellTrace CFSE cell proliferation kit (Invitrogen) following the manufacturer's instructions, seeded in 96 well plates (10^5 cells/well) and then stimulated with T1317 (1 µM) or DMSO for 18 h at 37 ºC. As a technical negative control for flow cytometry, unstained cells were seeded separately. Cell proliferation was stimulated with the mouse T cell activation/expansion kit (Miltenyi Biotec), in the presence of IL-2 (100 U/ml). The cells were allowed to proliferate for 48h. CFSE dispersion was analyzed through flow cytometry compared to non-activated Tregs. Murine IL-2 was expressed in *Escherichia coli* and purified from as described in Izquierdo *et al*., 2018.

Suppression of T cell proliferation

TAMs or Tregs, isolated as indicated above, were seeded in 96 well plates (200,000 TAMs/well or 100,000 Tregs/well in RPMI-10 % FBS) and stimulated with T1317 (1 µM) or DMSO for 18 h at 37 ºC. In experiments using TAMs, the medium was replaced without the LXR agonist. In experiments using Tregs, the cells were recovered, washed in PBS, and resuspended in fresh medium without LXR agonist. Total splenocytes were obtained from the spleens of C57BL/6J mice as indicated above and stained with the CellTrace CFSE cell proliferation kit following the manufacturer's specifications. Splenocytes non-stained with CFSE were grown separately as a negative technical control for cytometry. CFSE-stained splenocytes were either grown alone in RPMI-10 % FBS or co-incubated with TAMs or Treg at a 1:1 ratio. T cell proliferation was induced using the mouse T cell activation/expansion kit. The cells were allowed to proliferate for 48 h at 37 ºC and CFSE dispersion was analyzed by flow cytometry.

Phagocytosis assay

$MHCII^{high}$ TAMs and $MHCII^{low}$ TAMs, were seeded in 24 well plates (500,000 cells/well) and stimulated with T1317 1 µM or DMSO for 18 h at 37 ºC. The cells were incubated with 3-µm fluorescent microspheres (Fluoresbrite YG microspheres, Polysciences) at a ratio of 20 beads/cell for 30 min at 37 ºC. After this time, the plates were placed on ice and the cells were washed three times with ice-cold PBS. The cells were recovered and fixed in PBS-2 % PFA. The phagocytosis of microspheres was analyzed by flow cytometry.

RNA extraction, cDNA synthesis and quantitative real-time PCR analysis

For RNA extraction, the cells were lysed using Tri-reagent (Invitrogen) and total RNA was isolated by phenol-chloroform extraction. RNA samples were quantified using a ND-2000 Spectrophotometer (NanoDrop Technologies). For the synthesis of cDNA, 1µg of total RNA from each sample was reverse transcribed using M-MLV Reverse transcriptase RNase H Minus, Point Mutant, $oligo(dT)_{15}$ primer and deoxy-nucleotide mix (Promega).

Quantitative real time PCR (qPCR) was performed using the Power SYBR Green Reagent Kit (Applied Biosystems) following the manufacturer's recommendations. The sequences of primers used for qPCR analysis are shown in Table 2. Annealing for all primers was performed at 60 ºC. Real-time monitoring of PCR amplification was performed using the CFX384 Real-Time PCR Detection System (Bio-Rad). The data were expressed as mRNA levels relative to ribosomal *L14* or to *Gapdh* expression in murine and human cells, respectively.

Gene	Species	Forward primer	Reverse primer
Abca1	Mouse	5'-GCGAGGGCTCATCGACAT	5'-GAAGCGGTTCTCCCCAAAC
Abcg1	Mouse	5'-TCACCCAGTTCTGCATCCTCTT	5'-GCAGATGTGTCAGGACCGAGT
Angptl3	Mouse	5'-GCACCAAGAACTACTCCCCC	5'-CATGGACTGCCTGATTGGGT
Apoe	Mouse	5'-CTGACAGGATGCCTAGCCG	5'-CGCAGGTAATCCCAGAAGC
Arg1	Mouse	5'-TTGCCAGACGTAGACCCTGG	5'-CAAAGCTCAGGTGAATCGGC
Cd24a	Mouse	5'-CCACGCAGATTTACTGCAAC	5'-AGACGTTTCCTGGCCTGAGT
Foxp3	Mouse	5'-CCCATCCCCAGGAGTCTTG	5'-ACCATGACTAGGGGCACTGTA
Il1b	Mouse	5'-TGGGCCTCAAAGGAAAGAAT	5'-CAGGCTTGTGCTCTGCTTGT
Il10	Mouse	5'-AGCCTTATCGGAAATGATCCAGT	5'-GGCCTTGTAGACACCTTGGT
L14	Mouse	5'-TCCCAGGCTGTTAACGCGGT	5'-GCGCTGGCTGAATGCTCTG
Lxra	Mouse	5'-CCTTCCTCAAGGACTTCAGTTACAA	5'-CATGGCTCTGGAGAACTCAAAGAT
Lxrb	Mouse	5'-CATTGCGACTCCAGGACAAGA	5'-CCCAGATCTCGGACAGCAAG
Mgl2	Mouse	5'-AGGCACCCTAAGAGCCATTT	5'-CCCTCTTCTCCAGTGTGCTC
Nos2	Mouse	5'-GCCACCAACAATGGCAACA	5'-CGTACCGGATGAGCTGTGAATT

Ptgs2	Mouse	5'-ATTCTTTGCCCAGCACTTCA	5'-GGGATACACCTCTCCACCAA
Retnla	Mouse	5'-CCCTTCTCATCTGCATCTCC	5'-CAGTAGCAGTCATCCCAGCA
S100a8	Mouse	5'-ACTTCGAGGAGTTCCTTGCG	5'-TACTCCTTGTGGCTGTCTTTGT
Srebf1	Mouse	5'-AGGCCATCGACTACATCCG	5'-ATCCATAGACACATCTGTGCCTC
Tgfb1	Mouse	5'-GAGCCCGAAGCGGACTACTA	5'-TGGTTTTCTCATAGATGGCGTTG
Ccl17	Human	5'-CTCCAGGGATGCCATCGTTT	5'-TCTCTTGTTGTTGGGGTCCG
Ccl22	Human	5'-ATGGATCGCCTACAGACTGC	5'-GGATCGGCACAGATCTCCTT
Gapdh	Human	5'-GTGAAGGTCGGAGTCAACGG	5'-TTGAGGTCAATGAAGGGGTCA
Irf4	Human	5'-CCCGGAAATCCCGTACCAAT	5'-AGGTGGGGCACAAGCATAAA

Table 2. List of sequences of primers used for qPCR analyses

Gene expression profiling

TAMs were purified by cell sorting and stimulated *ex vivo* with T1317 as indicated in previous sections. Total RNA from TAMs was purified using the RNAeasy Kit (Qiagen) following the manufacturer's instructions. The quantity and purity of the RNA samples were determined using an ND-2000 Spectrophotometer (NanoDrop Technologies) and RNA integrity was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies). Only samples with acceptable purity and integrity were subsequently used in microarray experiments. The amplification and labelling of the samples were performed according to the protocol in GeneChip WT Plus reagent kit (Affymetrix) and using an input of 100 ng of total RNA. Processed samples were hybridized to GeneChip Mouse Clariom S Array (Affymetrix) in a GeneChip Hybridization Oven 640 (Affymetrix). Washing and scanning were performed using the Expression Wash and Stain and the GeneChip System of Affymetrix (GeneChip Fluidics Station 450 and GeneChip Scanner 3000 7G). Data quality control was assessed using Affymetrix Expression Console software. All arrays met the

quality control criteria. For each sample, expression estimates were calculated from probe intensities and represented as log2 values. Heat maps were produced with Heatmapper (Wishart Research Group, University of Alberta, Canada). Gene ontology (GO) analysis was carried out with the PANTHER Classification System. Microarray data have been deposited at the ArrayExpress database with accession number E-MTAB-9707.

Protein extraction and western blot analysis

The cells were washed twice in cold PBS and lysed on ice with RIPA lysis solution (1 % Triton X-100, 10 % glycerol, 50 mM HEPES, pH 7.5, 250 mM NaCl, protease inhibitors, 1 mM sodium orthovanadate). Insoluble material was removed by centrifugation at 13,000 x g for 8 min at 4 °C. Cell lysates (50–100 µg) were boiled at 95 °C in Laemmli SDS-loading buffer, separated by SDS-PAGE and electrophoretically transferred to PVDF membranes (Immobilon-FL). The membranes were blocked in 1 X Odissey blocking buffer (Li-Cor) in TBS-0.1 % Tween 20 (TBS-T) and then incubated with primary antibodies (table 3). The membranes were washed three times in TBS-T and then incubated for 1 h with fluorescently-labelled secondary antibodies (table 3). After three washes of 15 min with TBS-T, fluorescence was detected at 800 nm using an Odyssey® FC Imaging System (Li-Cor). In some cases, the same blot was used repeatedly for two different sets of antibodies after the treatment of the membrane with 1x NewBlot PVDF Stripping Buffer (LiCor) for 20 min, followed by three washes in TBS.

Antibody	P/S	Clone	Company
Goat polyclonal anti-mouse IRF4	Primary	M17	Santa Cruz Biotechnology
Mouse monoclonal anti-β-actin	Primary	AC-15	Sigma-Aldrich
Donkey anti-goat IRDye 800CW	Secondary		Li-Cor
Goat anti-mouse IRDye 800CW	Secondary		Li-Cor

Table 3. Antibodies used for western blot analyses.

ELISA

The supernatants from macrophage cultures were recovered and stored at -80 ºC. ELISA kits from Thermo Scientific (Mouse MDC (CCL22) ELISA kit and Mouse TARC (CCL17) ELISA kit) were used for quantitative measurement of secreted mouse CCL22 and CCL17, respectively, using the manufacturer's recommendations. Briefly, dilutions of the supernatants or a chemokine standard solution in sample diluent buffer were incubated in 96-well plates pre-coated with the specific monoclonal antibody (room temperature, 2.5 h). Sequentially, the wells were incubated with biotinylated detection antibodies (room temperature, 1 h) and avidin-biotin-peroxidase complexes (room temperature, 45 min). After each of these steps, the wells were washed four times in 1X wash buffer. The wells were incubated with peroxidase substrate TMB color developing agent (room temperature in the dark). Stop solution was added into each well and the absorbance at 450 nm was determined in a microplate reader within 30 min after adding the stop solution. Each sample was analyzed in duplicate and the concentration of secreted chemokines in the supernatants was interpolated from the standard curve.

Statistical Analysis

The GraphPad Prism 6.0 software was used to perform all statistical analyses. Differences in tumor volume in the 3LL-R model were analyzed by a two-way repeated-measure ANOVA with a Bonferroni *post hoc* test. The log-rank and Gehan-Wilcoxon tests were used to compare tumor latency curves in the PyMT model. The rest of the data was analyzed using either one way ANOVA, or two-tailed Student's *t*-test for data with normal distribution, or the non-parametric Kruskal Wallis-Dunn's test or *Mann–Whitney* U test for data not following normal distribution.

RESULTS

1. Effects of pharmacological LXR activation on tumor progression.

In previous experiments in our laboratory, we established a model of experimental cancer in C57BL/6J mice based on the subcutaneous injection of the Lewis lung carcinoma 3LL-R cell line. As mentioned in the introduction ("Previous results from the group" section), treatment with the LXR agonist T1317 reduced tumor growth. Importantly, functional expression of LXRs in host cells was required for this effect. In those studies, the LXR agonist was administered through intraperitoneal injection once the tumor was stablished.

To analyze if intraperitoneal administration of the LXR agonist results on effective activation of the LXR pathway within the tumor the expression of canonical LXRs target genes was evaluated in whole tumors. The LXRs agonist induced significatively the mRNA expression of the sterol transporter *Abca1*. A tendency for increased expression was also observed for *Abcg1* and *Srebf1* (SREBP1c). Of note, T1317 did not alter the expression of *ApoE* in whole tumors (Figure 9).

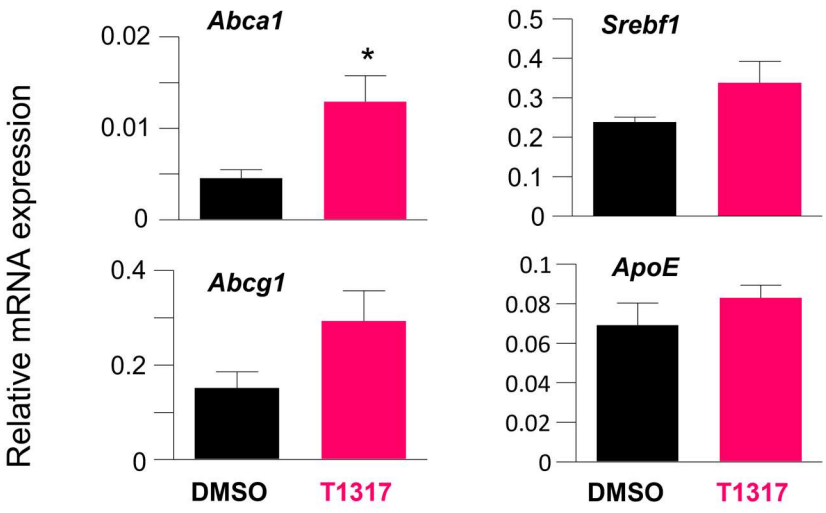


Figure 9. Expression of LXRs target genes in whole tumors. Mice carrying 3LL-R tumors were administered daily an intraperitoneal injection of either T1317 (15mg/kg) or DMSO for 7 days. Total RNA was recovered from whole tumors at day 15. Gene expression was evaluated by qPCR. Mean±SEM; n=4mice/group. T-test; *, p <0.05.

Next, we compared the effects of T1317 on tumor progression in male and female C57BL/6J mice. As shown in Figure 10A-B, T1317 treatment in 3LL-R tumor-bearing male mice resulted in significantly reduced tumor progression, in line with the previous results from the group. In tumor-bearing female mice, higher variability was observed between the tumoral growth curves in the experimental group treated with vehicle. However, the LXR agonist was able to reduce tumor growth also in female mice (Figure 10B). It is possible that the growth of 3LL-R tumors female C57BL/6J is highly influenced by hormonal factors, thus displaying higher variability within experimental groups. In addition, we compared the growth of 3LL-R tumors in WT and LXR-deficient male mice (Figure 10C). Tumor growth was enhanced in the absence of functional LXRs in the host.

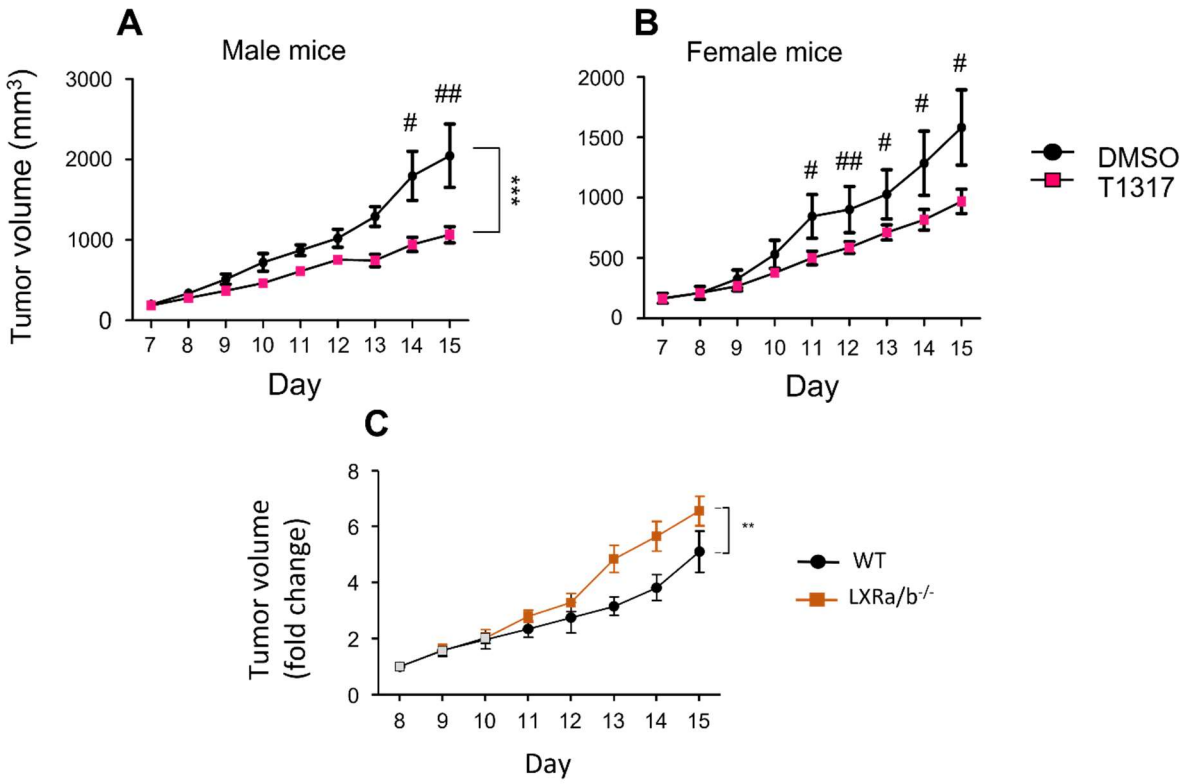


Figure 10. Pharmacological activation of LXR reduces the growth of 3LL-R tumors. C57BL/6J male (A-C) or female (B) mice were subjected to subcutaneous injection of 3LL-R cells (3x10^6 3LL-R cells/mouse). In A-B, from day 7 post-cancer cell injection, once the tumors were stablished, the mice were treated daily with DMSO or T1317 (15 mg/kg) through intraperitoneal injection. Tumor volume was measured daily up to day 15. In C, the tumors were allowed to

grow without treatment with an LXR agonist or vehicle. Graphics display tumor volume progression curves. Mean ± SEM; In A-B, n = 6–7 mice/group. In C, n = 10-11 mice/group. Two-way ANOVA-repeated measures. **, $p < 0,01$; ***, $p < 0.001$. T test. #, $p < 0.05$; ##, $p < 0.01$.

In collaboration with Dr. Joan Carles Escolà Gil and Dr. Lidia Cedó (Institut d'Investigacions Biomèdiques (IIB) Sant Pau, Barcelona, Spain), the effects of LXR activation were also evaluated in PyMT transgenic female mice, which spontaneously develop breast adenocarcinoma. In these studies, the LXR agonist was administered orally with the diet. Tumor latency (the amount of time elapsed until the tumors were detectable) was not altered by the LXR agonist, compared to the control mice (Figure 11A). However, treatment with T1317 decreased mammary gland weight at week 22 of age, when the mice were sacrificed (Figure 11B-C). These observations suggest that LXR activation in this model does not interfere with tumor initiation and establishment, but it does impact tumor progression.

Taking together the data obtained after injection of 3LL-R cells and the data from spontaneous tumor development, our results suggest that pharmacological LXR activation is effective in preventing tumor growth once the tumors are stablished. These findings argue for a major role of the LXR pathway in the TME of stablished tumors, rather than in the interference with cancer cell proliferation or early-stage carcinogenesis.

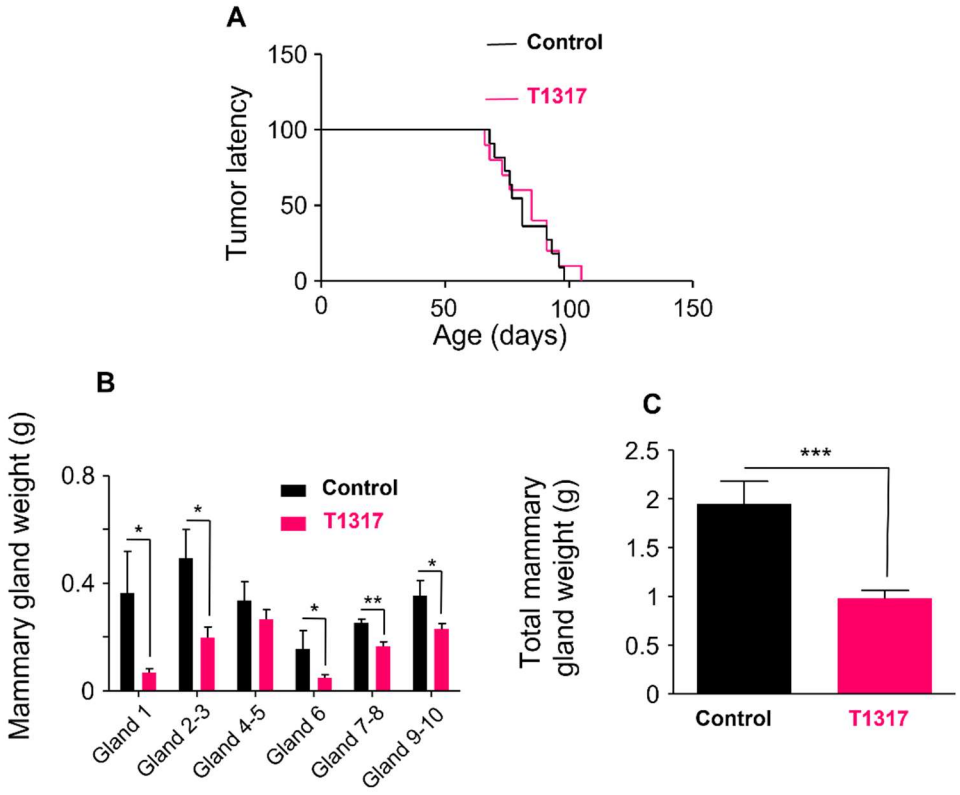


Figure 11. Effects of LXR activation on spontaneous tumor development and progression. PyMT female mice were administered a chow diet with or without supplementation with T1317. In A, tumor latency was evaluated daily. In B, at week 22 of age, the mice were sacrificed, and the mammary glands were excised and weighted. In C, total mammary gland weight. Mean ± SEM; n = 8 (Control), n = 10 (T1317). In A, Log-Rank-Wilcoxon test. In B-C, Mann–Whitney U test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

2. Effects of pharmacological LXR activation in the frequency and abundance of intratumoral immune cell populations.

The TME is a complex network of cells, mostly immune cells, that display either pro-tumoral or antitumoral roles. To determine if the effects on tumor growth observed upon T1317 administration depend on alterations in the tumor microenvironment, we analyzed by flow cytometry the frequency of several intratumoral immune cell populations with prognostic value (see gating strategy in Figure 8). These studies were

performed using the model of subcutaneous 3LL-R cancer cell injection. To minimize intra-group variability factors, we carried out the experiments in male mice.

In the myeloid compartment five different populations were targeted: CD11b+/Ly6C^{high}/Ly6G- cells (that are enriched in Ly6C^{high} monocytes and monocytic MDSCs), CD11b+/Ly6C^{low}/Ly6G+ cells (compatible with neutrophils and polymorphonuclear MDSCs), tumor associated DCs (TADC) identified as CD11b+/Ly6C-/Ly6G-/MHCII^{bright}, and two subpopulations of TAMs expressing different levels of MHC II (described in the introduction section), namely MHCII^{low} TAMs and MHCII^{high} TAMs (both populations are CD11b+/Ly6C-/Ly6G-).

Activation of the LXR pathway did not have any impact on the intratumoral frequencies of the populations enriched in MDSCs, TADC or MHCII^{low} TAMs. However, a reduced intratumoral frequency in MHCII^{high} TAMs was observed upon administration of T1317 (Figure 12).

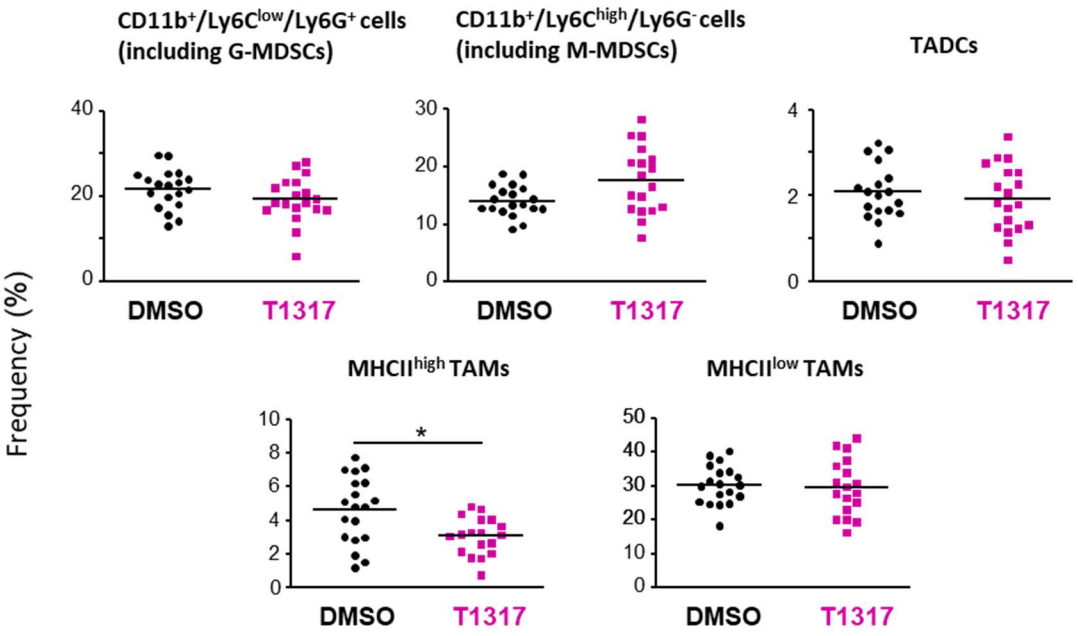


Figure 12. Effects of LXR activation on the frequency of intratumoral myeloid cell populations. C57BL/6J male mice were subjected to subcutaneous injection of 3LL-R cells. The mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 post-cancer cell injection. The tumors were collected at day 15 post-cancer cell inoculation. The frequency (percentage of gated live

singlets) of different myeloid cell populations was measured by flow cytometry. Horizontal bars indicate mean values in each experimental group. Pooled data from three independent experiments; n = 18–19 mice/group. Mann–Whitney test. *, $p < 0.05$.

We also analyzed the abundance of these myeloid cell populations by determining total cell numbers in each tumor and normalizing the data by tumor weight. The reduction in the frequency of MHCII^{high} TAMs was accompanied by only a tendency toward a decreased amount of these cells in the tumors from T1317 treated mice. These observations suggest that the decrease in the relative frequency of MHCII^{high} TAMs in response to T1317 does not consistently translate in reduced numbers of this cells in the tumor (Figure 13). In LXR-deficient mice, no significant changes were observed in the abundance of several myeloid cell populations when compared to the WT control counterparts (Figure 13).

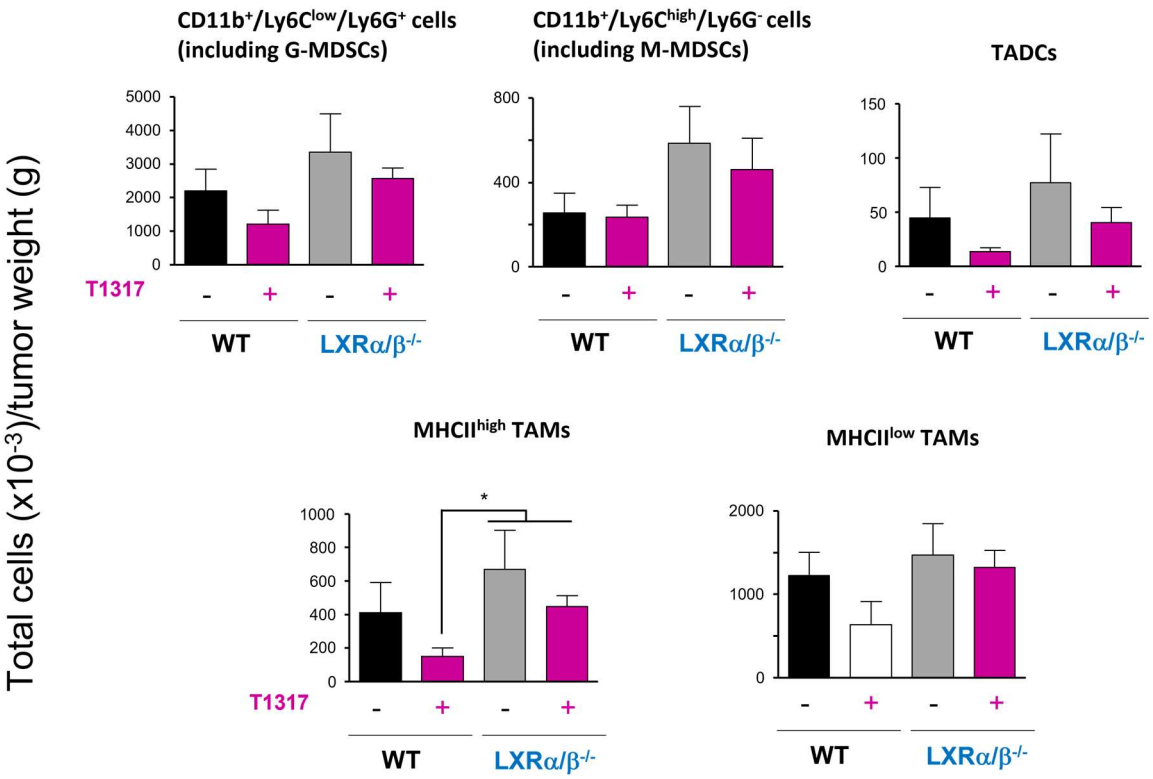


Figure 13. Total numbers of intratumoral myeloid cell populations in WT and LXR-deficient mice. WT (C57BL/6J) or LXR-deficient male mice were subjected to subcutaneous injection of

3LL-R cells. The mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 post-cancer cell injection. The tumors were collected at day 15 post-cancer cell inoculation. The abundance of different immune cell populations was measured by flow cytometry. The absolute numbers of cells were normalized to the tumor weight. Similar results were obtained when normalization was done using tumor volume. n = 5 mice/group. One-way ANOVA test followed by Newman–Keuls post hoc. *, $p < 0.05$.

In the intratumoral lymphoid cells compartment, three different populations were targeted: CD8+ lymphocytes (considered cytotoxic T cells), CD4+ FoxP3- lymphocytes (considered Th cells), and CD4+ FoxP3+ lymphocytes (considered Treg). As shown in Figure 14, the LXR ligand T1317 reduced the frequency of Tregs in wild type mice, while not affecting the frequency of cytotoxic or Th cells.

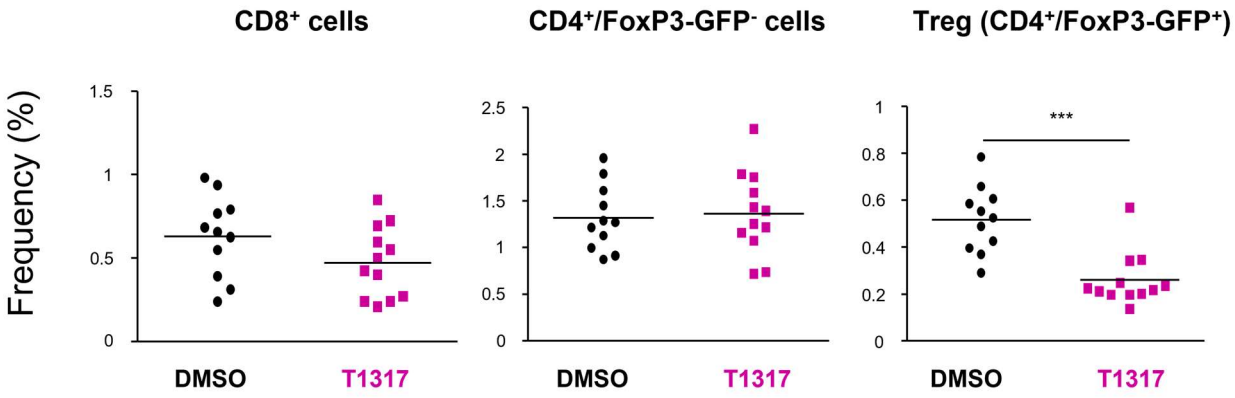


Figure 14. Frequency of intratumoral lymphoid cell populations. C57BL/6J male mice were subjected to subcutaneous injection of 3LL-R cells. The mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 post-cancer cell injection. The tumors were collected at day 15 post-cancer cell inoculation. The frequency (percentage of gated live singlets) of the targeted cell populations was measured by flow cytometry. Horizontal bars indicate mean values in each experimental group. Pooled data from three independent experiments; n = 18–19 mice/group. Mann–Whitney test. ***, $p < 0.001$.

The LXR ligand T1317 also reduced the total number of Treg in WT mice (Figure 15A). This reduction was not observed in LXR-deficient mice, indicating that the effects of T1317 on Treg abundance are specific and LXR-dependent (Figura 15B). Of note, a tendency for increased levels of Tregs was observed in the tumors growing in LXR-deficient mice.

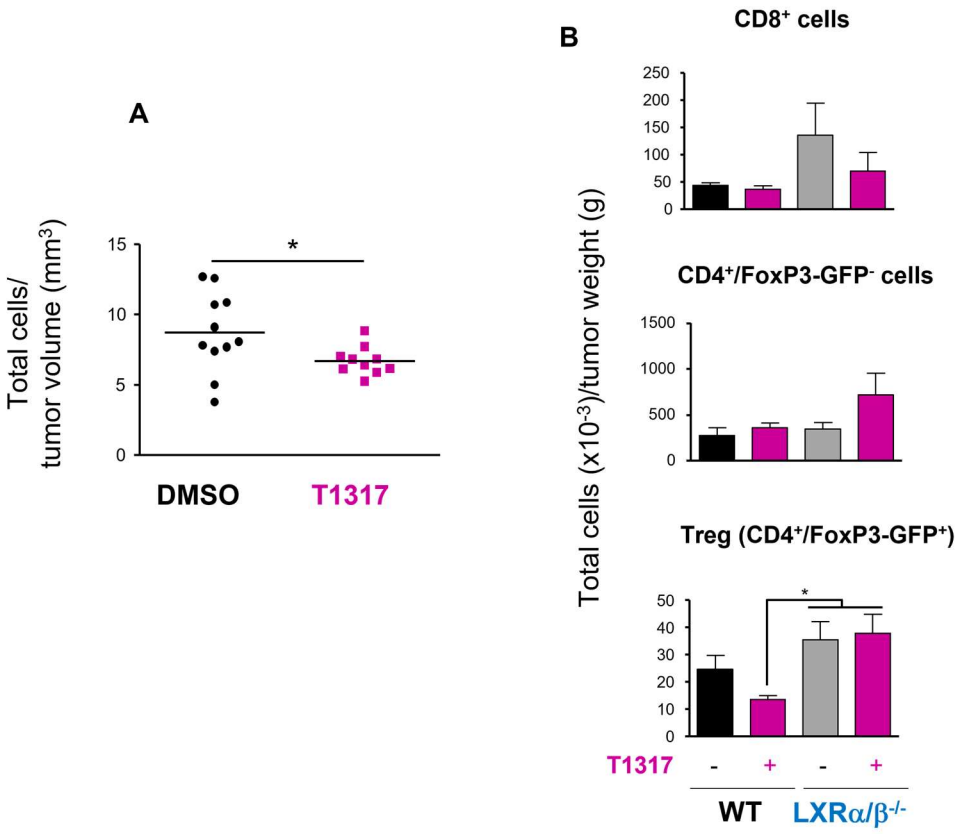


Figure 15. Effects of pharmacological LXR activation on the abundance of intratumoral lymphoid cell populations. WT (C57BL/6J) (A-B) or LXR-deficient (B) mice were subjected to subcutaneous injection of 3LL-R cells. The mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 post-cancer cell injection. The tumors were collected at day 15 post-cancer cell inoculation. The abundance of lymphoid cell populations was measured by flow cytometry. In A, the absolute amount of Tregs was determined in each tumor and normalized to the tumor volume. In B, the absolute numbers of lymphoid cells were normalized to the tumor weight. In A, pooled data from two independent experiments; n = 10–11 mice/group (T test). In B, n = 5 mice/group. One-way ANOVA test followed by Newman–Keuls post hoc. *, $p < 0.05$.

The effect of the LXR agonist on the frequency of Tregs was also analyzed using a transgenic mouse strain FoxP3-EGFP+ which expresses the transcription factor FoxP3 coupled to the green fluorescence protein, having as a result fluorescent Tregs (Figure 16). Again, T1317 reduced Treg frequency in the tumoral tissue, thus confirming the results obtained in WT mice.

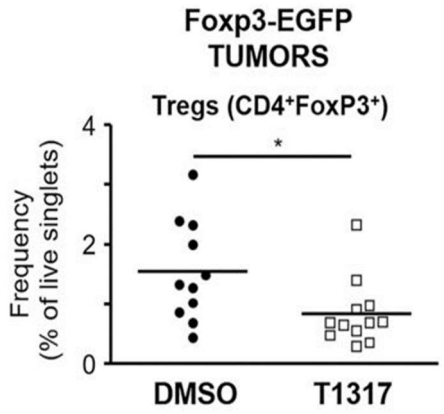


Figure 16. LXR activation reduces the frequency of intratumoral Treg in FoxP3-EGFP transgenic mice. Male mice were subjected to subcutaneous injection of 3LL-R cells. The mice were treated daily with DMSO or T1317 (15 mg/kg) from day 5 post-cancer cell injection. The tumors were collected at day 10 after tumor inoculation. The graphics represent the frequency of Tregs (percentage of gated live singlets) in the tumors. Horizontal bars indicate mean values from each experimental group. Pooled data from three independent experiments; n = 11–12 mice/group (Mann–Whitney test). *, $p < 0.05$.

We next studied whether activation of the LXR pathway was also able to affect the abundance of Treg in the PyMT model. In this case, the expression of FoxP3 was analyzed by qPCR in total RNA from whole mammary glands. Supplementation of the diet with T1317 resulted in a significant reduction in FoxP3 expression in the mammary gland, compared to the mammary glands from the mice fed a regular diet (Figure 17). These results suggest that LXR activation downregulates Treg infiltration also in spontaneous breast adenocarcinoma. Overall, the results obtained in this section encouraged us to further investigate the mechanisms through which LXR activation negatively affects the intratumoral Treg population.

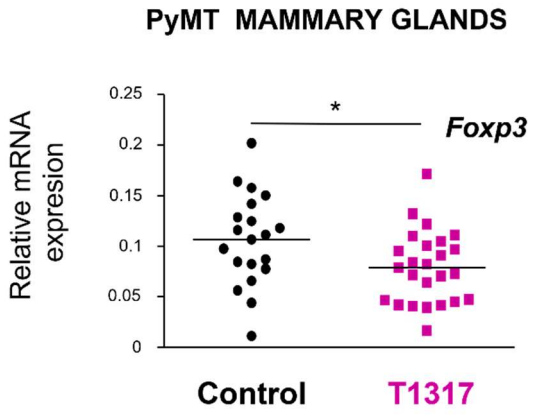


Figure 17. LXR activation decreased the levels of *Foxp3* expression in mammary glands from PyMT mice. PyMT female mice were fed a chow diet supplemented with T1317 (50 mg/Kg) or nor (Control). At week 22 of age the mice were sacrificed, and the mammary glands were excised. The samples (100 mg/each) were homogenized, and total RNA was extracted. cDNA was synthesized by retro-transcriptase PCR and gene expression was evaluated by qPCR. The graphic represents *Foxp3* expression normalized to the levels of *L14* expression. Mean ± SEM; n = 20 (Control), n = 25 (T1317). Mann–Whitney U test. *, $p < 0.05$.

3. Evaluation of the effects of LXR activation in Treg activity and proliferation.

As described above, LXR activation reduced the frequency and abundance of intratumoral Tregs. Since it has been extensively documented that LXR agonists interfere with T cell proliferation (Bensinger *et al*., 2008; Geyeregger *et al*., 2009; Cui *et al*., 2011), we next evaluated if the reduction in intratumoral Tregs was due to general modulation of Treg proliferation. First Treg frequency was analyzed in spleens from 3LL-R tumor-bearing mice (Figure 18). Treatment with T1317 did not reduce the frequency of these cells in the spleen, indicating that the inhibitory effects of the LXR agonist occur specifically in the TME. Of note, the frequency of splenic CD4+ FoxP3- T cells were downregulated upon pharmacologic LXR activation, in line with previous reports showing a role for LXRs in the negative control of central T cell proliferation (Bensinger *et al*., 2008; Geyeregger *et al*., 2009; Cui *et al*., 2011).

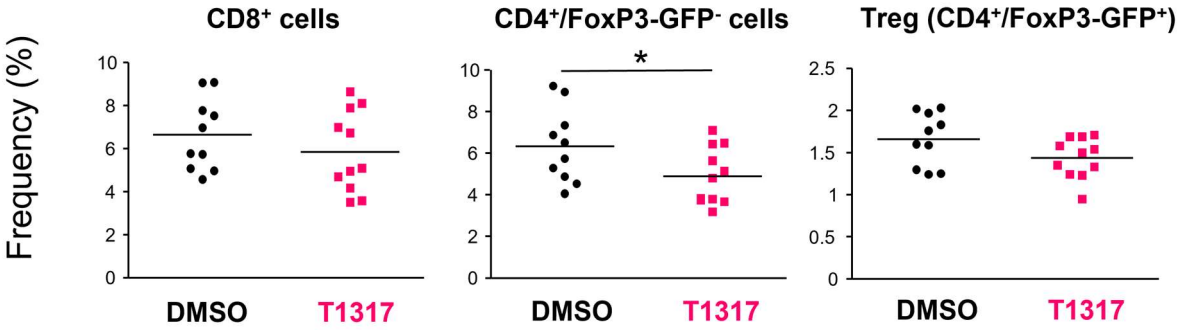


Figure 18. Effects of LXR activation on the frequency of T cells in the spleen. C57BL/6J male mice were subjected to subcutaneous injection of 3LL-R cells. The mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 post-cancer cell injection. The spleens were collected on day 15. The frequency of lymphoid cell populations was evaluated by flow cytometry. The graphics represent the frequencies of lymphocyte subtypes (percentage of gated live singlets) in the spleen. Horizontal bars indicate mean values from each experimental group. Pooled data from two independent experiments; n = 10–11 mice/group (Mann–Whitney test). *, $p < 0.05$.

We also evaluated if LXR agonists were capable of inhibiting Treg proliferation *in vitro.* Tregs were sorted out from the spleen of FoxP3-EGFP mice and stimulated *in vitro* with T1317 or vehicle. Next, polyclonal Treg proliferation was induced with anti-CD3 and anti-CD28 antibodies, in the presence of IL-2. As shown in Figure 19, the LXR agonist T1317 did not affect Treg proliferation *in vitro*, at least at the dose used in these studies.

Next, we assessed if the activation of the LXR pathway in Tregs interfered with their immunosuppressive capacity. On one hand, we performed T cell proliferation suppression assays using Tregs obtained from either tumors or splenic tissue from 3LL-R tumor-bearing FoxP3-EGFP mice. As it is shown in Figure 20A, T1317 had no effect on the ability of tumor or splenic Tregs to suppress T cell proliferation. On the other hand, the expression of the immuno-modulatory cytokines IL-10 and TGFβ was analyzed from total RNA from purified Treg stimulated *in vitro* with T1317 or vehicle. As shown in Figure 20B, T1317 did not affect the mRNA expression of these cytokines.

Taken together, the results presented in this section suggests that LXR activation does not interfere with the proliferation or the immunosuppressive capacity of Tregs in general. Therefore, a plausible explanation for the inhibitory effect of LXRs on the intratumoral Treg numbers should be sought within the TME.

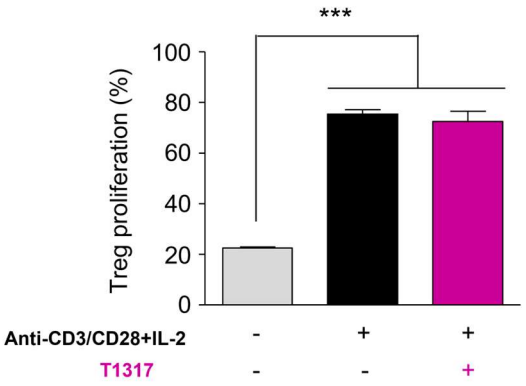


Figure 19. LXR activation does not inhibit Treg proliferation. Purified splenic Treg were stained with CFSE and then, incubated with T1317 (1 µM) or vehicle (DMSO) for 18 hours. Polyclonal Treg proliferation was induced by a combination of anti-CD3/CD28 antibodies and murine recombinant IL-2 (100 U/mL) for 48 hours. Treg proliferation was analyzed by flow cytometry. The graphic represents the percentage of Treg with CFSE dispersion compared with non-stimulated Treg. Mean ± SD, n = 3 (one-way ANOVA). ***, $p < 0.001$.

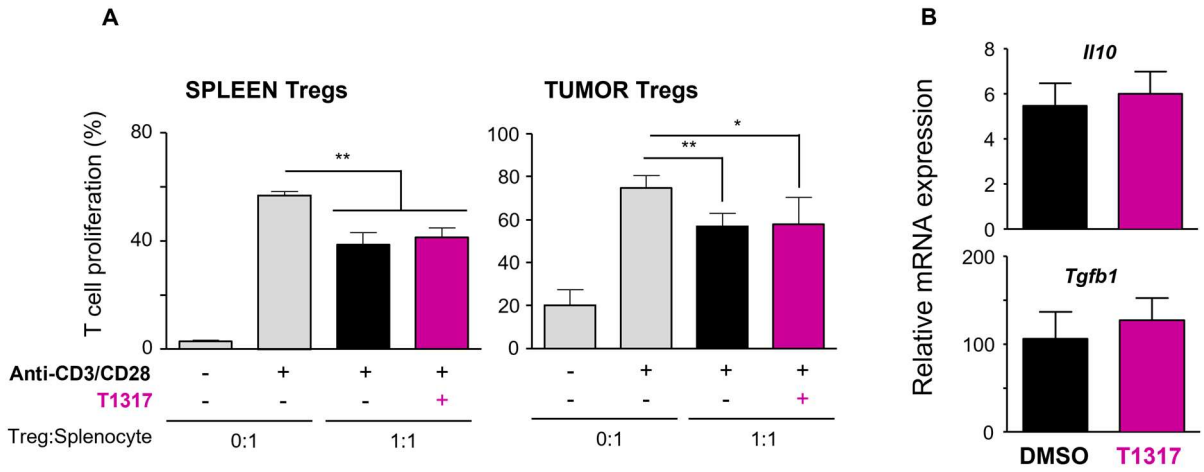


Figure 20. LXR activation in Treg does not impair their immunosuppressive capacity. In A, T1317 does not impair the capability of Tregs to suppress T-cell proliferation. Tregs were isolated from the spleens (left) or tumors (right) of tumor-bearing FoxP3-EGFP mice. In either case, Tregs were treated with T1317 (1 μM) or DMSO for 18 hours. Total splenocytes were isolated from the spleens of WT mice. The splenocytes were stained with CFSE, and co-incubated with Tregs at a 1:1 (Treg:splenocyte) ratio. Control splenocytes were grown in the absence of Tregs. T-cell proliferation was induced *in vitro* for 48 hours using antibodies against CD3 and CD28 and analyzed by flow cytometry. The graphics represent the percentage of splenocytes with CFSE dispersion compared with non-activated splenocytes. Mean ± SD. Left, $n = 3$ biological replicates. Right, $n = 5$ biological replicates; *t* test. In B, Tregs were isolated from the spleen of FoxP3-EGFP mice. Purified Tregs were incubated with T1317 (1 μM) or DMSO for 18 hours. Total RNA was obtained and the expression of *Il10* and *Tgfb* was analyzed by qPCR; mean ± SD, $n = 3$ (one-way ANOVA). *, $p < 0.05$; **, $p < 0.01$.

4. Role of Treg in the antitumoral action of pharmacological LXR activation.

Tregs are essential cells that modulate the immune response and suppress undesired responses against self-antigens. In the context of cancer, however, they can promote the inhibition of the antitumoral immune response. Indeed, a high infiltration of Tregs in the TME is often linked to a worse prognosis in many types of cancers (Tanaka & Sakaguchi, 2017). In this sense, there is multiple evidence supporting that their reduction may enhance the antitumoral response. To dissect the importance of Tregs in the antitumoral actions of the LXR agonist T1317, the levels of Treg were reduced in FoxP3-EGFP mice during tumor development. Treg depletion was carried out through the intraperitoneal injection of anti-CD25 antibodies. As shown in Figure 21A injection of antibodies against CD25 resulted in significant downregulation of the levels of Treg both in the spleen and within the tumor, in comparison with the level of Treg in mice injected with the control isotype antibody. The mice undergoing anti-CD25-mediated Treg depletion displayed reduced tumor growth compared with mice treated with the isotype control (Figure 21B), which suggests that Treg do mediate an antitumoral role in 3LL-R tumors. In the control mice injected with isotype antibodies, treatment with the LXR agonist reduced the intratumoral frequency of Tregs, while not affecting the frequency of splenic Tregs (Figure 21A), in line with the results described in Figures 14,

15, 16 and 18. In control mice injected with isotype antibodies, LXR activation resulted in inhibited tumor growth (Figure 21B). In mice injected with anti-CD25 antibodies, however, T1317 did not reduce tumor progression in comparison with the DMSO-treated mice (Figure 21B), suggesting that the effect of T1317 on tumor progression is Treg-dependent. In addition, tumor progression was lower in the isotype/T1317 group than in either of the experimental groups injected with anti-CD25 antibodies, (Figure 21B), indicating that T1317 may be affecting other components in the TME beyond the effect on intratumoral Treg abundance.

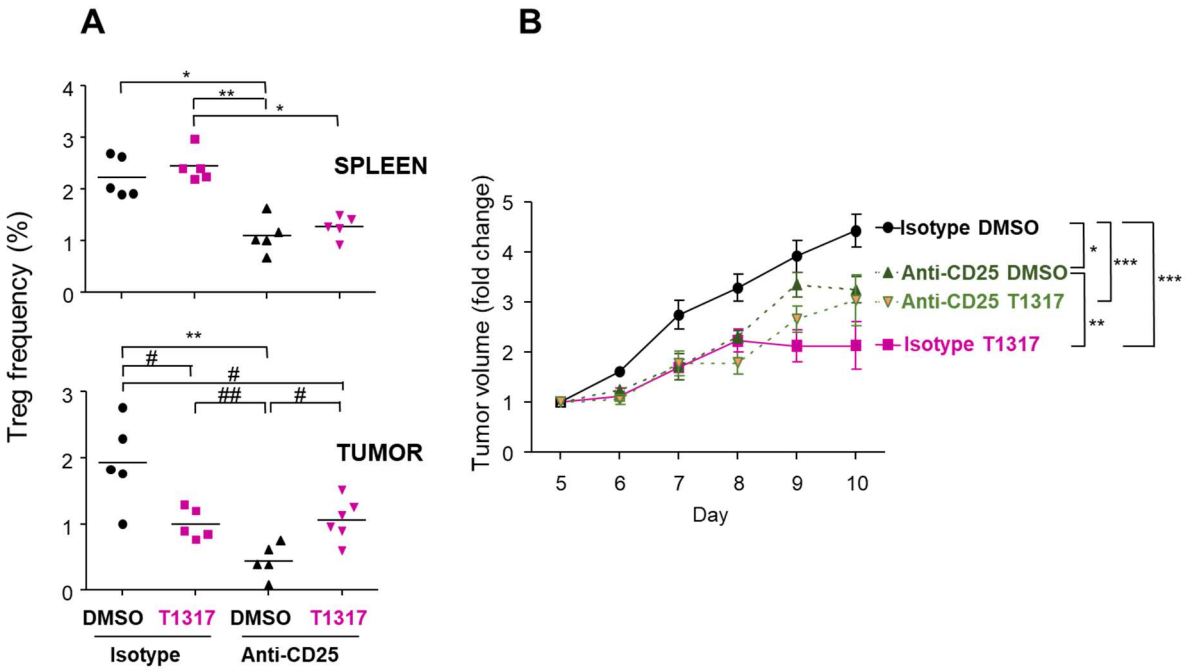


Figure 21. Effects of LXR activation in mice undergoing Treg depletion. A and B, male FoxP3-EGFP reporter mice were subjected to subcutaneous injection of 3LL-R cells. At days 2, 5, and 8 post cancer cell injection, the mice were administered either anti-CD25 antibodies or control isotype antibodies (200 µg per animal in PBS by intraperitoneal injection). At day 5, and until day 9, the mice received a daily dose of T1317 (15 mg/kg) or vehicle (DMSO). n = 5–6 animals/group. On day 10, the mice were euthanized. In A, the frequency of FoxP3-GFP^{+} Treg in spleens (top) and tumors (bottom) was evaluated by flow cytometry. Horizontal bars represent mean values. Kruskal–Wallis test followed by Dunn multiple comparison test; *, $p < 0.05$; **, $p < 0.01$. Selected experimental conditions were also compared using a Mann–Whitney test (#, $p < 0.05$; ##, $p <$

0.01). In B, tumor volumes were measured from day 5 to day 10 and represented as fold change. Two-way ANOVA with repeated measures; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

5. Effects of T1317 on the transcriptional profile and immunosuppressive capacity of TAMs.

The chemokines CCL17 and CCL22 are key mediators of Treg recruitment to tissues. Both MHCII^{high} TAM and MHCII^{low} TAM subpopulations (specially MHCII^{high} TAMs), are important producers of these chemokines in the TME (Movahedi *et al*., 2010). As indicated in the "Previous results from the group" section, previous studies from our group had shown that LXR activation strongly inhibited the expression of *Ccl17*, and to a lesser extent of *Ccl22*, in MHCII^{high} TAMs. A tendency for downregulation of *Ccl17* was also observed in MHCII^{low} TAMs in T1317-treated mice. In addition, LXR activation *ex vivo* strongly inhibited the expression of *Ccl17* and *Ccl22* in MHCII^{low} TAMs (data included in the doctoral thesis defended by Dr. JM Carbó, 2017).

To further study the global effects of LXR activation in MHCII^{high} TAMs and MHCII^{low} TAMs, gene expression profiling was performed in these TAM subpopulations stimulated *ex vivo* with T1317 or DMSO. The processing of the microarray data is detailed in Materials and methods. First, the expression of the LXR isoforms *Lxra* and *Lxrb*, as well as the RXR isoforms *Rxra* and *Rxrb* was confirmed in both TAM subpopulations, and the LXR agonist T1317 did not alter their expression (Figure 22). Low levels of expression of *Rxrg* were also detected (Figure 22).

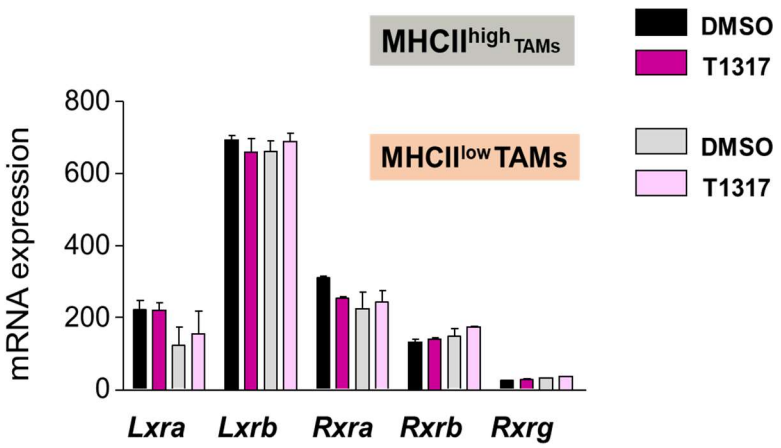


Figure 22. Expression of LXR and RXR isoforms in TAMs. MHCII^{high} TAMs and MHCII^{low} TAMs were stimulated *ex vivo* with T1317 (1 µM) or DMSO for 24 hours. Changes in gene expression were analyzed by expression profiling. The graphic displays the expression levels of LXR and RXR isoforms in TAMs. Results from three (MHCII^{high} TAMs) or two (MHCII^{low} TAMs) independent experiments. Paired T-test.

The expression levels of well-established LXR target genes were also analyzed (Figure 23). Stimulation with T1317 induced the expression of several LXR targets as the sterol transporters ABCA1 and ABCG1, the transcription factor SREBP1c (*Srebf1*), the ubiquitin ligase IDOL (*Mylip*), and the multifunctional enzyme CD38 in both TAM subpopulations (Figure 23A).

The induction of selected genes in TAMs was also validated through qPCR in independent experiments (Figure 23B). Of note, LXR activation did not induce the expression of *ApoE* in these cells, in contrast with previous observations in other cell types (Tavazoie *et al.*, 2018).

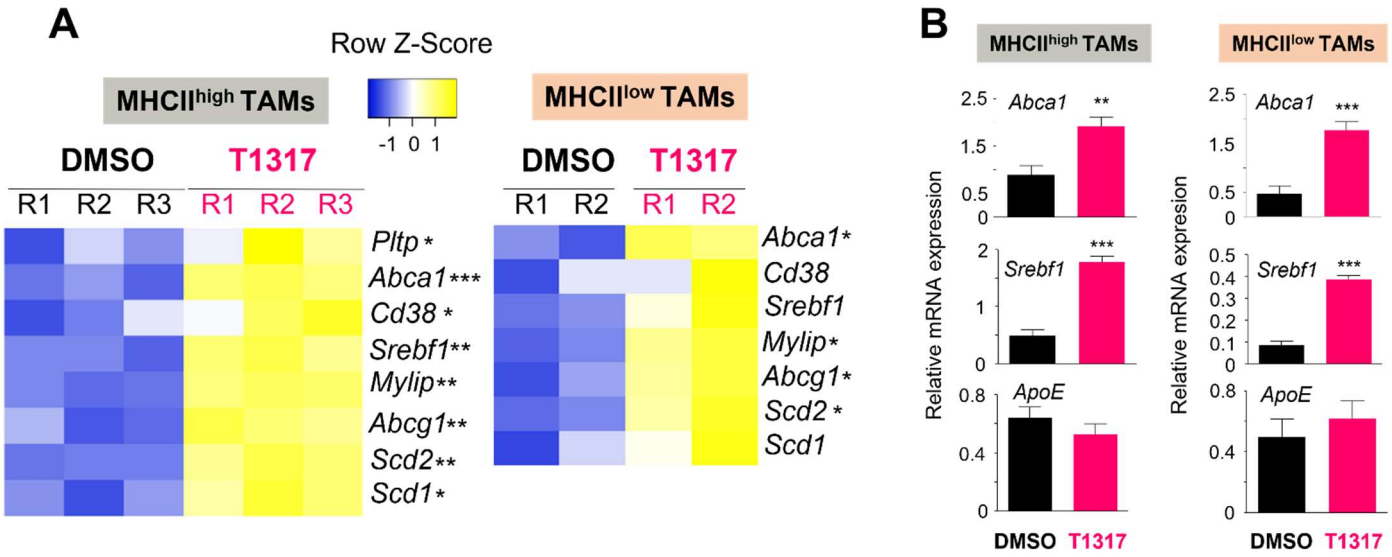


Figure 23. T1317 induces the expression of canonical LXR target genes in TAMs. MHCII^{high} TAMs and MHCII^{low} TAMs were isolated from 3LL-R tumors and stimulated *ex vivo* with T1317 (1 µM) or DMSO for 24h. In A, changes in gene expression were analyzed by gene profiling. The graphics display heatmaps showing that T1317 induces the expression of several canonical LXR target genes in TAMs. In B, validation of LXR target genes expression in TAMs by qPCR. In independent experiments, TAMs were isolated and processed as described in A. The expression of selected LXR targets was evaluated by qPCR in MHCII^{high} TAMs (left) and MHCII^{low} TAMs (right). In A, results from three (MHCII^{high} TAMs) or two (MHCII^{low} TAMs) independent experiments. In B, mean±SEM; n = 3 independent experiments. In A, paired T-test (T1317 versus DMSO). In B, T-test; *, p <0.05; **, p <0.01; ***, p <0.001. R, replicate.

The expression profile data was analyzed to further characterize the effects of LXR activation on selected markers of macrophage activation, including surface markers, enzymes, cytokines, and chemokines, other than CCL17 and CCL22 (Figure 24). MHCII^{high} TAMs and MHCII^{low} TAMs differ in their expression pattern of several markers of activation, in line with previous work reported by Movahedi and collaborators (Movahedi *et al*., 2010). Most of the selected markers were unaffected by LXR activation in both TAM subpopulations. However, in MHCII^{high} TAMs, a significant reduction in the expression of *Cd80*, *Nos2*, *Il1b*, *Il10*, *Il4ra*, *Mmp9* and *Ptges* was observed upon LXR activation (Figure 24).

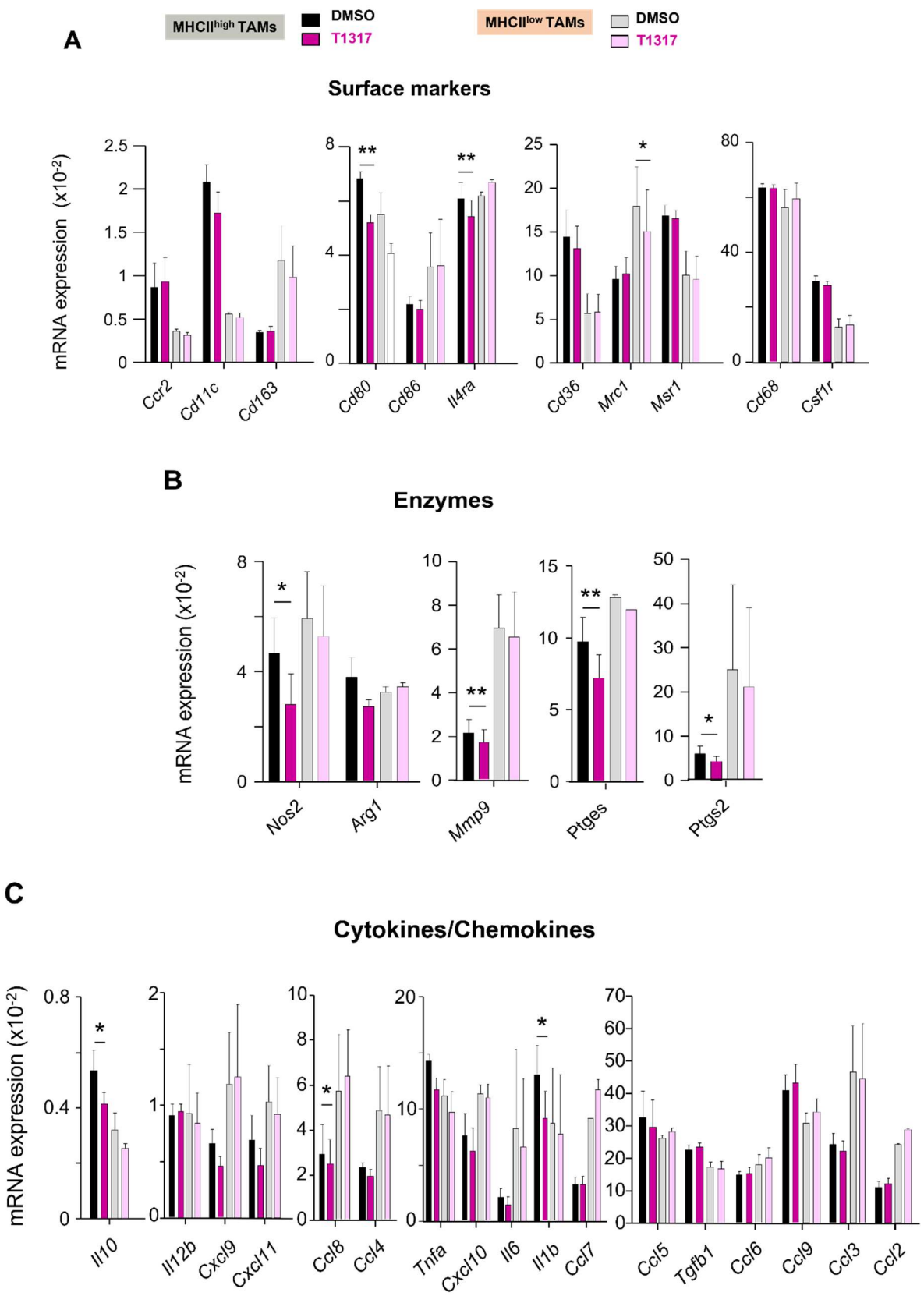


Figure 24. Analysis of the effects of T1317 on selected markers of macrophage activation. MHCII^{high} TAMs and MHCII^{low} TAMs were sorted out from 3LL-R tumors and stimulated *ex vivo*

with T1317 (1 µM) or DMSO for 24h. Changes in gene expression were analyzed by gene profiling. The graphics display relative expression values of selected surface markers (A), enzymes (B), and cytokines and chemokines (C). Results from three (MHCII^{high} TAMs) or two (MHCII^{low} TAMs) independent experiments. Paired T-test; *, p <0.05; **, p <0.01.

In parallel, we investigated if LXR activation affects two main functions of TAMs, their immunosuppressive capacity, and their ability to exert phagocytosis. Functional assays were performed *ex vivo* with MHCII^{high} TAMs and MHCII^{low} TAMs. The ability to inhibit polyclonal T cell proliferation was assessed through suppression assays, measuring CFSE dispersion by flow cytometry. In these assays, TAMs were treated or not with LXR agonists and co-incubated with CFSE-stained splenocytes. T cell proliferation was stimulated with anti-CD3 and anti-CD28 antibodies. Interestingly, both subpopulations of TAMs were able to suppress T cell proliferation, and the stimulation of TAMs with T1317 partially reduced this inhibitory effect (Figure 25).

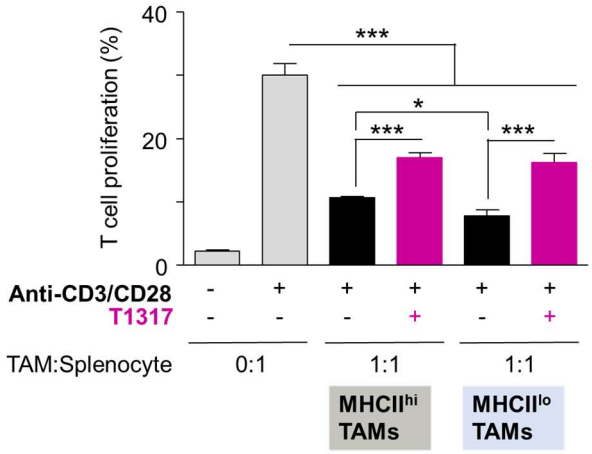


Figure 25. LXR activation inhibits the capability of TAMs to suppress T-cell proliferation. MHCII^{high} TAMs and MHCII^{low} TAMs were isolated from 15-day 3LL-R tumors and stimulated *ex vivo* with T1317 (1 µM) or DMSO for 18 hours. In parallel, freshly isolated splenocytes were stained with CFSE and co-incubated with TAMs at a 1:1 (TAM:splenocyte) ratio. Control cells were maintained in the absence of TAMs. T-cell proliferation was induced for 48 hours using antibodies against CD3 and CD28.CFSE dispersion was analyzed by flow cytometry. The graphic

represents the percentage of cells with CFSE dispersion using non activated splenocytes as reference. Mean ± SD; n = 3 biological replicates. One-way ANOVA; *, $p < 0.05$; ***, $p < 0.001$.

On the other hand, the effects of LXR activation on the phagocytic capacity of TAMs was measured using fluorescent latex microspheres. In contrast with the effects on immunosuppression, T1317 did not affect the phagocytic activity of TAMs (Figure 26). Altogether, these results suggest that LXR activation impacts the immunosuppressive capacity of MHCII^{high} TAMs and MHCII^{low} TAMs without affecting their phagocytic activity, which may contribute to the general inhibitory effect of the LXR agonists on tumor progression.

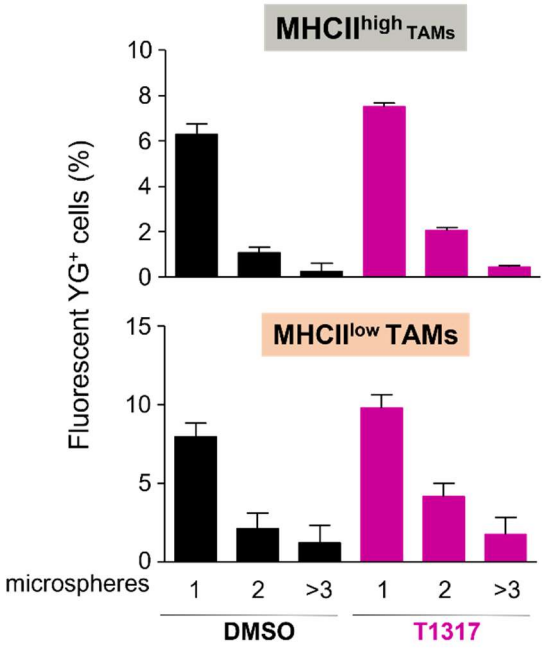
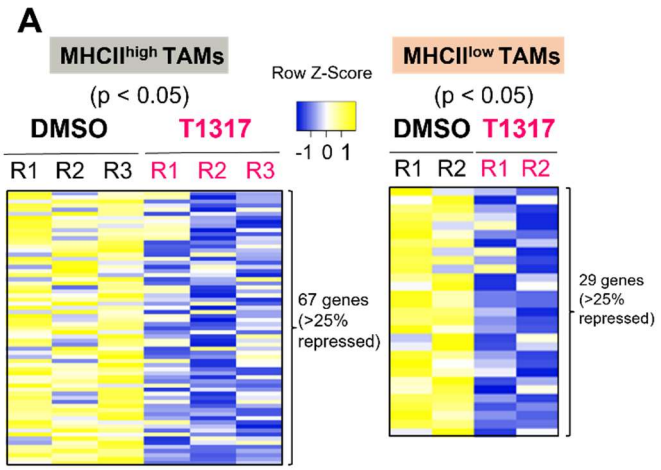


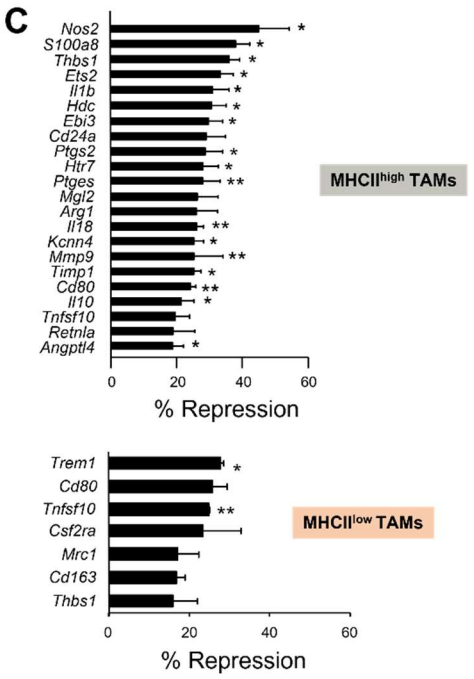
Figure 26. LXR activation does not alter the phagocytic capacity of TAMs. MHCII^{high} TAMs and MHCII^{low} TAMs were isolated from 15-day 3LL-R tumors and stimulated *ex vivo* with T1317 (1 µM) or DMSO for 18 hours. TAMs were incubated with 3-µm fluorescent microspheres (20 beads/cell) for 30 minutes. The percentage of TAMs that had phagocyted fluorescent microspheres was analyzed by flow cytometry. Mean ± SD; *n* = 2 biological replicates.

Based on the inhibitory effects of LXR activation on the capacity of TAMs to suppress T cell proliferation, a deeper analysis was performed on the expression profiling data now focusing on genes reportedly involved in the immunosuppressive mechanisms of TAMs or in the acquisition of an alternatively activated phenotype in macrophages. First, the analysis revealed that different subsets of genes were repressed (>25% repression) by T1317 in MHCII^{high} TAMs and MHCII^{low} TAMs (Figure 27A). An unbiased gene ontology analysis was performed with the repressed genes in each TAM subpopulation. In MHCII^{high} TAMs, the analysis showed several biological processes affected, including the biosynthesis of ROS and the positive regulation of nitric oxide (Figure 27B), both important processes for the immunosuppressive activities of TAMs. No significant enrichment of specific biological processes was observed among the genes repressed in MHCII^{low} TAMs. In addition, we further selected a list of genes involved in immunosuppression in tumors or in macrophage alternative activation and analyzed them separately (Figure 27C). Importantly, the expression of several genes that are part of the pro-tumoral program of TAMs were downregulated upon LXR activation (Figure 27C). The effects on some of these genes were further validated by qPCR in independent experiments (Figure 27D). Of note, the LXR agonist was able to repress a larger set of genes in MHCII^{high} TAMs than in MHCII^{low} TAMs, suggesting differences between both cell subpopulations in the repertoire of genes susceptible to LXR-mediated repression.



B

GO biological process	Fold enrichment	Fisher p-value	False Discovery Rate	Genes
Cellular response to fluid shear stress (GO:0071498)	63.24	2.31E-05	5.15E-02	*Ass1, Ptgs2, Ptk2b*
Positive regulation of nitric oxide biosynthetic process (GO:0045429)	29.98	1.33E-05	4.16E-02	*Ass1, Ptgs2, Ptk2b, Il1b*
Positive regulation of reactive oxygen species biosynthetic process (GO:2000379)	19.65	8,44E-07	6.58E-03	*Ass1, Il18, Thbs1, Ptgs2, Ptk2b, Il1b*
Inflammatory response (GO:0006954)	7.96	5.54E-07	8.65E-03	*S100a8, Il18, Timp1, Thbs1, Ppbp, Ptgs2, Fpr1, Il1b, Nfkbiz, Smpdl3b*
Defense response (GO:0006952)	3.96	4.17E-06	2.17E-02	*S100a8, Il18, Timp1, Thbs1, Ppbp, Ptgs2, Fpr1, Ptk2b, Penk, Il1b, Nfkbiz, Smpdl3b, Trim62, Coro1a, Sh2d1b*
Positive regulation of immune system process (GO:0002684)	4.28	9.06E-06	3.53E-02	*Tnfsf9, Il18, Thbs1, Hcar2, Kcnn4, Fpr1, Ptk2b, Il1b, Nfkbiz, H2-M2, Stap1, Coro1a, Sh2d1b*



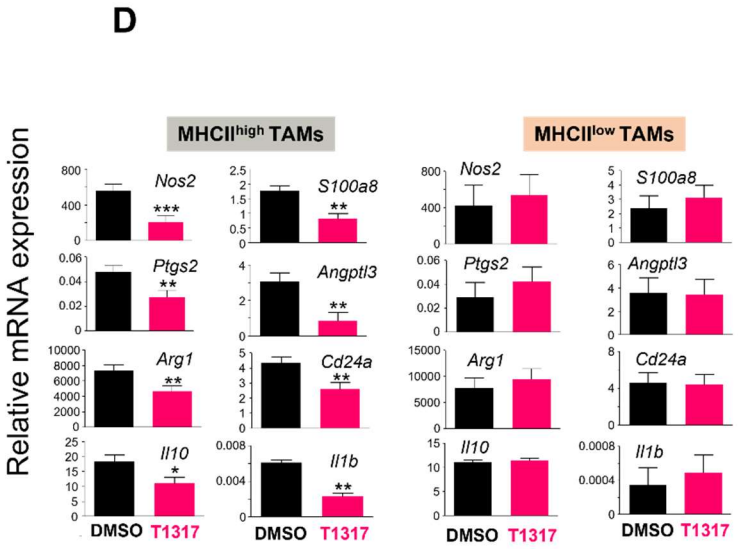


Figure 27. LXR activation downregulates the expression of genes that are part of the pro-tumoral program of TAMs. TAMs were isolated from 15-day 3LL-R tumors and stimulated *ex vivo* with T1317 (1 µM) or DMSO for 24 hours. Changes in gene expression were analyzed by expression profiling. In A, graphics display heatmaps showing the expression profile of genes that underwent significant down-regulation (≥ 25 % repression) by T1317. Results from three (MHCII^{high} TAMs, left) or two (MHCII^{low} TAMs, right) independent experiments. Paired T-test. R, replicate. In B, biological processes affected by T1317 in MHCII^{high} TAMs. The table displays the results of gene ontology analysis of the list of genes significantly downregulated by T1317 in

MHCII^{high} TAMs, with enrichment of several biological processes and the biological processes enriched. The results from three independent experiments are included. In C, the graphics show the percentage of repression by T1317 of genes that have been selected because of their reported involvement in immunosuppressive functions in the TME or in macrophage alternative activation. The results from three (MHCII^{high} TAMs) or two (MHCII^{low} TAMs) independent experiments are included. Paired T test; *, $p < 0.05$; **, $p < 0.01$. (T1317-treated vs. control cells). In D, gene expression was evaluated by qPCR TAMs in MHCII^{high} TAMs (left) and MHCII^{low} TAMs (right). The graphics display gene expression levels of several genes associated to the immunosuppressive capabilities of. Mean ± SEM; n=3; T-test. *, $p < 0,05$; *, $p < 0,01$; ***, $p < 0,001$ (T1317 *versus* DMSO).

6. LXR activation represses the secretion of CCL17 and CCL22 in bone-marrow derived macrophages.

As mentioned in the introduction (see section "Previous results from the group"), our group had previously observed inhibitory effects of the LXR pathway on the mRNA expression of the chemokines CCL17 and CCL22. Since these chemokines are important inducers of Treg recruitment to tumors, we evaluated whether LXR activation also affects their secretion. These studies were performed using BMDM and the secretion of chemokines was stimulated with two different factors commonly produced in the TME, IL-4 and GM-CSF. Macrophages were treated with the LXR agonist T1317 or vehicle and then stimulated with IL-4 or GM-CSF. As shown in Figure 28, IL-4 and GM-CSF stimulated the secretion of abundant levels of both chemokines and T1317 inhibited this increase. The inhibitory effect of the LXR agonist, was not observed in macrophages deficient for LXRs (Figure 28). Indicating that the actions of T1317 were specific for LXRs. In addition, the induction of CCL17 and CCL22 secretion was significantly higher in LXR-deficient macrophages which suggests that LXRs can perform basal repression of these chemokines in the absence of synthetic agonists.

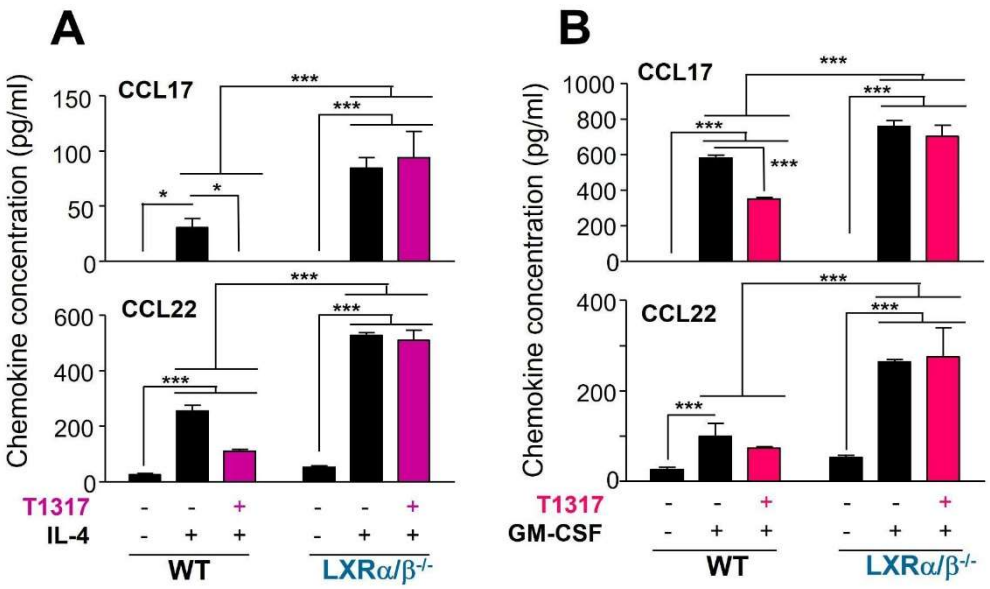


Figure 28. LXR activation inhibits the secretion of CCL17 and CCL22 in macrophages. BMDM were obtained from WT mice or LXR-deficient mice. The cells were pretreated with T1317 (1 µM; 16 hours) and then stimulated with IL4 (10 ng/mL; 24 hours) or B) GM-CSF (5 ng/mL; 24 hours). The supernatants were collected and the levels of secreted CCL17 and CCL22 were determined by ELISA. Mean ± SD; *n* = 3 biological replicates. Two-way ANOVA–Bonferroni. *, $p < 0.05$; ***, $p < 0.001$.

7. Effects of LXR activation in the macrophage response to GM-CSF.

GM-CSF produced in the TME contributes to the acquisition of pro-tumoral activities by TAMs (Mantovani *et al*., 2017). We therefore investigated if LXR agonists modulate the expression of several genes that are part of the GM-CSF-induced signature in macrophages. In these studies, two different LXR agonists were used, T1317 and GW3965. The expression of the genes *Arg1*, *Il1b*, resistin-like alpha (*Retnla)* and macrophage galactose n-acetyl-galactosamine-specific lectin (*Mgl)2* was stimulated by GM-CSF in BMDM (Figure 29). In addition, we confirmed the induction of the chemokines *Ccl17* and *Ccl22*. In all cases, pre-treatment with the LXR agonists T1317 or GW3965 lead to a reduced expression of the GM-CSF-induced genes compared to the macrophages pre-treated with vehicle (DMSO). This inhibitory effect was severely impaired in macrophages from LXR-deficient mice (Figure 29A). Moreover, GM-CSF

stimulation downregulated the expression of *Lxra* (Figure 29B), suggesting general reciprocal negative interaction between GM-CSF signaling and the LXR pathway.

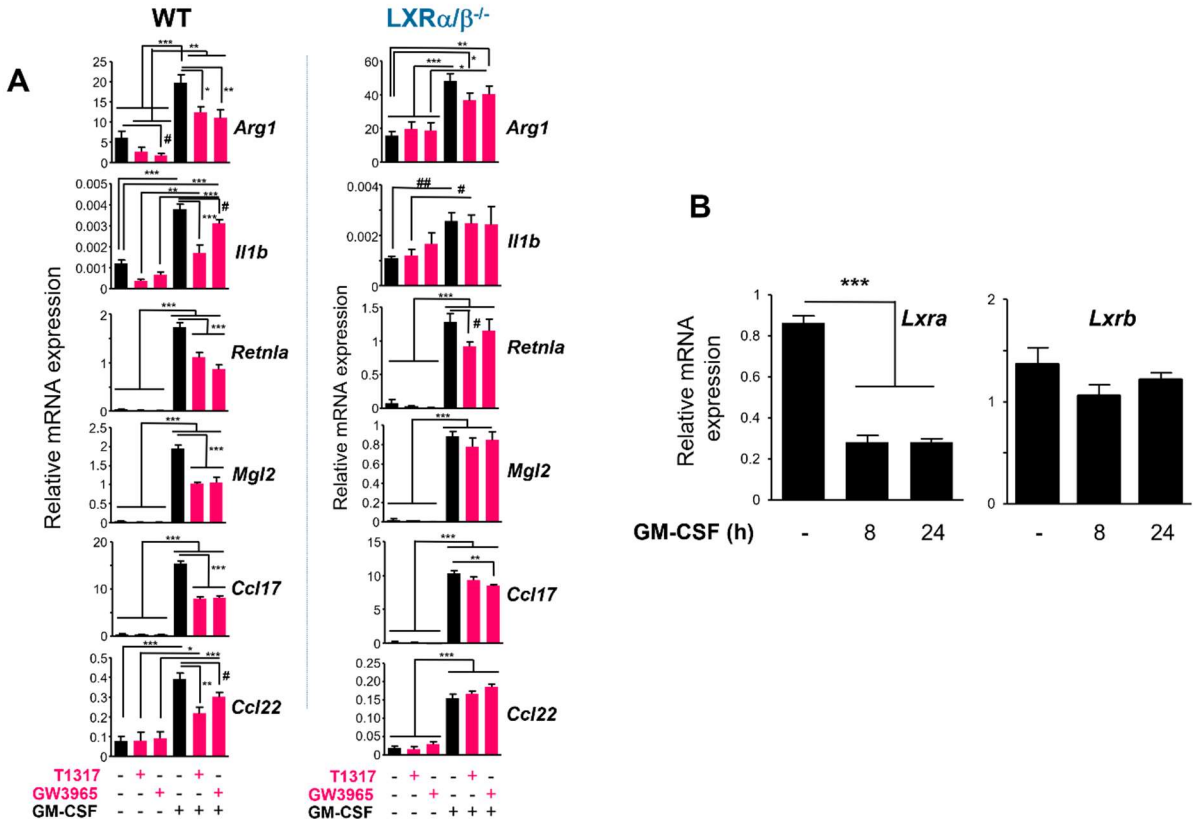


Figure 29. Effects of LXR activation on the expression of genes representative of the GM-CSF signature in macrophages. In A, macrophages were differentiated from bone marrow cells obtained from either WT (left) or LXR-deficient mice (right). Macrophages were pretreated with T1317 or GW3965 (1 µM; 16 hours) and then stimulated with GM-CSF (5 ng/mL; 24 hours). Total RNA was extracted, and gene expression was evaluated by qPCR. Mean ± SEM. *n* = 3–8 (WT) *n* = 4 (LXRα/β$^{-/-}$) independent experiments using 1–2 biological replicates for each experiment (ANOVA–Bonferroni); In B, effects of GM-CSF on the expression of LXR isoforms. BMDM were obtained from WT mice. Macrophages were stimulated with GM-CSF (5 ng/ml; 8 or 24 hours). Gene expression was evaluated by qPCR. Mean ± SEM, *n* = 3-4 independent experiments using 1–2 biological replicates for each experiment (one way ANOVA–Bonferroni); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Selected experimental conditions were also compared using a *t* test (#, $p < 0.05$; ##, $p < 0.01$).

8. Role of IRF4 in the antitumoral actions of the LXR pathway.

The transcription factor IRF4 is a common component in both IL-4 and GM-CSF signaling pathways (El Chartouni *et al*., 2010; Lacey *et al*., 2012). As mentioned in the Introduction (see "Previous results from the group" section), our group had demonstrated that IRF4 activity is required for the induction of CCL17 and CCL22 expression in response to IL-4 or GM-CSF. Here, we analyzed the effects of LXR activation on IRF4 expression at different levels. BMDM were pretreated with LXR agonists and stimulated with IL-4 or GM-CSF. IRF4 mRNA and protein expression were analyzed qPCR and western blotting, respectively. The results show an inhibitory effect of LXR agonists on *Irf4* mRNA expression (Figures 30A-B), confirming previous observations from the group. These effects were specific for the LXR pathway, as they were abolished in LXR-deficient macrophages (Figures 30A-B). In addition, the inhibitory effect of the LXR pathway translated in reduced IRF4 protein levels during the macrophage response to either IL-4 or GM-CSF (Figures 30C-D)

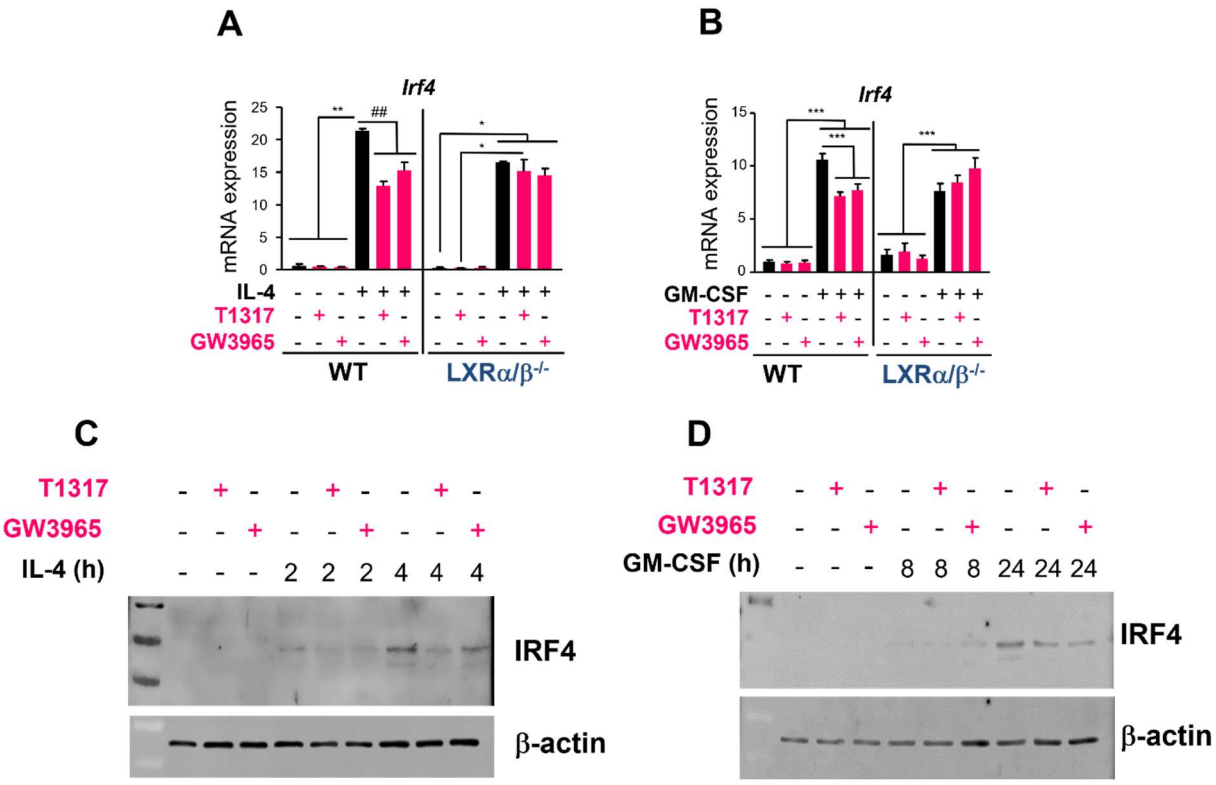


Figure 30. LXR agonists inhibit the expression levels of IRF4 mRNA and protein level. BMDM were obtained from WT (A-D) or LXR-deficient (A, B) mice. Macrophages were pretreated with T1317 or GW3965 (1 µM; 18 hours) and then stimulated with IL4 (A, C) (10 ng/mL; in A, 4 hours; in C, 2 and 4 hours) or GM-CSF (B, D) (5 ng/mL; in B, 24 hours; In D, 8 and 24 hours). IRF4 expression was analyzed by qPCR (A-B) or by Western blotting (7 µg whole cell extract/lane) (C-D). In A-B, Mean ± SEM, *n* = 6. In C-D, representative experiments. In A, Kruskal–Wallis; In B, ANOVA-Bonferroni. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. In A, a Mann–Whitney test was also used for selected comparisons (##, $P < 0.01$).

Taking together our previous observations of the inhibitory effects of LXR activation on IRF4 expression in TAMs, and the results presented here from BMDM, we hypothesized that downregulation of IRF4 expression and the subsequent decrease in CCL17 expression in the TME could be the mechanism by which LXR activation results in reduced intratumoral Treg numbers. To investigate the relevance of IRF4 in tumor progression, we compared the growth of 3LL-R tumors in WT and IRF4-deficient mice

treated with T1317 or vehicle. In addition, intratumoral lymphoid cell populations were evaluated by flow cytometry.

As shown in figure 31A, tumors developing in IRF4-deficient mice reached a larger size when compared to the ones growing in WT mice. Moreover, treatment with T1317 was less effective in inhibiting tumor growth in the IRF4-deficient background. These results suggested that the functional expression of IRF4 in the TME is important for the antitumoral effects of the LXR agonist. Notably, there was lower infiltration of lymphocytes in general (including Tregs) in the tumors developed in IRF4-deficient mice (figure 31B) which may explain the increased tumor growth in these mice. In addition, treatment with T1317 specifically downregulated the numbers of the Treg population in WT mice, but not in IRF4-deficient mice (figure 31B), in line with the diminished responsiveness of these tumors to the LXR agonist. In conclusion, these data support the importance of IRF4 as a mediator of the antitumoral actions of pharmacological LXR activation.

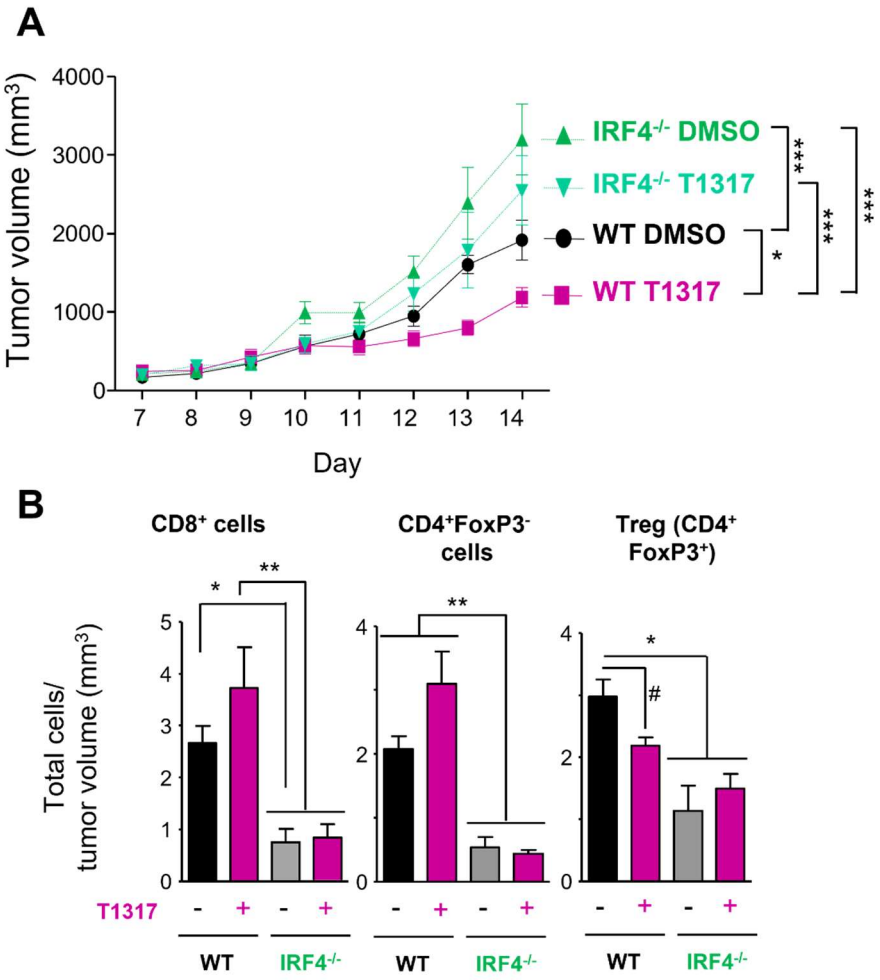


Figure 31. Evaluation of the antitumoral response of the LXR pathway in WT versus IRF4-deficient mice. WT or IRF4-deficient mice were subjected to subcutaneous injection of 3LL-R cells and treated daily with DMSO or T1317 (15 mg/kg) from day 7 post-cancer cell injection. In A, the graphic displays tumor volume progression curves. In B, abundance of intratumoral lymphoid cells in WT versus IRF4-deficient mice. Absolute numbers of intratumoral lymphocytes were measured by flow cytometry and normalized to tumor volume. In A-B, Mean ± SEM; n = 6–7 mice/group. In A, two-way ANOVA-repeated measures; in B, one-way ANOVA – Bonferroni; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. In addition, in B, selected conditions were compared with a T-test; #, $p < 0.05$.

9. Effects of LXR activation over on IRF4-CCL17/CCL22 axis in human macrophages.

Based on the inhibitory actions of LXR agonists on the expression of IRF4, CCL17 and CCL22 in mice, we explored whether such effects were also active in the human system. Human macrophages were differentiated from peripheral blood mononuclear cells from healthy donors. Macrophages were pretreated with T1317 and then stimulated with IL-4. LXR activation inhibited the induction of the three genes tested (Figure 32), suggesting that the crosstalk between LXRs and the IRF4-CCL17/CCL22 axis is evolutionary conserved and may be also relevant in humans.

In this sense, Kaplan-Meier analyses were performed on metadata from the R2: Genomics Analysis and Visualization platform (http://r2.amc.nl) to evaluate the association of chemokine expression with the probability of overall survival in patient cohort of different types of cancer. The analyses showed that a high CCL17 expression associated with lower survival in non-small cell lung cancer and in kidney clear cell carcinoma (Figure 33).

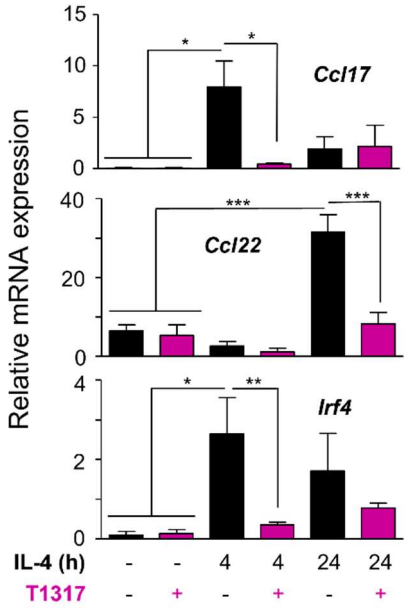


Figure 32. Effects of LXR agonists on the IRF4-CCL17/CCL22 axis in human macrophages. Human macrophages were differentiated *in vitro* from peripheral blood mononuclear cells from healthy donors. The cells were pretreated with T1317 (1 µM; 18 hours) or vehicle (DMSO) and

stimulated with IL4 (10 ng/mL; 4 and 24 hours) or left untreated. Gene expression was evaluated by qPCR. n = 3 independent experiments. One-way ANOVA; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

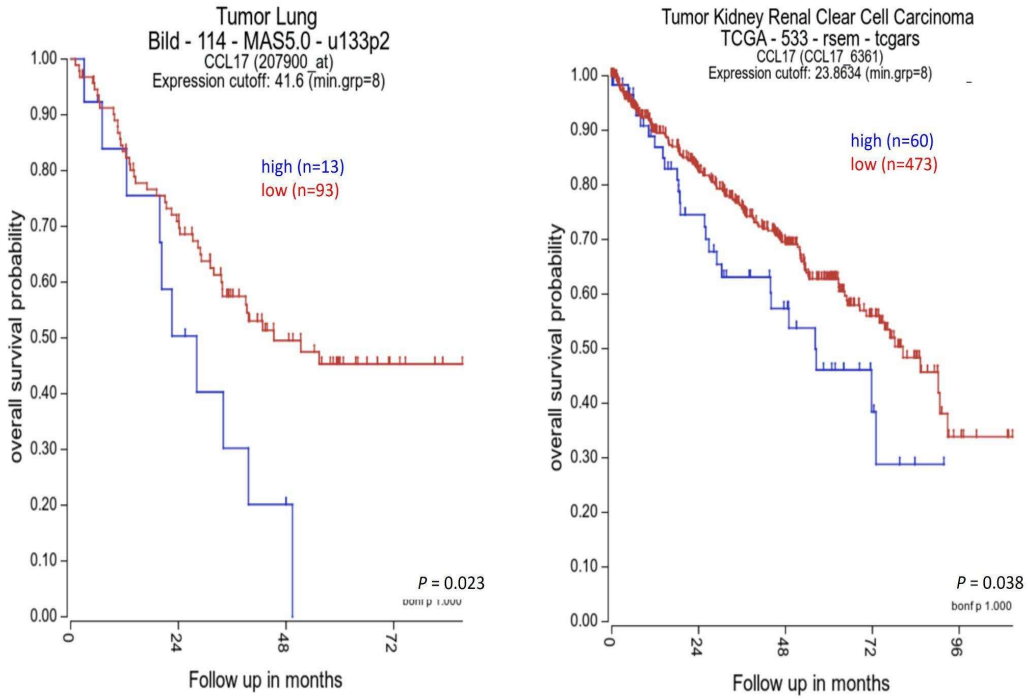


Figure 33. High expression of CCL17associates with poorer overall survival probability in different types of cancer. Kaplan-Meier analysis was performed on data from the R2: Genomics Analysis and Visualization Platform to assess the probability of overall survival in patient cohorts of non-small cell lung cancer (Bild AH *et al*., Nature, 2006;439:353) (left) and of kidney renal clear cell carcinoma (The Cancer Genome Atlas (TCGA) project) (right).

Taking together all the data shown in the results section so far, this work provides more insights about the biological actions of LXR agonists in the TME, supporting their use as antitumoral drugs. All the observations described here are included in the publication Carbó *et al*., 2021 (see Annex), in which I am co-first author.

10. Role of CD38 in the T1317-mediated effects on tumor progression.

Our group has identified in the past the existence of negative crosstalk between inflammatory mediators and the LXR pathway (Pascual-García *et al*., 2013). To evaluate if factors secreted by tumor cells affect the expression of LXR target genes, we treated BMDM with conditioned medium from 3LL-R cells in the presence of the LXR agonist T1317 or vehicle. The expression of several conventional LXR targets evaluated here was not affected by the 3LL-R cell conditioned medium. However, the expression of *Cd38* dramatically increased when the macrophages were exposed to 3LL-R conditioned medium in combination with T1317 (Figure 34). This result suggested a synergistic effect of T1317 with undefined mediators produced by 3LL-R cells selectively affecting *Cd38* expression in macrophages. The next step was to evaluate potential inflammatory signals that could be driving this effect. To this end, the effect of T1317 and the 3LL-R conditioned medium on *Cd38* expression was evaluated in BMDM from either STAT1-deficient mice or p38α-deficient mice (Figure 35). In STAT1-deficient macrophages, T1317 induced more potently the expression of *Cd38* than in WT macrophages. However, the synergy between the LXR agonist and the 3LL-R conditioned medium on *Cd38* expression was lost in STAT1-deficient macrophages (Figure 35A). Likewise, the synergistic effect was also abolished in p38α-deficient macrophages, although the lack of functional p38α did not influence the induction of *Cd38* expression by the LXR agonist alone (Figure 35B). Taken together, these results indicate that both STAT1 and p38α signaling pathways contribute to the synergistic effect of T1317 and 3LL-R conditioned medium on *Cd38* expression.

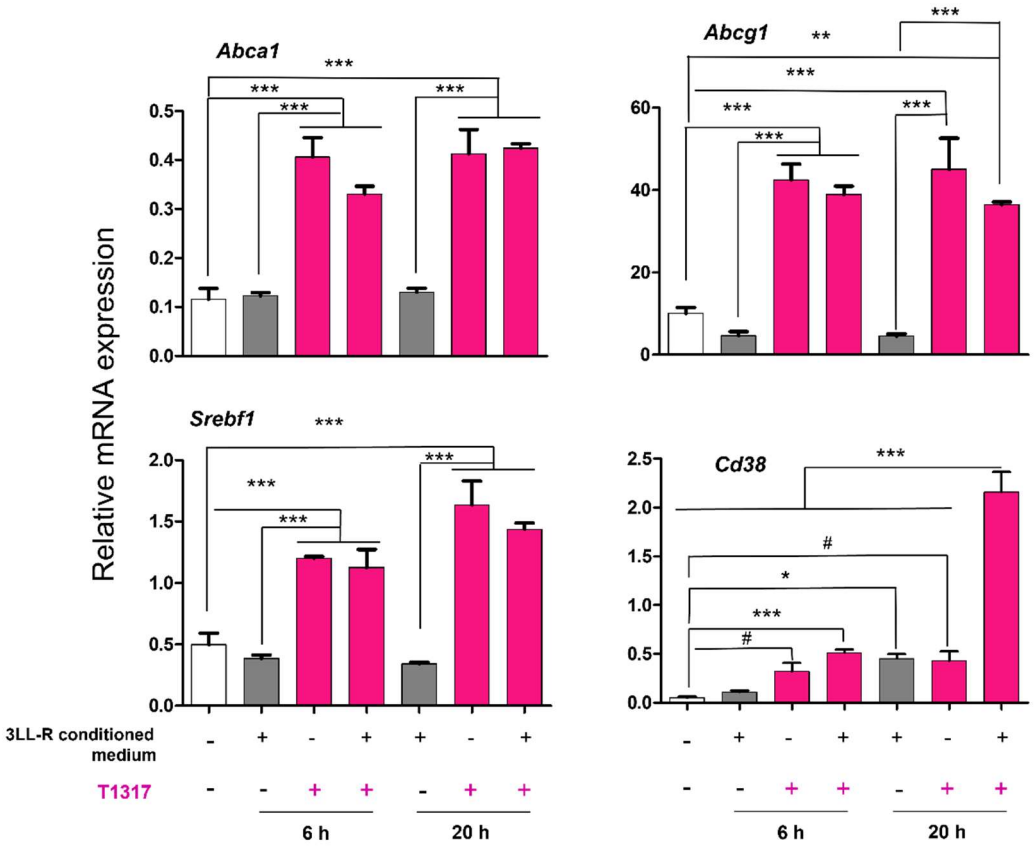


Figure 34. Effects of the LXR agonist T1317 and 3LL-R cell conditioned medium on the expression of LXR target genes. BMDM were obtained from WT mice. The cells were incubated with T1317 (1 µM) and 3LL-R conditioned medium (½ dilution in complete medium) either alone or in combination during the indicated periods of time. Control cells were left untreated. Gene expression was evaluated by qPCR. n = 3 independent experiments. One-way ANOVA; *, $p < 0,05$; **, $p < 0.01$; ***, $p < 0.001$. Selected conditions were also compared with a T-test; #, $p < 0.05$.

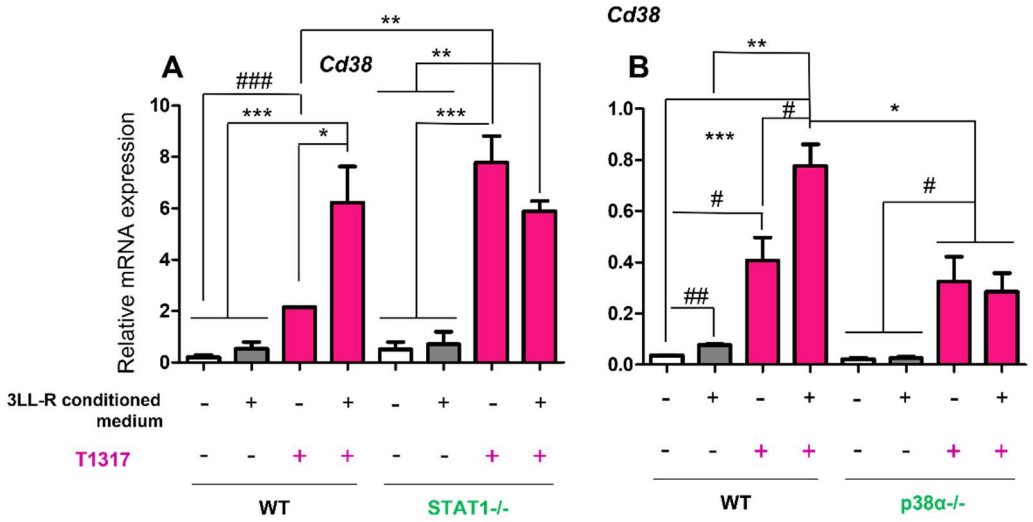


Figure 35. Roles of inflammatory signaling pathways on *Cd38* expression. BMDM were obtained from WT mice (A-B), STAT1-deficient mice (A) or from mice with specific ablation in p38α in myeloid cells (B). The cells were incubated for 20 hours with T1317 (1 µM), 3LL-R conditioned medium (½ dilution in complete medium), or a combination of both. Control cells were left untreated. Gene expression was evaluated by qPCR. n = 3 (A) or n = 2 (B) independent experiments. One-way ANOVA; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Selected conditions were also compared by T-test; #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$.

To investigate the importance of CD38 for the T1317-mediated antitumoral effects, 3LL-R tumor progression studies were conducted in WT and CD38-deficient mice. Of note, no significant differences were observed on tumor progression between WT and CD38-deficient mice treated with DMSO. In WT mice, T1317 mediated reduction of tumor progression as expected (Figure 36). The tumor growth curves in CD38-deficient mice suggested that T1317 was effective until day 10 and lost the inhibitory effect during the second half of the curve (Figure 36).

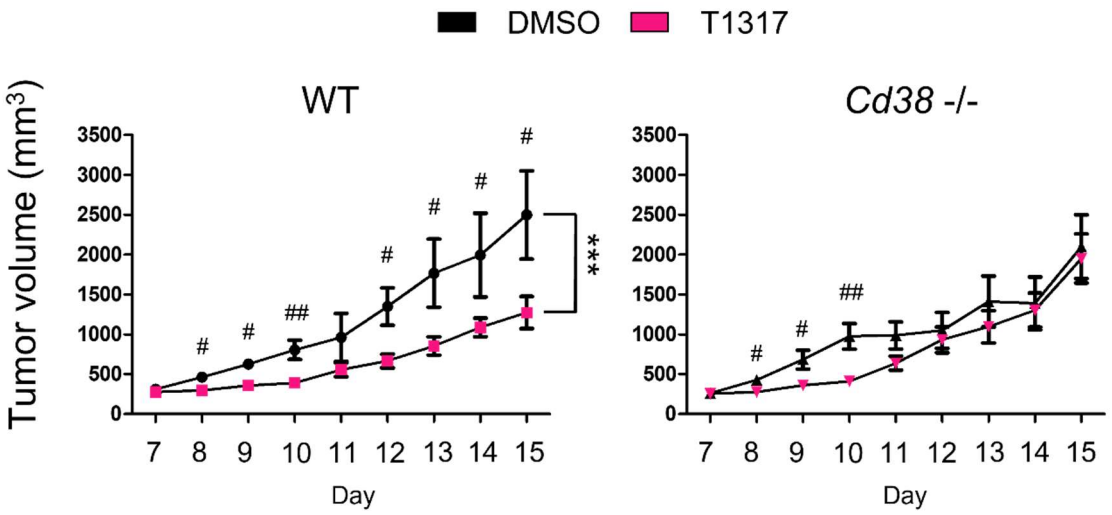


Figure 36. Role of CD38 in the antitumoral action of the LXR pathway. WT (left) or CD38-deficient (right) male mice were subjected to subcutaneous injection of 3LL-R cells and treated daily with DMSO or T1317 (15 mg/kg) from day 7 post-cancer cells injection. Graphic displays tumor volume progression curves. Mean ± SEM; n = 5–9 mice/group. Two-way ANOVA-repeated measures; ***, $p < 0.001$. In addition, T test. #, $p < 0.05$; ##, $p < 0.01$

Taken together, the results in this section suggest that factors secreted by cancer cells act in combination with LXR agonists to induce the expression of CD38 in macrophages. Furthermore, functional expression of CD38 in the host is important for the maintenance of the inhibitory potential of the LXR pathway during tumor progression. Whether the loss of effectiveness of the LXR agonist in the CD38-deficient background is associated with changes in the TME needs to be addressed in future studies.

DISCUSSION

The investigation on LXRs as therapeutic targets in a variety of diseases is particularly interesting. On one hand, LXRs are involved directly or indirectly in many physiological processes. On the other hand, they can be activated through the treatment with synthetic agonists that display high affinity and specificity. In the context of cancer, it has been extensively documented that LXR agonists exert potent anti-proliferative and pro-apoptotic effects on many types of tumoral cell lines *in vitro* and also antitumoral activities in several murine models *in vivo* (reviewed in Font-Díaz *et al*., 2021). In this work, we have identified novel roles of activated LXRs in the control of tumor growth, which are based on interference with various macrophage-mediated mechanisms that help maintain an immunosuppressive TME (Figure 37).

In our hands, the LXR agonist T1317 proved to be effective as an antitumoral drug in two different models of tumor growth, a syngeneic model of Lewis lung carcinoma based on subcutaneous injection of 3LL-R cells and a spontaneous model of breast adenocarcinoma in MMTV-PyMT female mice. Since different responses to LXR activation have been described in male and female mice in several processes, such as atherosclerosis (Joseph *et al*., 2002; Chen *et al*., 2015), circadian rhythm-controlled mechanisms (Feillet *et al*., 2016), or neutrophil function in a myocardial infarction context (DeLeon-Pennell *et al*., 2018), the influence of sex was evaluated in the 3LL-R lung carcinoma model. Control female mice displayed higher variability in tumor growth than the male counterparts, but the LXR agonist was able to inhibit the size of tumors in both sexes. Our conclusion that the drug is effective in females is further supported by the fact that the LXR agonist inhibited tumor development in the mammary glands of MMTV-PyMT female mice. However, since the effects in 3LL-R lung carcinoma tumors were more solid in male mice, subsequent mechanistic studies in this model were carried out in males. Another remarkable aspect is that pharmacological LXR activation was effective on tumors that are already established, thus supporting the therapeutical use of LXR agonists in cancer.

We have dissected the effects of LXR activation in the intratumoral abundance of immune cell populations that have a prognostic value. Recently, Tavazoie and collaborators published a study placing the actions of LXR agonists in the TME in the spotlight. In that work, high doses of a newly developed LXR agonist, RGX-104, resulted

in depletion of MDSCs from the tumor and in an increased cytotoxic T cell response, which inhibited tumor progression. Mechanistic studies using B16F10 melanoma tumors suggested that LXR-induced ApoE expression promoted the apoptosis of MDSCs in the TME through its interaction with the LDL receptor related protein 8 (also known as LRP8) receptor (Tavazoie *et al*., 2018). In our study, the analysis of intratumoral myeloid cells included two populations, CD11b+/Ly6C^{high}/Ly6G- cells and CD11b+/Ly6C^{low}/Ly6G+ cells, enriched in monocytic MDSCs and granulocytic MDSCs, respectively. However, treatment with the LXR agonist T1317 did not alter the frequency or total abundance of these cell populations. These differences might be attributed to several factors, such as the type of tumor, the characteristics of the LXR agonist (including the dose), or the stage of tumor progression at the time of agonist administration. In this sense, the doses of LXR agonists used by Tavazoie and collaborators were of 80-100 mg/kg/day (Tavazoie *et al*., 2018), whereas the doses used in our experiments were remarkably lower (15mg/kg/day). Of note, *ApoE* expression was not upregulated in whole tumors from mice treated with the dose of the LXR agonist used in our study, nor in TAMs exposed to the agonist *in vitro*, whereas the expected increase was observed for other well established LXR targets.

In contrast with the lack of effects in MDSCs, administration of T1317 did result in a significant reduction in the frequency of MHCII^{high} TAMs. Local proliferation within the tumor has been described for TAMs derived either from tissue resident macrophages (Zhu *et al*., 2017) or from blood monocytes (Tymoszuk *et al*., 2014). An important mediator of local TAM expansion is M-CSF (Tymoszuk *et al*., 2014). Our group has previously described that LXR activation inhibits macrophage proliferation induced by M-CSF or GM-CSF (Pascual-García *et al*., 2011). While we cannot discard the possibility that LXRs contribute to control local TAM proliferation, the reduction in the frequency of MHCII^{high} TAMs was not consistently accompanied by a significant reduction in the absolute numbers of these cells within the tumor. These considerations suggest that the changes in the percentage of these cells might be mostly influenced by the relative distribution of other intratumoral cells.

The results from this work strongly suggest that combined actions on the modulation of TAM responses contribute to the antitumoral effects of pharmacological LXR activation

(Figure 37). In fact, the LXR agonist repressed several genes that play key roles in the pro-tumoral program of TAMs. This conclusion is based, in part, on the analysis of the effects of T1317 on the transcriptional profile of MHCII^{low} TAMs and MHCII^{high} TAMs. Interestingly, the LXR agonist repressed different subsets of genes in these two cell populations, with a higher proportion of genes being affected in MHCII^{high} TAMs. For example, the expression of *Nos2*, which reduces the availability of arginine for activated T cells (Martí i Líndez & Reith, 2021), was only repressed in MHCII^{high} TAMs, while the expression of *Trem1* was selectively inhibited in MHCII^{low} TAMs. The explanation for such differences is unclear. Indeed, these subpopulations are different in their expression profile and intratumoral localization, and in the mechanisms used for immunosuppression, with MHCII^{high} TAMs being more dependent on NOS2 activity (Movahedi *et al*., 2010). We cannot discard that MHCII^{low} TAMs, which are found in hypoxic areas within the tumor, may undergo cellular/biochemical adaptations resulting in increased protection of several genes against LXR-mediated repression. Although further understanding of the relative contribution of some of these genes to the suppressive capacity of TAM subpopulations is required, functional assays indicated that LXR activation was effective in inhibiting the capacity of both MHCII^{low} TAMs and MHCII^{high} TAMs to suppress polyclonal T cell proliferation.

In addition to direct suppressive mechanisms, MHCII^{high} TAMs are major producers of the chemokines CCL17 and CCL22 in the TME (Movahedi *et al*., 2010), which are important signals for the recruitment of Treg to tissues. Indeed, migration and accumulation of Treg into tumoral tissue is favored by their expression of the chemokine receptor CCR4, which binds to CCL17 and CCL22 produced in the TME (Marshall *et al*., 2020). As indicated in the introduction, the group had already generated data (Carbó JM. Doctoral thesis, 2017) showing an inhibitory effect of the LXR pathway on the expression of these chemokines by TAMs. In the current study, a significant reduction in the frequency and abundance of intratumoral Treg was detected in T1317-treated mice. Treg infiltrating tumoral tissue are mostly in a potently activated and proliferative state (Tanaka & Sakaguchi, 2017). In order to understand if the pharmacological activation of LXRs was directly affecting Treg functionality, we performed functional assays in both tumoral and splenic Treg populations from 3LL-R tumor-bearing mice. Our results did

not show any inhibitory effect of T1317 on the immunosuppressive potential or the proliferative capacity of Treg from either origin. Moreover, contrary to the observations in tumors, activation of LXRs did not impact the frequency of Treg in the spleen of 3LL-R tumor-bearing mice. These observations suggest that the inhibitory effect of the LXR agonist on the intratumoral abundance of Treg most probably reflects decreased recruitment of these cells to the tumor, instead of diminished central proliferation or survival of these cells. Other studies available in the literature shed contrasting conclusions on the effects of LXR agonists on Treg biology. As an example, the LXR agonist GW3965 inhibited the differentiation of several Treg subpopulations, reducing their presence in the intestine draining mesenteric lymph nodes under homeostatic conditions (Parigi *et al*., 2021). Conversely, the same agonist, GW3965, induced and activated Treg populations in the gut, even enhancing their immunosuppressive capabilities (Herold *et al*., 2017). In a cancer context, Carpenter and collaborators reported that treatment *in vitro* with LXR agonists increased the viability and induced FoxP3 and CTLA-4 expression in Treg stimulated with conditioned media from breast cancer cells (Carpenter *et al*., 2019). They also described that treatment with the LXR inverse agonist SR9243, which acts as an LXR inhibitor, reduced the intratumoral levels of several immune cell populations, namely TADCs, G-MDSCs and Treg (Carpenter *et al*., 2019). Whether or not the actions of SR9243 leading to these results are specifically mediated through LXR inhibition is unclear. In our model, the inhibitory effect of T1317 on Treg abundance was specifically mediated by the LXR pathway, as it was abolished in LXR-deficient mice. In addition, in MMTV-PyMT transgenic mice, even though we did not perform an exhaustive evaluation of intratumoral immune cell populations, administration of the LXR agonist led to a significant reduction in mammary gland *Foxp3* expression, which we interpret as an indication of reduced Treg abundance.

Because Treg exist in low numbers in LLC tumors, we further evaluated the relevance of this immune cell population in our model. Treg depletion using anti-CD25 antibodies resulted in a substantial reduction in tumor progression, thus indicating a pro-tumoral role of this immune cell population in our cancer setting. This result is in line with many evidences showing that Treg infiltration and accumulation in tumors often leads to a dampening in antitumoral responses and worse prognosis (Tanaka & Sakaguchi, 2017)

(Shang *et al*., 2015). Importantly, the LXR agonist did not efficiently reduce tumor growth in Treg-depleted mice. Therefore, taking together the different pieces of evidence from this work, we propose that decreased production of Treg-attracting chemokines by TAMs and subsequent reduction of Treg infiltrates is a mechanism contributing to the suppressive actions of the LXR agonist on tumor growth. However, it should be noted that tumor progression was more profoundly inhibited in control mice treated with the LXR agonist than in mice undergoing Treg depletion. This result reinforces the idea that inhibitory effects on additional aspects of the macrophage pro-tumoral program, as described above, also contribute to the antitumoral activity of the LXR pathway.

At the molecular level, the results from this work propose the targeting of the transcription factor IRF4 as a relevant mechanism that links pharmacologic LXR activation with the downregulation of CCL17 (Figure 37). Previous work by Achuthan and collaborators showed that the GM-CSF-mediated induction of CCL17 was dependent on IRF4 (Achuthan *et al*., 2016). Similar results were obtained by our group for both *Ccl17* and *Ccl22* in the context of the macrophage response to GM-CSF or IL-4 (Carbó JM. Doctoral thesis, 2017). In this work, we have further deepened into this type of studies to show that LXR activation represses the protein expression levels of IRF4 protein and the subsequent production and secretion of CCL17 and CCL22. The relevance of IRF4 in this context was supported by a reduced efficiency of the LXR agonist in suppressing tumor growth in IRF4-deficient mice. Of note, however, these studies were carried out using a model of systemic IRF4 deficiency and the interpretation of the data should be done with caution. IRF4 is an important transcription factor for CD4+ T cell maturation (Mittrücker *et al*., 1997) and for the establishment of Th1 responses, not only for Treg biology (reviewed in Crepeau & Ford, 2020). In fact, the tumors growing in IRF4-deficient mice showed a reduction in all the T cell populations evaluated, not only in Treg, which might compromise the development of antitumoral immune responses. These considerations help explain the increase in tumor growth in IRF4-deficient mice (as also described in Metzger *et al*., 2020), despite Treg numbers being downregulated. For this reason, we are aware that a deficiency in IRF4 specifically in macrophages will better

help evaluate the relevance of IRF4 in the antitumoral actions of pharmacological LXR activation.

Importantly, our results indicate that LXR activation also exerts inhibitory effects on the induction of IRF4, CCL17 and CCL22 in human macrophages. Whether or not the expression levels of IRF4 specifically in TAMs have prognostic value remains elusive. Several studies suggest the pro-tumoral effects of CCL22 and, particularly, CCL17 in different types of human cancer (Liu *et al*., 2015; Li *et al*., 2019; Jorapur *et al*., 2022). Indeed, Treg recruitment mediated by CCL17- and CCL22-producing TAMs has been described to be a marker for worse prognosis in human cancer (Zhu *et al*., 2016; Kinoshita *et al*., 2017; Maolake *el at*., 2017; Wei *et al*., 2019). Our Kaplan-Meier analysis using the R2: Genomics platform for analysis and visualization of genomic data indicated that a higher expression of CCL17 was also associated with a lower overall survival probability in renal clear cell carcinoma and non-small lung cancer. In mice models, interference with CCL17 or its receptor CCR4 reduces Treg recruitment to the tumor (Biragyn *et al*., 2013; Hirata *et al*., 2019). All these observations suggest that targeting Treg recruitment to the tumor through the inhibition of CCL17 might be a promising antitumor strategy.

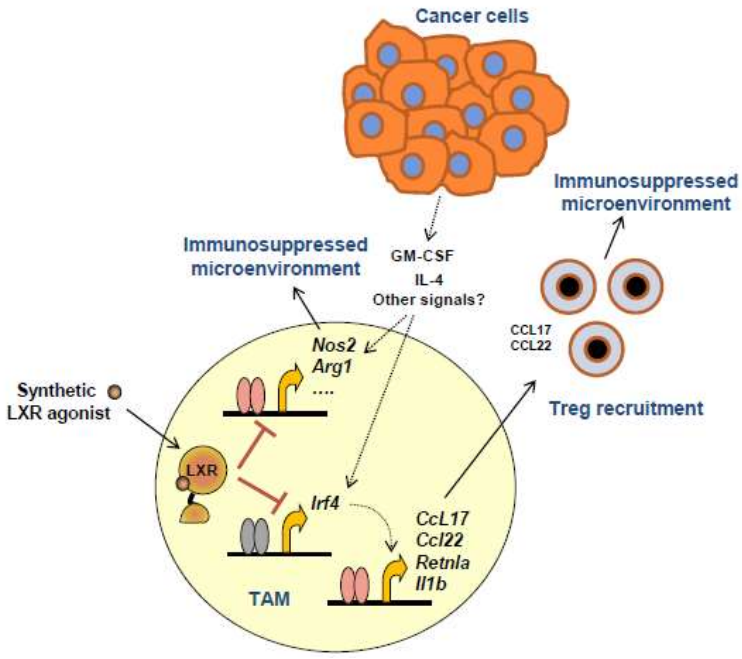


Figure 37. Working model: Pharmacological LXR activation inhibits several macrophage-mediated mechanisms that are important for the maintenance of an immunosuppressed tumor microenvironment. Treatment with an LXR agonist represses several genes that play key roles in the pro-tumoral program of TAMs (*e.g.*, *Nos2*, *Arg1*). Among these, repression of the *Irf4*/*Ccl17* pathway correlates with a reduction in the abundance of Tregs within the tumor.

In the last part of this thesis, we have opened the door to new questions by exploring the specific involvement of a recently identified LXR target, the multifunctional enzyme CD38 (Matalonga *et al* 2017). The role of CD38 in cancer biology is very complex and different studies have shed contrasting observations. On one hand, there are evidences that a low expression of CD38 in tumoral cells from prostatic and pancreatic carcinomas is associated with higher tumor cell survival and aggressiveness, and induction of CD38 expression in these cells reduces cell growth and increases apoptosis (Chini *et al*., 2014; Mottahedeh *et al*., 2018). On the other hand, the expression of CD38 in the tumor has been described to have several pro-tumoral effects. The CD38-mediated production of ADP ribose followed by the concerted action of CD203a/polycystin-1 (PC-1) and CD73,

generates adenosine. The accumulation of adenosine in the TME interferes with immune cell activation and causes immune suppression (Chillemi *et al*., 2017). Another CD38 metabolic byproduct, NAADP, participates in the VEGF-VEGFR2 angiogenic pathway in the TME through its role in Ca+ signaling (Favia *et al*., 2016; Baruch *et al*., 2018). MDSCs and Treg displaying high CD38 expression in the TME have a more potent immunosuppressive activity and, in the case of MDSCs, a higher capacity to promote tumor growth (Karakasheva *et al*., 2015; Feng *et al*., 2017). In addition, inhibition of CD38 in the context of antitumoral adoptive T-cell transfer causes a metabolic reprogramming in T cells, improving their antitumor potential through an increase of intracellular NAD+ levels and sirtuin 1 activity (Chatterjee et al., 2018).

In our studies, combined treatment with the LXR agonist T1317 and conditioned medium from 3LL-R cancer cells resulted in synergistic induction of CD38 expression in macrophages. Interestingly, key pathways mediating inflammatory signaling, such as STAT1 and p38α MAPK, are involved in the synergistic upregulation of CD38 expression under these conditions. STAT1 is a key transcription factor that regulates target genes in response to IFNγ or IFNα/β signaling (Darnell et al., 1994), whereas p38 MAPKs are activated by a plethora of factors (including environmental stress factors, growth factors and pro-inflammatory cytokines) and mediate the downstream activation of many transcription factors, including STAT1 among others (reviewed in Cuadrado & Nebreda, 2010). These observations suggest that cytokines or other proinflammatory soluble factors produced at the TME synergize with the LXR pathway to induce macrophage CD38 expression and are in line with previous work by our group describing the synergistic upregulation of CD38 by LXR agonists and inflammatory mediators, such as LPS, TNFα, and IFNγ (Matalonga *et al*., 2017). In addition, CD38 came out as highly upregulated gene in both $MHCII^{low}$ TAMs and $MHCII^{high}$ TAMs treated with the LXR agonist.

Based on the predominant pro-tumoral roles of CD38, we evaluated tumor progression in the context of a CD38-deficiency in host cells, expecting to obtain an increase in the antitumoral effects of the LXR pathway. A tendency for ameliorated tumor growth was observed in CD38-deficient mice, as compared to WT mice, in line with other reports (Bu *et al*., 2018). To our surprise, however, the treatment with T1317 was less effective in

CD38-deficient mice. Indeed, the response to the agonist was biphasic in these mice, with a first stage in which the drug was able to inhibit tumor progression followed by a second phase in which the agonist was not effective. This type of response suggests either the acquisition of resistance mechanisms that blunt the effect of the treatment in the CD38-deficient background, or the existence of a combination of CD38-independent and CD38-dependent mechanisms operating at different stages of tumor development. Future experiments need to be addressed to evaluate intratumoral immune cell populations and quantification of specific mediators at different time points of tumor progression in CD38-deficient mice. Cell type-specific deletion of CD38 in immune cells is also an interesting approach that will be addressed in the future.

Taken together, this work has characterized deeply the antitumoral actions of the LXR pathway, revealing novel insights about the mechanisms of action of LXR agonists as therapeutic drugs against cancer.

CONCLUSIONS

- Pharmacological activation of the LXR pathway with the agonist T1317 reduces the growth of established tumors in mice. This conclusion is supported by the results obtained in two cancer settings, a model of syngeneic lung carcinoma and a model of spontaneous breast adenocarcinoma.
- In 3LL-R lung carcinoma, the antitumoral effects of pharmacological LXR activation are largely dependent on Treg. LXR activation reduces the intratumoral numbers of Treg, without affecting their frequency in the spleen, their proliferation, or their immunosuppressive capacity in general.
- LXR activation represses several genes that are part of the pro-tumoral program of TAMs and reduced the capacity of TAMs to suppress T cell proliferation.
- LXR activation reduces the expression of the transcription factor IRF4 and the subsequent production of the chemokine CCL17 during the macrophage response to IL-4 or GM-CSF. A similar tendency is observed for CCL22.
- Tumors growing in IRF4-deficient mice do not respond as efficiently to the LXR agonist T1317 as those in WT mice, suggesting that functional expression of IRF4 is important for the antitumoral effects of LXR activation.
- The inhibitory effect of the LXR pathway on the IRF4-CCL17/CCL22 axis is conserved in human macrophages stimulated with IL-4. In addition, high CCL17 expression is associated to poorer survival of patients with non-small lung cancer or renal clear cell carcinoma.
- Factors secreted by cancer cells synergize with LXR activation to induce the expression of the multifunctional enzyme CD38 in macrophages. Inflammatory signaling through the STAT1 and p38 MAP kinase pathways mediate this effect.
- Functional expression of CD38 in host cells is required for sustained antitumoral effects of the LXR pathway.

BIBLIOGRAPHY

- A Study of RGX-104 in Patients With Advanced Solid Malignancies and Lymphoma - Full Text View - ClinicalTrials.gov, (n.d.). https://clinicaltrials.gov/ct2/show/NCT02922764. (Accessed 18 June 2020).

- Achuthan, A., Cook, A. D., Lee, M. C., Saleh, R., Khiew, H. W., Chang, M. W., ... & Hamilton, J. A. (2016). Granulocyte macrophage colony-stimulating factor induces CCL17 production via IRF4 to mediate inflammation. *The Journal of clinical investigation*, *126*(9), 3453-3466.

- Akagawa, K. S., Komuro, I., Kanazawa, H., Yamazaki, T., Mochida, K., & Kishi, F. (2006). Functional heterogeneity of colony-stimulating factor-induced human monocyte-derived macrophages. *Respirology*, *11*, S32-S36.

- Allard, B., Beavis, P. A., Darcy, P. K., & Stagg, J. (2016). Immunosuppressive activities of adenosine in cancer. *Current opinion in pharmacology*, *29*, 7-16.

- Anderson, N. M., & Simon, M. C. (2020). The tumor microenvironment. *Current Biology*, *30*(16), R921-R925.

- Arneth, B. (2020). Tumor microenvironment. *Medicina*, *56*(1), 15.

- Bär, E., Whitney, P. G., Moor, K., e Sousa, C. R., & LeibundGut-Landmann, S. (2014). IL-17 regulates systemic fungal immunity by controlling the functional competence of NK cells. *Immunity*, *40*(1), 117-127.

- Baruch, B. B., Blacher, E., Mantsur, E., Schwartz, H., Vaknine, H., Erez, N., & Stein, R. (2018). Stromal CD38 regulates outgrowth of primary melanoma and generation of spontaneous metastasis. *Oncotarget*, *9*(61), 31797.

- Bassler, K., Schulte-Schrepping, J., Warnat-Herresthal, S., Aschenbrenner, A. C., & Schultze, J. L. (2019). The myeloid cell compartment—cell by cell. *Annual review of immunology*, *37*, 269-293.

- Bayne, L. J., Beatty, G. L., Jhala, N., Clark, C. E., Rhim, A. D., Stanger, B. Z., & Vonderheide, R. H. (2012). Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. *Cancer cell*, *21*(6), 822-835.

- Beceiro, S., Pap, A., Czimmerer, Z., Sallam, T., Guillén, J. A., Gallardo, G., ... & Castrillo, A. (2018). Liver X receptor nuclear receptors are transcriptional regulators of dendritic cell chemotaxis. *Molecular and cellular biology*, *38*(10), e00534-17.

- Becher, B., Tugues, S., & Greter, M. (2016). GM-CSF: from growth factor to central mediator of tissue inflammation. *Immunity*, *45*(5), 963-973.

- Bensinger, S. J., Bradley, M. N., Joseph, S. B., Zelcer, N., Janssen, E. M., Hausner, M. A., ... & Tontonoz, P. (2008). LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell*, *134*(1), 97-111.

- Bingle, L., Brown, N. J., & Lewis, C. E. (2002). The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *196*(3), 254-265.

- Biragyn, A., Bodogai, M., Olkhanud, P. B., Denny-Brown, S. R., Puri, N., Ayukawa, K., ... & Lee-Chang, C. (2013). Inhibition of lung metastasis by chemokine CCL17-mediated in vivo silencing of genes in CCR4+ Tregs. *Journal of immunotherapy (Hagerstown, Md.: 1997)*, *36*(4), 258.

- Bischoff, E. D., Daige, C. L., Petrowski, M., Dedman, H., Pattison, J., Juliano, J., ... & Schulman, I. G. (2010). Non-redundant roles for LXRα and LXRβ in atherosclerosis susceptibility in low density lipoprotein receptor knockout mice [S]. *Journal of lipid research*, *51*(5), 900-906.

- Biswas, S. K. Metabolic reprogramming of immune cells in cancer progression. Immunity 43, 435–449 (2015).

- Bogunovic, M., Ginhoux, F., Helft, J., Shang, L., Hashimoto, D., Greter, M., ... & Merad, M. (2009). Origin of the lamina propria dendritic cell network. *Immunity*, *31*(3), 513-525.

- Bonavita, E., Galdiero, M. R., Jaillon, S., & Mantovani, A. (2015). Phagocytes as corrupted policemen in cancer-related inflammation. *Advances in cancer research*, *128*, 141-171.

- Borriello, F., Iannone, R., Di Somma, S., Loffredo, S., Scamardella, E., Galdiero, M. R., ... & Marone, G. (2017). GM-CSF and IL-3 modulate human monocyte TNF-α production and renewal in in vitro models of trained immunity. *Frontiers in immunology*, *7*, 680.

- Bronte, V., Brandau, S., Chen, S. H., Colombo, M. P., Frey, A. B., Greten, T. F., ... & Gabrilovich, D. I. (2016). Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun 7: 12150.

- Bruns, H., Büttner, M., Fabri, M., Mougiakakos, D., Bittenbring, J. T., Hoffmann, M. H., ... & Gerbitz, A. (2015). Vitamin D–dependent induction of cathelicidin in human macrophages results in cytotoxicity against high-grade B cell lymphoma. *Science translational medicine*, *7*(282), 282ra47-282ra47.

- Bu, X., Kato, J., Hong, J. A., Merino, M. J., Schrump, D. S., Lund, F. E., & Moss, J. (2018). CD38 knockout suppresses tumorigenesis in mice and clonogenic growth of human lung cancer cells. *Carcinogenesis*, *39*(2), 242-251.

- Bunda, S., Kommaraju, K., Heir, P., & Ohh, M. (2013). SOCS-1 mediates ubiquitylation and degradation of GM-CSF receptor. *PLoS one*, *8*(9), e76370.

- Burgess, A. W., Camakaris, J. A. M. E. S., & Metcalf, D. O. N. A. L. D. (1977). Purification and properties of colony-stimulating factor from mouse lung-conditioned medium. *Journal of Biological Chemistry*, *252*(6), 1998-2003.

- Burris, T. P., Solt, L. A., Wang, Y., Crumbley, C., Banerjee, S., Griffett, K., ... & Kojetin, D. J. (2013). Nuclear receptors and their selective pharmacologic modulators. *Pharmacological reviews*, *65*(2), 710-778.

- Campbell, I. K., Rich, M. J., Bischof, R. J., Dunn, A. R., Grail, D., & Hamilton, J. A. (1998). Protection from collagen-induced arthritis in granulocyte-macrophage colony-stimulating factor-deficient mice. *The Journal of Immunology*, *161*(7), 3639-3644.

- Candelaria, N. R., Addanki, S., Zheng, J., Nguyen-Vu, T., Karaboga, H., Dey, P., ... & Lin, C. Y. (2014). Antiproliferative effects and mechanisms of liver X receptor ligands in pancreatic ductal adenocarcinoma cells. *PloS one*, *9*(9), e106289.

- Carbó, J. M., León, T. E., Font-Díaz, J., De la Rosa, J. V., Castrillo, A., Picard, F. R., ... & Valledor, A. F. (2021). Pharmacologic Activation of LXR Alters the Expression Profile of Tumor-Associated Macrophages and the Abundance of Regulatory T Cells in the Tumor Microenvironment. *Cancer research*, *81*(4), 968-985.

- Carbó Marqués, José Mª. 2017. Papel del receptor nuclear LXR en la proliferación y perfil metastático de células tumorales y en la actividad de macrófagos asociados a tumor. Universitat de Barcelona. http://hdl.handle.net/2445/120369

- Carmona-Fontaine, C., Deforet, M., Akkari, L., Thompson, C. B., Joyce, J. A., & Xavier, J. B. (2017). Metabolic origins of spatial organization in the tumor microenvironment. *Proceedings of the National Academy of Sciences*, *114*(11), 2934-2939.

- Carpenter, K. J., Valfort, A. C., Steinauer, N., Chatterjee, A., Abuirqeba, S., Majidi, S., ... & Flaveny, C. A. (2019). LXR-inverse agonism stimulates immune-mediated tumor destruction by enhancing CD8 T-cell activity in triple negative breast cancer. *Scientific reports*, *9*(1), 1-18.

- Cedó, L., García-León, A., Baila-Rueda, L., Santos, D., Grijalva, V., Martínez-Cignoni, M. R., ... & Blanco-Vaca, F. (2016). ApoA-I mimetic administration, but not increased apoA-I-containing HDL, inhibits tumour growth in a mouse model of inherited breast cancer. *Scientific reports*, *6*(1), 1-11.

- Chatterjee, S., Daenthanasanmak, A., Chakraborty, P., Wyatt, M. W., Dhar, P., Selvam, S. P., ... & Mehrotra, S. (2018). CD38-NAD+ axis regulates immunotherapeutic anti-tumor T cell response. *Cell metabolism*, *27*(1), 85-100.

- Chen, Y., Duan, Y., Yang, X., Sun, L., Liu, M., Wang, Q., ... & Han, J. (2015). Inhibition of ERK1/2 and activation of LXR synergistically reduce atherosclerotic lesions in ApoE-deficient mice. *Arteriosclerosis, thrombosis, and vascular biology*, *35*(4), 948-959.

- Chillemi, A., Quarona, V., Antonioli, L., Ferrari, D., Horenstein, A. L., & Malavasi, F. (2017). Roles and modalities of ectonucleotidases in remodeling the multiple myeloma niche. *Frontiers in Immunology*, *8*, 305.

- Chini, C. C., Guerrico, A. M. G., Nin, V., Camacho-Pereira, J., Escande, C., Barbosa, M. T., & Chini, E. N. (2014). Targeting of NAD metabolism in pancreatic cancer cells: potential novel therapy for pancreatic tumors. *Clinical cancer research*, *20*(1), 120-130.

- Chini, E. N. (2009). CD38 as a regulator of cellular NAD: a novel potential pharmacological target for metabolic conditions. Current pharmaceutical design, 15(1), 57-63.

- Chu, K., Miyazaki, M., Man, W. C., & Ntambi, J. M. (2006). Stearoyl-coenzyme A desaturase 1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by liver X receptor activation. *Molecular and cellular biology*, *26*(18), 6786-6798.

- Clausen, B. E., Burkhardt, C., Reith, W., Renkawitz, R., & Förster, I. J. T. R. (1999). Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic research*, *8*(4), 265-277.

- Cockayne, D. A., Muchamuel, T., Grimaldi, J. C., Muller-Steffner, H., Randall, T. D., Lund, F. E., ... & Howard, M. C. (1998). Mice deficient for the ecto-nicotinamide adenine dinucleotide glycohydrolase CD38 exhibit altered humoral immune responses. Blood, The Journal of the American Society of Hematology, 92(4), 1324-1333.

- Codarri, L., Gyülvészi, G., Tosevski, V., Hesske, L., Fontana, A., Magnenat, L., ... & Becher, B. (2011). RORγt drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nature immunology*, *12*(6), 560-567.

- Commerford, S. R., Vargas, L., Dorfman, S. E., Mitro, N., Rocheford, E. C., Mak, P. A., ... & Saez, E. (2007). Dissection of the insulin-sensitizing effect of liver X receptor ligands. *Molecular Endocrinology*, *21*(12), 3002-3012.

- Condamine, T., Dominguez, G. A., Youn, J. I., Kossenkov, A. V., Mony, S., Alicea-Torres, K., ... & Gabrilovich, D. I. (2016). Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. Science immunology, 1(2), aaf8943-aaf8943.

- Condamine, T., Ramachandran, I., Youn, J. I., & Gabrilovich, D. I. (2015). Regulation of tumor metastasis by myeloid-derived suppressor cells. Annual review of medicine, 66, 97-110.

- Corzo, C. A., Condamine, T., Lu, L., Cotter, M. J., Youn, J. I., Cheng, P., ... & Gabrilovich, D. I. (2010). HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. Journal of Experimental Medicine, 207(11), 2439-2453.

- Crepeau, R. L., & Ford, M. L. (2020). Programmed T cell differentiation: Implications for transplantation. *Cellular immunology*, *351*, 104099.

- Cuadrado, A., & Nebreda, A. R. (2010). Mechanisms and functions of p38 MAPK signalling. *Biochemical Journal*, *429*(3), 403-417.

- Cui, G., Qin, X., Wu, L., Zhang, Y., Sheng, X., Yu, Q., ... & Zang, Y. Q. (2011). Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. *The Journal of clinical investigation*, *121*(2), 658-670.

- Cullen, S. P., Brunet, M., & Martin, S. J. (2010). Granzymes in cancer and immunity. *Cell Death & Differentiation*, *17*(4), 616-623.

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

- Curran, C. S., & Bertics, P. J. (2012). Lactoferrin regulates an axis involving CD11b and CD49d integrins and the chemokines MIP-1α and MCP-1 in GM-CSF-treated human primary eosinophils. *Journal of Interferon & Cytokine Research*, *32*(10), 450-461.

- Curran, M. A., Montalvo, W., Yagita, H., & Allison, J. P. (2010). PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proceedings of the National Academy of Sciences*, *107*(9), 4275-4280.

- Cynamon, M. H., Sorg, T. B., & Patapow, A. (1988). Utilization and metabolism of NAD by Haemophilus parainfluenzae. Microbiology, 134(10), 2789-2799.

- Däbritz, J., Weinhage, T., Varga, G., Wirth, T., Walscheid, K., Brockhausen, A., ... & Foell, D. (2015). Reprogramming of monocytes by GM-CSF contributes to regulatory immune functions during intestinal inflammation. *The Journal of Immunology*, *194*(5), 2424-2438.

- Dai, X. M., Ryan, G. R., Hapel, A. J., Dominguez, M. G., Russell, R. G., Kapp, S., ... & Stanley, E. R. (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, The Journal of the American Society of Hematology*, *99*(1), 111-120.

- Darnell Jr, J. E., Kerr, L. M., & Stark, G. R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*, *264*(5164), 1415-1421.

- De Medina, P., Paillasse, M. R., Segala, G., Voisin, M., Mhamdi, L., Dalenc, F., ... & Poirot, M. (2013). Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties. *Nature communications*, *4*(1), 1-10.

- DeLeon-Pennell, K. Y., Mouton, A. J., Ero, O. K., Ma, Y., Padmanabhan Iyer, R., Flynn, E. R., ... & Lindsey, M. L. (2018). LXR/RXR signaling and neutrophil

phenotype following myocardial infarction classify sex differences in remodeling. *Basic research in cardiology*, *113*(5), 1-19.

- Dennis, K. L., Blatner, N. R., Gounari, F., & Khazaie, K. (2013). Current status of IL-10 and regulatory T-cells in cancer. *Current opinion in oncology*, *25*(6), 637.

- Derangere, V., Chevriaux, A., Courtaut, F., Bruchard, M., Berger, H., Chalmin, F., ... & Rébé, C. (2014). Liver X receptor β activation induces pyroptosis of human and murine colon cancer cells. *Cell Death & Differentiation*, *21*(12), 1914-1924.

- Dixon, E. D., Nardo, A. D., Claudel, T., & Trauner, M. (2021). The role of lipid sensing nuclear receptors (PPARs and LXR) and metabolic lipases in obesity, diabetes and NAFLD. *Genes*, *12*(5), 645.

- Dougan, M., Dranoff, G., & Dougan, S. K. (2019). GM-CSF, IL-3, and IL-5 family of cytokines: regulators of inflammation. *Immunity*, *50*(4), 796-811.

- Downs-Canner, S., Berkey, S., Delgoffe, G. M., Edwards, R. P., Curiel, T., Odunsi, K., ... & Obermajer, N. (2017). Suppressive IL-17A+ Foxp3+ and ex-Th17 IL-17AnegFoxp3+ Treg cells are a source of tumour-associated Treg cells. Nat Commun 8: 14649.

- Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., ... & Mulligan, R. C. (1993). Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proceedings of the National Academy of Sciences*, *90*(8), 3539-3543.

- El Chartouni, C., Schwarzfischer, L., & Rehli, M. (2010). Interleukin-4 induced interferon regulatory factor (Irf) 4 participates in the regulation of alternative macrophage priming. *Immunobiology*, *215*(9-10), 821-825.

- El Roz, A., Bard, J. M., Huvelin, J. M., & Nazih, H. (2012). LXR agonists and ABCG1-dependent cholesterol efflux in MCF-7 breast cancer cells: relation to proliferation and apoptosis. *Anticancer research*, *32*(7), 3007-3013.

- El-Behi, M., Ciric, B., Dai, H., Yan, Y., Cullimore, M., Safavi, F., ... & Rostami, A. (2011). The encephalitogenicity of TH 17 cells is dependent on IL-1-and IL-23-induced production of the cytokine GM-CSF. *Nature immunology*, *12*(6), 568-575.

- Engelhardt, J. J., Boldajipour, B., Beemiller, P., Pandurangi, P., Sorensen, C., Werb, Z., ... & Krummel, M. F. (2012). Marginating dendritic cells of the tumor

microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. *Cancer cell*, *21*(3), 402-417.

- Epelman, S., Lavine, K. J., & Randolph, G. J. (2014). Origin and functions of tissue macrophages. *Immunity*, *41*(1), 21-35.

- Estrada-Figueroa, L. A., Ramírez-Jiménez, Y., Osorio-Trujillo, C., Shibayama, M., NAVARRO-GARCÍA, F., GARCÍA-TOVAR, C., & TALAMÁS-ROHANA, P. (2011). Absence of CD38 delays arrival of neutrophils to the liver and innate immune response development during hepatic amoebiasis by Entamoeba histolytica. Parasite immunology, 33(12), 661-668.

- Favia, A., Pafumi, I., Desideri, M., Padula, F., Montesano, C., Passeri, D., ... & Filippini, A. (2016). NAADP-dependent Ca2+ signaling controls melanoma progression, metastatic dissemination and neoangiogenesis. *Scientific reports*, *6*(1), 1-12.

- Feillet, C., Guérin, S., Lonchampt, M., Dacquet, C., Gustafsson, J. Å., Delaunay, F., & Teboul, M. (2016). Sexual Dimorphism in Circadian Physiology Is Altered in LXR α Deficient Mice. *PLoS One*, *11*(3), e0150665.

- Feng, X., Zhang, L., Acharya, C., An, G., Wen, K., Qiu, L., ... & Anderson, K. C. (2017). Targeting CD38 suppresses induction and function of T regulatory cells to mitigate immunosuppression in multiple myeloma. *Clinical cancer research*, *23*(15), 4290-4300.

- Flaveny, C. A., Griffett, K., El-Gendy, B. E. D. M., Kazantzis, M., Sengupta, M., Amelio, A. L., ... & Burris, T. P. (2015). Broad anti-tumor activity of a small molecule that selectively targets the Warburg effect and lipogenesis. *Cancer cell*, *28*(1), 42-56.

- Fleetwood, A. J., Lawrence, T., Hamilton, J. A., & Cook, A. D. (2007). Granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF-dependent macrophage phenotypes display differences in cytokine profiles and transcription factor activities: implications for CSF blockade in inflammation. *The Journal of immunology*, *178*(8), 5245-5252.

- Font-Díaz, J., Jiménez-Panizo, A., Caelles, C., dM Vivanco, M., Pérez, P., Aranda, A., ... & Valledor, A. F. (2021, August). Nuclear receptors: Lipid and hormone sensors with essential roles in the control of cancer development. In *Seminars in Cancer Biology* (Vol. 73, pp. 58-75). Academic Press.

- Francisco, L. M., Salinas, V. H., Brown, K. E., Vanguri, V. K., Freeman, G. J., Kuchroo, V. K., & Sharpe, A. H. (2009). PD-L1 regulates the development,

maintenance, and function of induced regulatory T cells. *Journal of Experimental Medicine*, *206*(13), 3015-3029.

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

- Frasca, L., Fedele, G., Deaglio, S., Capuano, C., Palazzo, R., Vaisitti, T., ... & Ausiello, C. M. (2006). CD38 orchestrates migration, survival, and Th1 immune response of human mature dendritic cells. Blood, 107(6), 2392-2399.

- Fu, C., & Jiang, A. (2018). Dendritic cells and CD8 T cell immunity in tumor microenvironment. *Frontiers in immunology*, *9*, 3059.

- Fu, L. Q., Du, W. L., Cai, M. H., Yao, J. Y., Zhao, Y. Y., & Mou, X. Z. (2020). The roles of tumor-associated macrophages in tumor angiogenesis and metastasis. *Cellular Immunology*, *353*, 104119.

- Fu, W., Yao, J., Huang, Y., Li, Q., Li, W., Chen, Z., ... & Yan, J. (2014). LXR agonist regulates the carcinogenesis of PCa via the SOCS3 pathway. *Cellular Physiology and Biochemistry*, *33*(1), 195-204.

- Fuentes-Prior, P., Rojas, A., Hagler, A. T., & Estébanez-Perpiñá, E. (2019). Diversity of quaternary structures regulates nuclear receptor activities. *Trends in biochemical sciences*, *44*(1), 2-6.

- Fukuchi, J., Kokontis, J. M., Hiipakka, R. A., Chuu, C. P., & Liao, S. (2004). Antiproliferative effect of liver X receptor agonists on LNCaP human prostate cancer cells. *Cancer research*, *64*(21), 7686-7689.

- Funaro, A., Horenstein, A. L., Calosso, L., Morra, M., Tarocco, R. P., Franco, L., ... & Malavasi, F. (1996). Identification and characterization of an active soluble form of human CD38 in normal and pathological fluids. International immunology, 8(11), 1643-1650.

- Futosi, K., Fodor, S., & Mócsai, A. (2013). Reprint of Neutrophil cell surface receptors and their intracellular signal transduction pathways. *International immunopharmacology*, *17*(4), 1185-1197.

- Gelman, L., Deterre, P., Gouy, H., Boumsell, L., Debré, P., & Bismuth, G. (1993). The lymphocyte surface antigen CD38 acts as a nicotinamide adenine dinucleotide glycohydrolase in human T lymphocytes. European journal of immunology, 23(12), 3361-3364.

- Geyeregger, R., Shehata, M., Zeyda, M., Kiefer, F. W., Stuhlmeier, K. M., Porpaczy, E., ... & Stulnig, T. M. (2009). Liver X receptors interfere with cytokine-induced proliferation and cell survival in normal and leukemic lymphocytes. *Journal of leukocyte biology*, *86*(5), 1039-1048.

- Geyeregger, R., Zeyda, M., Bauer, W., Kriehuber, E., Säemann, M. D., Zlabinger, G. J., ... & Stulnig, T. M. (2007). Liver X receptors regulate dendritic cell phenotype and function through blocked induction of the actin-bundling protein fascin. *Blood*, *109*(10), 4288-4295.

- Ghisletti, S., Huang, W., Ogawa, S., Pascual, G., Lin, M. E., Willson, T. M., ... & Glass, C. K. (2007). Parallel SUMOylation-dependent pathways mediate gene-and signal-specific transrepression by LXRs and PPARγ. *Molecular cell*, *25*(1), 57-70.

- Glaría, E., Letelier, N. A., & Valledor, A. F. (2020). Integrating the roles of liver X receptors in inflammation and infection: Mechanisms and outcomes. *Current Opinion in Pharmacology*, *53*, 55-65.

- Glaría, E., & Valledor, A. F. (2020). Roles of CD38 in the Immune Response to Infection. *Cells*, *9*(1), 228.

- Goossens, P., Rodriguez-Vita, J., Etzerodt, A., Masse, M., Rastoin, O., Gouirand, V., ... & Lawrence, T. (2019). Membrane cholesterol efflux drives tumor-associated macrophage reprogramming and tumor progression. *Cell metabolism*, *29*(6), 1376-1389.

- Gorina, R., Font-Nieves, M., Márquez-Kisinousky, L., Santalucia, T., & Planas, A. M. (2011). Astrocyte TLR4 activation induces a proinflammatory environment through the interplay between MyD88-dependent NFκB signaling, MAPK, and Jak1/Stat1 pathways. *Glia*, *59*(2), 242-255.

- Greter, M., Helft, J., Chow, A., Hashimoto, D., Mortha, A., Agudo-Cantero, J., ... & Merad, M. (2012). GM-CSF controls nonlymphoid tissue dendritic cell homeostasis but is dispensable for the differentiation of inflammatory dendritic cells. *Immunity*, *36*(6), 1031-1046.

- Groot, P. H., Pearce, N. J., Yates, J. W., Stocker, C., Sauermelch, C., Doe, C. P., ... & Jaye, M. (2005). Synthetic LXR agonists increase LDL in CETP species. *Journal of lipid research*, *46*(10), 2182-2191.

- Grosso, J. F., & Jure-Kunkel, M. N. (2013). CTLA-4 blockade in tumor models: an overview of preclinical and translational research. *Cancer Immunity Archive*, *13*(1).

- Gschwend, J., Sherman, S. P., Ridder, F., Feng, X., Liang, H. E., Locksley, R. M., ... & Schneider, C. (2021). Alveolar macrophages rely on GM-CSF from alveolar epithelial type 2 cells before and after birth. *Journal of Experimental Medicine*, *218*(10), e20210745.

- Guilliams, M., De Kleer, I., Henri, S., Post, S., Vanhoutte, L., De Prijck, S., ... & Lambrecht, B. N. (2013). Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *Journal of Experimental Medicine*, *210*(10), 1977-1992.

- Guy, C. T., Cardiff, R. D., & Muller, W. J. (1992). Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. *Molecular and cellular biology*, *12*(3), 954-961.

- Hambardzumyan, D., Gutmann, D. H., & Kettenmann, H. (2016). The role of microglia and macrophages in glioma maintenance and progression. *Nature neuroscience*, *19*(1), 20-27.

- Hamilton, J. A. (2019). GM-CSF-dependent inflammatory pathways. *Frontiers in immunology*, *10*, 2055.

- Han, S., Zhuang, H., Shumyak, S., Wu, J., Xie, C., Li, H., ... & Reeves, W. H. (2018). Liver X receptor agonist therapy prevents diffuse alveolar hemorrhage in murine lupus by repolarizing macrophages. *Frontiers in Immunology*, *9*, 135.

- Hanada, T., Nakagawa, M., Emoto, A., Nomura, T., Nasu, N., & Nomura, Y. (2000). Prognostic value of tumor-associated macrophage count in human bladder cancer. *International Journal of Urology*, *7*(7), 263-269.

- Haque, A. S. M., Moriyama, M., Kubota, K., Ishiguro, N., Sakamoto, M., Chinju, A., ... & Nakamura, S. (2019). CD206+ tumor-associated macrophages promote proliferation and invasion in oral squamous cell carcinoma via EGF production. *Scientific reports*, *9*(1), 1-10.

- Harden, J. L., & Egilmez, N. K. (2012). Indoleamine 2, 3-dioxygenase and dendritic cell tolerogenicity. *Immunological investigations*, *41*(6-7), 738-764.

- Heinrichsdorff, J., Luedde, T., Perdiguero, E., Nebreda, A. R., & Pasparakis, M. (2008). p38α MAPK inhibits JNK activation and collaborates with IκB kinase 2 to prevent endotoxin-induced liver failure. *EMBO reports*, *9*(10), 1048-1054.

- Herbert, M., Sauer, E., Smethurst, G., Kraiβ, A., Hilpert, A. K., & Reidl, J. (2003). Nicotinamide ribosyl uptake mutants in Haemophilus influenzae. Infection and immunity, 71(9), 5398-5401.

- Herndler-Brandstetter, D., & Flavell, R. A. (2014). Producing GM-CSF: a unique T helper subset?. *Cell research*, *24*(12), 1379-1380.

- Herold, M., Breuer, J., Hucke, S., Knolle, P., Schwab, N., Wiendl, H., & Klotz, L. (2017). Liver X receptor activation promotes differentiation of regulatory T cells. *PLoS One*, *12*(9), e0184985.

- Hindinger, C., Hinton, D. R., Kirwin, S. J., Atkinson, R. D., Burnett, M. E., Bergmann, C. C., & Stohlman, S. A. (2006). Liver X receptor activation decreases the severity of experimental autoimmune encephalomyelitis. *Journal of neuroscience research*, *84*(6), 1225-1234.

- Hirata, A., Hashimoto, H., Shibasaki, C., Narumi, K., & Aoki, K. (2019). Intratumoral IFN-α gene delivery reduces tumor-infiltrating regulatory T cells through the downregulation of tumor CCL17 expression. *Cancer gene therapy*, *26*(9), 334-343.

- Hirata, Y., Egea, L., Dann, S. M., Eckmann, L., & Kagnoff, M. F. (2010). GM-CSF-facilitated dendritic cell recruitment and survival govern the intestinal mucosal response to a mouse enteric bacterial pathogen. *Cell host & microbe*, *7*(2), 151-163.

- Hodi, F. S., Lee, S., McDermott, D. F., Rao, U. N., Butterfield, L. H., Tarhini, A. A., ... & Kirkwood, J. M. (2014). Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. *Jama*, *312*(17), 1744-1753.

- Hogan, K. A., Chini, C., & Chini, E. N. (2019). The multi-faceted ecto-enzyme CD38: roles in immunomodulation, cancer, aging, and metabolic diseases. Frontiers in immunology, 10, 1187.

- Hong, I. S. (2016). Stimulatory versus suppressive effects of GM-CSF on tumor progression in multiple cancer types. *Experimental & molecular medicine*, *48*(7), e242-e242.

- Honvo-Houéto, E., & Truchet, S. (2015). Indirect immunofluorescence on frozen sections of mouse mammary gland. JoVE (Journal of Visualized Experiments), (106), e53179.

- Hu, C., Liu, D., Zhang, Y., Lou, G., Huang, G., Chen, B., ... & He, F. (2014). LXRα-mediated downregulation of FOXM1 suppresses the proliferation of hepatocellular carcinoma cells. *Oncogene*, *33*(22), 2888-2897.

- Huang, W., Ghisletti, S., Saijo, K., Gandhi, M., Aouadi, M., Tesz, G. J., ... & Glass, C. K. (2011). Coronin 2A mediates actin-dependent de-repression of inflammatory response genes. *Nature*, *470*(7334), 414-418.

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

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

- Iwai, Y., Ishida, M., Tanaka, Y., Okazaki, T., Honjo, T., & Minato, N. (2002). Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proceedings of the National Academy of Sciences*, *99*(19), 12293-12297.

- Izquierdo, C., Ortiz, A. Z., Presa, M., Malo, S., Montoya, A., Garabatos, N., ... & Stratmann, T. (2018). Treatment of T1D via optimized expansion of antigen-specific Tregs induced by IL-2/anti-IL-2 monoclonal antibody complexes and peptide/MHC tetramers. *Scientific reports*, *8*(1), 1-14.

- Jalil, A., Bourgeois, T., Ménégaut, L., Lagrost, L., Thomas, C., & Masson, D. (2019). Revisiting the role of LXRs in PUFA metabolism and phospholipid homeostasis. *International Journal of Molecular Sciences*, *20*(15), 3787.

- Jinushi, M., Sato, M., Kanamoto, A., Itoh, A., Nagai, S., Koyasu, S., ... & Tahara, H. (2009). Milk fat globule epidermal growth factor–8 blockade triggers tumor destruction through coordinated cell-autonomous and immune-mediated mechanisms. *Journal of Experimental Medicine*, *206*(6), 1317-1326.

- Jorapur, A., Marshall, L. A., Jacobson, S., Xu, M., Marubayashi, S., Zibinsky, M., ... & Cutler, G. (2022). EBV+ tumors exploit tumor cell-intrinsic and-extrinsic mechanisms to produce regulatory T cell-recruiting chemokines CCL17 and CCL22. *PLoS pathogens*, *18*(1), e1010200.

- Joseph, S. B., Bradley, M. N., Castrillo, A., Bruhn, K. W., Mak, P. A., Pei, L., ... & Tontonoz, P. (2004). LXR-dependent gene expression is important for macrophage survival and the innate immune response. *Cell*, *119*(2), 299-309.

- Joseph, S. B., McKilligin, E., Pei, L., Watson, M. A., Collins, A. R., Laffitte, B. A., ... & Tontonoz, P. (2002). Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proceedings of the National Academy of Sciences*, *99*(11), 7604-7609.

- Kang, B. N., Tirumurugaan, K. G., Deshpande, D. A., Amrani, Y., Panettieri, R. A., Walseth, T. F., ... & Karman, M. S. (2006). Transcriptional regulation of CD38 expression by tumor necrosis factor-α in human airway smooth muscle cells: role of NF-κB and sensitivity to glucocorticoids. The FASEB journal, 20(7), 1000-1002.

- Karakasheva, T. A., Waldron, T. J., Eruslanov, E., Kim, S. B., Lee, J. S., O'Brien, S., ... & Rustgi, A. K. (2015). CD38-expressing myeloid-derived suppressor cells promote tumor growth in a murine model of esophageal cancer. *Cancer research*, *75*(19), 4074-4085.

- Kawahara, A., Hattori, S., Akiba, J., Nakashima, K., Taira, T., Watari, K., ... & Ono, M. (2010). Infiltration of thymidine phosphorylase-positive macrophages is closely associated with tumor angiogenesis and survival in intestinal type gastric cancer. *Oncology reports*, *24*(2), 405-415.

- Kim, K. H., Lee, G. Y., Kim, J. I., Ham, M., Lee, J. W., & Kim, J. B. (2010). Inhibitory effect of LXR activation on cell proliferation and cell cycle progression through lipogenic activity [S]. *Journal of lipid research*, *51*(12), 3425-3433.

- Kinoshita, T., Kudo-Saito, C., Muramatsu, R., Fujita, T., Saito, M., Nagumo, H., ... & Kawakami, Y. (2017). Determination of poor prognostic immune features of tumour microenvironment in non-smoking patients with lung adenocarcinoma. *European Journal of Cancer*, *86*, 15-27.

- Kirchgessner, T. G., Sleph, P., Ostrowski, J., Lupisella, J., Ryan, C. S., Liu, X., ... & Frost, R. J. (2016). Beneficial and adverse effects of an LXR agonist on human lipid and lipoprotein metabolism and circulating neutrophils. *Cell metabolism*, *24*(2), 223-233.

- Kitamura, T., Hayashida, K., Sakamaki, K., Yokota, T., Arai, K. I., & Miyajima, A. (1991). Reconstitution of functional receptors for human granulocyte/macrophage colony-stimulating factor (GM-CSF): evidence that the protein encoded by the AIC2B cDNA is a subunit of the murine GM-CSF receptor. *Proceedings of the National Academy of Sciences*, *88*(12), 5082-5086.

- Ko, H. J., Brady, J. L., Ryg-Cornejo, V., Hansen, D. S., Vremec, D., Shortman, K., ... & Lew, A. M. (2014). GM-CSF–responsive monocyte-derived dendritic cells are pivotal in Th17 pathogenesis. *The Journal of Immunology*, *192*(5), 2202-2209.

- Kohanbash, G., McKaveney, K., Sakaki, M., Ueda, R., Mintz, A. H., Amankulor, N., ... & Okada, H. (2013). GM-CSF promotes the immunosuppressive activity of glioma-infiltrating myeloid cells through interleukin-4 receptor-α. *Cancer research*, *73*(21), 6413-6423.

- Korach-André, M., Archer, A., Barros, R. P., Parini, P., & Gustafsson, J. Å. (2011). Both liver-X receptor (LXR) isoforms control energy expenditure by regulating brown adipose tissue activity. *Proceedings of the National Academy of Sciences*, *108*(1), 403-408.

- Krausgruber, T., Blazek, K., Smallie, T., Alzabin, S., Lockstone, H., Sahgal, N., ... & Udalova, I. A. (2011). IRF5 promotes inflammatory macrophage polarization and TH 1-TH 17 responses. *Nature immunology*, *12*(3), 231-238.

- Kryczek, I., Wei, S., Zou, L., Zhu, G., Mottram, P., Xu, H., ... & Zou, W. (2006). Cutting edge: induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells. *The Journal of Immunology*, *177*(1), 40-44.

- A) Kumar, V., Cheng, P., Condamine, T., Mony, S., Languino, L. R., McCaffrey, J. C., ... & Gabrilovich, D. I. (2016). CD45 phosphatase inhibits STAT3 transcription factor activity in myeloid cells and promotes tumor-associated macrophage differentiation. *Immunity*, *44*(2), 303-315.

- B) Kumar, V., Patel, S., Tcyganov, E., & Gabrilovich, D. I. (2016). The nature of myeloid-derived suppressor cells in the tumor microenvironment. Trends in immunology, 37(3), 208-220.

- Kwong, A. K. Y., Chen, Z., Zhang, H., Leung, F. P., Lam, C. M. C., Ting, K. Y., ... & Lee, H. C. (2012). Catalysis-based inhibitors of the calcium signaling function of CD38. Biochemistry, 51(1), 555-564.

- Lacey, D. C., Achuthan, A., Fleetwood, A. J., Dinh, H., Roiniotis, J., Scholz, G. M., ... & Hamilton, J. A. (2012). Defining GM-CSF–and macrophage-CSF–dependent macrophage responses by in vitro models. *The Journal of Immunology*, *188*(11), 5752-5765.

- Laffitte, B. A., Repa, J. J., Joseph, S. B., Wilpitz, D. C., Kast, H. R., Mangelsdorf, D. J., & Tontonoz, P. (2001). LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proceedings of the National Academy of Sciences*, *98*(2), 507-512.

- Lammel, V., Stoeckle, C., Padberg, B., Zweifel, R., Kienle, D. L., Reinhart, W. H., & Simon, H. U. (2012). Hypereosinophilia driven by GM-CSF in large-cell carcinoma of the lung. *Lung Cancer*, *76*(3), 493-495.

- Langers, I., Renoux, V. M., Thiry, M., Delvenne, P., & Jacobs, N. (2012). Natural killer cells: role in local tumor growth and metastasis. *Biologics: targets & therapy*, *6*, 73.

- Larocca, C. A., LeBoeuf, N. R., Silk, A. W., & Kaufman, H. L. (2020). An update on the role of talimogene laherparepvec (T-VEC) in the treatment of melanoma: best practices and future directions. *American Journal of Clinical Dermatology*, 1-12.

- Lawson, D. H., Lee, S., Zhao, F., Tarhini, A. A., Margolin, K. A., Ernstoff, M. S., ... & Kirkwood, J. M. (2015). Randomized, placebo-controlled, Phase III trial of yeast-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) versus peptide vaccination versus GM-CSF plus peptide vaccination versus placebo in patients with no evidence of disease after complete surgical resection of locally advanced and/or stage IV melanoma: a trial of the Eastern Cooperative Oncology Group–American College of Radiology Imaging Network Cancer Research Group (E4697). *Journal of Clinical Oncology*, *33*(34), 4066.

- Lee, J. H., Park, S. M., Kim, O. S., Lee, C. S., Woo, J. H., Park, S. J., ... & Jou, I. (2009). Differential SUMOylation of LXRα and LXRβ mediates transrepression of STAT1 inflammatory signaling in IFN-γ-stimulated brain astrocytes. *Molecular cell*, *35*(6), 806-817.

- Lee, K. Y. (2019). M1 and M2 polarization of macrophages: A mini-review.

- Leek, R. D., Lewis, C. E., Whitehouse, R., Greenall, M., Clarke, J., & Harris, A. L. (1996). Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer research*, *56*(20), 4625-4629.

- Lei, C., Lin, R., Wang, J., Tao, L., Fu, X., Qiu, Y., & Lei, B. (2017). Amelioration of amyloid β-induced retinal inflammatory responses by a LXR agonist TO901317 is associated with inhibition of the NF-κB signaling and NLRP3 inflammasome. *Neuroscience*, *360*, 48-60.

- León Moreno, Theresa Elizabeth. 2013. Selective roles of the Nuclear Receptors LXR in the transcriptional control of classical and alternative macrophage activation = Efectos selectivos de los receptores nucleares LXR en el control transcripcional de la activación clásica y alternativa de macrófagos. Universitat de Barcelona. http://hdl.handle.net/2445/49088

- Li, P., Spann, N. J., Kaikkonen, M. U., Lu, M., Fox, J. N., Bandyopadhyay, G., ... & Olefsky, J. M. (2013). NCoR repression of LXRs restricts macrophage biosynthesis of insulin-sensitizing omega 3 fatty acids. *Cell*, *155*(1), 200-214.

- Li, J., Yang, C., Yang, J., & Zou, L. (2019). Down-regulation of CCL17 in cancer-associated fibroblasts inhibits cell migration and invasion of breast cancer through ERK1/2 pathway. *Cancer management and research*, *11*, 7439.

- Li, R., Rezk, A., Miyazaki, Y., Hilgenberg, E., Touil, H., Shen, P., ... & Canadian B Cells in MS Team. (2015). Proinflammatory GM-CSF–producing B cells in multiple sclerosis and B cell depletion therapy. *Science translational medicine*, *7*(310), 310ra166-310ra166.

- Liang, B., Workman, C., Lee, J., Chew, C., Dale, B. M., Colonna, L., ... & Clynes, R. (2008). Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *The Journal of Immunology*, *180*(9), 5916-5926.

- Liang, N., Jakobsson, T., Fan, R., & Treuter, E. (2019). The nuclear receptor—Co-repressor complex in control of liver metabolism and disease. *Frontiers in endocrinology*, *10*, 411.

- Lieschke, G. J., Grail, D., Hodgson, G., Metcalf, D., Stanley, E., Cheers, C., ... & Dunn, A. R. (1994). Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization.

- Lin, W. K., Bolton, E. L., Cortopassi, W. A., Wang, Y., O'Brien, F., Maciejewska, M., ... & Terrar, D. A. (2017). Synthesis of the Ca2+-mobilizing messengers NAADP and cADPR by intracellular CD38 enzyme in the mouse heart: Role in β-adrenoceptor signaling. Journal of Biological Chemistry, 292(32), 13243-13257.

- Liontos, L. M., Dissanayake, D., Ohashi, P. S., Weiss, A., Dragone, L. L., & McGlade, C. J. (2011). The Src-like adaptor protein regulates GM-CSFR signaling and monocytic dendritic cell maturation. *The Journal of Immunology*, *186*(4), 1923-1933.

- Lischke, T., Heesch, K., Schumacher, V., Schneider, M., Haag, F., Koch-Nolte, F., & Mittrücker, H. W. (2013). CD38 controls the innate immune response against Listeria monocytogenes. Infection and immunity, 81(11), 4091-4099.

- Liu, L. B., Xie, F., Chang, K. K., Shang, W. Q., Meng, Y. H., Yu, J. J., ... & Li, M. Q. (2015). Chemokine CCL17 induced by hypoxia promotes the proliferation of cervical cancer cell. *American journal of cancer research*, *5*(10), 3072.

- Liu, L. Y., Wang, H., Xenakis, J. J., & Spencer, L. A. (2015). Notch signaling mediates granulocyte-macrophage colony-stimulating factor priming-induced transendothelial migration of human eosinophils. *Allergy*, *70*(7), 805-812.

- Locati, M., Curtale, G., & Mantovani, A. (2020). Diversity, mechanisms, and significance of macrophage plasticity. *Annual Review of Pathology: Mechanisms of Disease*, *15*, 123-147.

- Ma, L., Dong, F., Zaid, M., Kumar, A., & Zha, X. (2012). ABCA1 protein enhances Toll-like receptor 4 (TLR4)-stimulated interleukin-10 (IL-10) secretion through protein kinase A (PKA) activation. *Journal of Biological Chemistry*, *287*(48), 40502-40512.

- Malavasi, F., Deaglio, S., Funaro, A., Ferrero, E., Horenstein, A. L., Ortolan, E., ... & Aydin, S. (2008). Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiological reviews, 88(3), 841-886.

- Man, K., Miasari, M., Shi, W., Xin, A., Henstridge, D. C., Preston, S., ... & Kallies, A. (2013). The transcription factor IRF4 is essential for TCR affinity–mediated metabolic programming and clonal expansion of T cells. *Nature immunology*, *14*(11), 1155-1165.

- Mantovani, A. (2016). Reflections on immunological nomenclature: in praise of imperfection. *Nature immunology*, *17*(3), 215-216.

- Mantovani, A., Marchesi, F., Malesci, A., Laghi, L., & Allavena, P. (2017). Tumour-associated macrophages as treatment targets in oncology. *Nature reviews Clinical oncology*, *14*(7), 399-416.

- Mao, Y., Sarhan, D., Steven, A., Seliger, B., Kiessling, R., & Lundqvist, A. (2014). Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity. Clinical cancer research, 20(15), 4096-4106.

- Maolake, A., Izumi, K., Shigehara, K., Natsagdorj, A., Iwamoto, H., Kadomoto, S., ... & Mizokami, A. (2017). Tumor-associated macrophages promote prostate cancer migration through activation of the CCL22–CCR4 axis. *Oncotarget*, *8*(6), 9739.

- Marshall, L. A., Marubayashi, S., Jorapur, A., Jacobson, S., Zibinsky, M., Robles, O., ... & Talay, O. (2020). Tumors establish resistance to immunotherapy by regulating Treg recruitment via CCR4. *Journal for immunotherapy of cancer*, *8*(2).

- Martí i Líndez, A. A., & Reith, W. (2021). Arginine-dependent immune responses. *Cellular and Molecular Life Sciences*, *78*(13), 5303-5324.

- Matalonga, J., Glaria, E., Bresque, M., Escande, C., Carbó, J. M., Kiefer, K., ... & Valledor, A. F. (2017). The nuclear receptor LXR limits bacterial infection of host macrophages through a mechanism that impacts cellular NAD metabolism. *Cell reports*, *18*(5), 1241-1255.

- Metzger, P., Kirchleitner, S. V., Boehmer, D. F., Hörth, C., Eisele, A., Ormanns, S., ... & König, L. M. (2020). Systemic but not MDSC-specific IRF4 deficiency promotes an immunosuppressed tumor microenvironment in a murine pancreatic cancer model. *Cancer Immunology, Immunotherapy*, *69*(10), 2101-2112.

- Mittal, S. K., Cho, K. J., Ishido, S., & Roche, P. A. (2015). Interleukin 10 (IL-10)-mediated immunosuppression: March-I induction regulates antigen presentation by macrophages but not dendritic cells. *Journal of Biological Chemistry*, *290*(45), 27158-27167.

- Mittrücker, H. W., Matsuyama, T., Grossman, A., Kündig, T. M., Potter, J., Shahinian, A., ... & Mak, T. W. (1997). Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. *Science*, *275*(5299), 540-543.

- Molon, B., Ugel, S., Del Pozzo, F., Soldani, C., Zilio, S., Avella, D., ... & Viola, A. (2011). Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. Journal of Experimental Medicine, 208(10), 1949-1962.

- Mottahedeh, J., Haffner, M. C., Grogan, T. R., Hashimoto, T., Crowell, P. D., Beltran, H., ... & Goldstein, A. S. (2018). CD38 is methylated in prostate cancer and regulates extracellular NAD+. *Cancer & metabolism*, *6*(1), 1-17.

- Movahedi, K., Laoui, D., Gysemans, C., Baeten, M., Stangé, G., Van den Bossche, J., ... & Van Ginderachter, J. A. (2010). Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C (high) monocytes. *Cancer research*, *70*(14), 5728-5739.

- Murdoch, C., Muthana, M., Coffelt, S. B., & Lewis, C. E. (2008). The role of myeloid cells in the promotion of tumour angiogenesis. *Nature reviews cancer*, *8*(8), 618-631.

- Murray, P. J., Allen, J. E., Biswas, S. K., Fisher, E. A., Gilroy, D. W., Goerdt, S., ... & Wynn, T. A. (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*, *41*(1), 14-20.

- Musso, T., Deaglio, S., Franco, L., Calosso, L., Badolato, R., Garbarino, G., ... & Malavasi, F. (2001). CD38 expression and functional activities are up-regulated

by IFN-γ on human monocytes and monocytic cell lines. Journal of leukocyte biology, 69(4), 605-612.

- Nagaraj, S., Gupta, K., Pisarev, V., Kinarsky, L., Sherman, S., Kang, L., ... & Gabrilovich, D. I. (2007). Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nature medicine*, *13*(7), 828-835.

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

- Noghero, A., Perino, A., Seano, G., Saglio, E., Sasso, G. L., Veglio, F., ... & Morello, F. (2012). Liver X receptor activation reduces angiogenesis by impairing lipid raft localization and signaling of vascular endothelial growth factor receptor-2. *Arteriosclerosis, thrombosis, and vascular biology*, *32*(9), 2280-2288.

- Noman, M. Z., Desantis, G., Janji, B., Hasmim, M., Karray, S., Dessen, P., ... & Chouaib, S. (2014). PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. Journal of Experimental Medicine, 211(5), 781-790.

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

- Onetto-Pothier, N., Aumont, N., Haman, A., Bigras, C., Wong, G. G., Clark, S. C., ... & Hoang, T. (1990). Characterization of granulocyte-macrophage colony-stimulating factor receptor on the blast cells of acute myeloblastic leukemia.

- Ouvrier, A., Cadet, R., Vernet, P., Laillet, B., Chardigny, J. M., Lobaccaro, J. M. A., ... & Saez, F. (2009). LXR and ABCA1 control cholesterol homeostasis in the proximal mouse epididymis in a cell-specific manner. *Journal of lipid research*, *50*(9), 1766-1775.

- Pan, P. Y., Ma, G., Weber, K. J., Ozao-Choy, J., Wang, G., Yin, B., ... & Chen, S. H. (2010). Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. Cancer research, 70(1), 99-108.

- Pan, Y., Yu, Y., Wang, X., & Zhang, T. (2020). Tumor-associated macrophages in tumor immunity. *Frontiers in immunology*, 3151.

- Pandiyan, P., Zheng, L., Ishihara, S., Reed, J., & Lenardo, M. J. (2007). CD4+ CD25+ Foxp3+ regulatory T cells induce cytokine deprivation–mediated apoptosis of effector CD4+ T cells. *Nature immunology*, *8*(12), 1353-1362.

- Pascual-García, M., Carbó, J. M., León, T., Matalonga, J., Out, R., Van Berkel, T., ... & Valledor, A. F. (2011). Liver X receptors inhibit macrophage proliferation through downregulation of cyclins D1 and B1 and cyclin-dependent kinases 2 and 4. *The Journal of Immunology*, *186*(8), 4656-4667.

- Parigi, S. M., Das, S., Frede, A., Cardoso, R. F., Tripathi, K. P., Doñas, C., ... & Villablanca, E. J. (2021). Liver X receptor regulates Th17 and RORγt+ Treg cells by distinct mechanisms. *Mucosal Immunology*, *14*(2), 411-419.

- Park, M. C., Kwon, Y. J., Chung, S. J., Park, Y. B., & Lee, S. K. (2010). Liver X receptor agonist prevents the evolution of collagen-induced arthritis in mice. *Rheumatology*, *49*(5), 882-890.

- Partida-Sanchez, S., Cockayne, D. A., Monard, S., Jacobson, E. L., Oppenheimer, N., Garvy, B., ... & Lund, F. E. (2001). Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. Nature medicine, 7(11), 1209-1216.

- Pascual-García, M., Rué, L., León, T., Julve, J., Carbó, J. M., Matalonga, J., ... & Valledor, A. F. (2013). Reciprocal negative cross-talk between liver X receptors (LXRs) and STAT1: effects on IFN-γ–induced inflammatory responses and LXR-dependent gene expression. *The Journal of Immunology*, *190*(12), 6520-6532.

- Peet, D. J., Turley, S. D., Ma, W., Janowski, B. A., Lobaccaro, J. M. A., Hammer, R. E., & Mangelsdorf, D. J. (1998). Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRα. *Cell*, *93*(5), 693-704.

- Perez, F. A., Fligner, C. L., & Yu, E. Y. (2009). Rapid clinical deterioration and leukemoid reaction after treatment of urothelial carcinoma of the bladder: possible effect of granulocyte colony-stimulating factor. *Journal of Clinical Oncology*, *27*(34), e215-e217.

- Petrina, M., Martin, J., & Basta, S. (2021). Granulocyte macrophage colony-stimulating factor has come of age: from a vaccine adjuvant to antiviral immunotherapy. *Cytokine & growth factor reviews*, *59*, 101-110.

- Plater-Zyberk, C., Helsen, M. M. A., Hepp, J., Baeuerle, P. A., & van den Berg, W. B. (2007). GM-CSF neutralisation suppresses inflammation and protects cartilage

in acute streptococcal cell wall arthritis of mice. Annals of the rheumatic diseases, 66(4), 452-457.

- Pommier, A. J. C., Alves, G., Viennois, E., Bernard, S., Communal, Y., Sion, B., ... & Lobaccaro, J. M. A. (2010). Liver X Receptor activation downregulates AKT survival signaling in lipid rafts and induces apoptosis of prostate cancer cells. *Oncogene*, *29*(18), 2712-2723.

- Ponomarev, E. D., Shriver, L. P., Maresz, K., Pedras-Vasconcelos, J., Verthelyi, D., & Dittel, B. N. (2007). GM-CSF production by autoreactive T cells is required for the activation of microglial cells and the onset of experimental autoimmune encephalomyelitis. *The Journal of Immunology*, *178*(1), 39-48.

- Pourcet, B., Gage, M. C., León, T. E., Waddington, K. E., Pello, O. M., Steffensen, K. R., ... & Pineda-Torra, I. (2016). The nuclear receptor LXR modulates interleukin-18 levels in macrophages through multiple mechanisms. *Scientific reports*, *6*(1), 1-12.

- Punt, J., Kuby, J., Stranford, S. A., Jones, P. P., & Owen, J. A. (2019). *Kuby immunology*. New York, N.Y: Macmillan Education.

- Pylayeva-Gupta, Y. (2016). Molecular pathways: interleukin-35 in autoimmunity and cancer. *Clinical Cancer Research*, *22*(20), 4973-4978.

- Raber, P., Ochoa, A. C., & Rodríguez, P. C. (2012). Metabolism of L-arginine by myeloid-derived suppressor cells in cancer: mechanisms of T cell suppression and therapeutic perspectives. Immunological investigations, 41(6-7), 614-634.

- Raber, P. L., Thevenot, P., Sierra, R., Wyczechowska, D., Halle, D., Ramirez, M. E., ... & Rodriguez, P. C. (2014). Subpopulations of myeloid-derived suppressor cells impair T cell responses through independent nitric oxide-related pathways. International journal of cancer, 134(12), 2853-2864.

- Ramón-Vázquez, A., de la Rosa, J. V., Tabraue, C., Lopez, F., Díaz-Chico, B. N., Bosca, L., ... & Castrillo, A. (2019). Common and differential transcriptional actions of nuclear receptors liver X receptors α and β in macrophages. *Molecular and cellular biology*, *39*(5), e00376-18.

- Rangaraju, S., Dammer, E. B., Raza, S. A., Rathakrishnan, P., Xiao, H., Gao, T., ... & Levey, A. I. (2018). Identification and therapeutic modulation of a pro-inflammatory subset of disease-associated-microglia in Alzheimer's disease. *Molecular neurodegeneration*, *13*(1), 1-25.

- Repa, J. J., Berge, K. E., Pomajzl, C., Richardson, J. A., Hobbs, H., & Mangelsdorf, D. J. (2002). Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors α and β. *Journal of Biological Chemistry*, *277*(21), 18793-18800.

- A) Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M. A., Shimomura, I., ... & Mangelsdorf, D. J. (2000). Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. *Genes & development*, *14*(22), 2819-2830.

- B) Repa, J. J., Turley, S. D., Lobaccaro, J. M., Medina, J., Li, L., Lustig, K., ... & Mangelsdorf, D. J. (2000). Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science*, *289*(5484), 1524-1529.

- Revoltella, R. P., Menicagli, M., & Campani, D. (2012). Granulocyte–macrophage colony-stimulating factor as an autocrine survival-growth factor in human gliomas. *Cytokine*, *57*(3), 347-359.

- Ribas, A., Dummer, R., Puzanov, I., VanderWalde, A., Andtbacka, R. H., Michielin, O., ... & Long, G. V. (2018). Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. *Cell*, *174*(4), 1031-1032.

- Romio, M., Reinbeck, B., Bongardt, S., Hüls, S., Burghoff, S., & Schrader, J. (2011). Extracellular purine metabolism and signaling of CD73-derived adenosine in murine Treg and Teff cells. *American Journal of Physiology-Cell Physiology*, *301*(2), C530-C539.

- Ruan, Q., Kameswaran, V., Zhang, Y., Zheng, S., Sun, J., Wang, J., ... & Chen, Y. H. (2011). The Th17 immune response is controlled by the Rel–RORγ–RORγT transcriptional axis. *Journal of Experimental Medicine*, *208*(11), 2321-2333.

- Sanjurjo, L., Aran, G., Téllez, É., Amézaga, N., Armengol, C., López, D., ... & Sarrias, M. R. (2018). CD5L promotes M2 macrophage polarization through autophagy-mediated upregulation of ID3. *Frontiers in immunology*, *9*, 480.

- Sasso, G. L., Bovenga, F., Murzilli, S., Salvatore, L., Di Tullio, G., Martelli, N., ... & Moschetta, A. (2013). Liver X receptors inhibit proliferation of human colorectal cancer cells and growth of intestinal tumors in mice. *Gastroenterology*, *144*(7), 1497-1507.

- Schiavoni, I., Scagnolari, C., Horenstein, A. L., Leone, P., Pierangeli, A., Malavasi, F., ... & Fedele, G. (2018). CD 38 modulates respiratory syncytial virus-driven proinflammatory processes in human monocyte-derived dendritic cells. Immunology, 154(1), 122-131.

- Schlecker, E., Stojanovic, A., Eisen, C., Quack, C., Falk, C. S., Umansky, V., & Cerwenka, A. (2012). Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. *The Journal of Immunology*, *189*(12), 5602-5611.

- Schulman, I. G. (2017). Liver X receptors link lipid metabolism and inflammation. *FEBS letters*, *591*(19), 2978-2991.

- Segala, G., David, M., de Medina, P., Poirot, M. C., Serhan, N., Vergez, F., ... & Silvente-Poirot, S. (2017). Dendrogenin A drives LXR to trigger lethal autophagy in cancers. *Nature communications*, *8*(1), 1-17.

- Segura, E., & Amigorena, S. (2013). Inflammatory dendritic cells in mice and humans. *Trends in immunology*, *34*(9), 440-445.

- Sever, R., & Glass, C. K. (2013). Signaling by nuclear receptors. *Cold Spring Harbor perspectives in biology*, *5*(3), a016709.

- Shang, B., Liu, Y., Jiang, S. J., & Liu, Y. (2015). Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. *Scientific reports*, *5*(1), 1-9.

- Shevyrev, D., & Tereshchenko, V. (2020). Treg heterogeneity, function, and homeostasis. *Frontiers in immunology*, 3100.

- Shi, Y., Liu, C. H., Roberts, A. I., Das, J., Xu, G., Ren, G., ... & Devadas, S. (2006). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. *Cell research*, *16*(2), 126-133.

- Shiomi, A., & Usui, T. (2015). Pivotal roles of GM-CSF in autoimmunity and inflammation. *Mediators of inflammation*, *2015*.

- Shrimp, J. H., Hu, J., Dong, M., Wang, B. S., MacDonald, R., Jiang, H., ... & Lin, H. (2014). Revealing CD38 cellular localization using a cell permeable, mechanism-based fluorescent small-molecule probe. Journal of the American Chemical Society, 136(15), 5656-5663.

- Shu, B., Feng, Y., Gui, Y., Lu, Q., Wei, W., Xue, X., ... & Dai, C. (2018). Blockade of CD38 diminishes lipopolysaccharide-induced macrophage classical activation and acute kidney injury involving NF-κB signaling suppression. *Cellular Signalling*, *42*, 249-258.

- Sierra-Filardi, E., Puig-Kröger, A., Blanco, F. J., Nieto, C., Bragado, R., Palomero, M. I., ... & Corbí, A. L. (2011). Activin A skews macrophage polarization by promoting a proinflammatory phenotype and inhibiting the acquisition of anti-

inflammatory macrophage markers. *Blood, The Journal of the American Society of Hematology*, *117*(19), 5092-5101.

- Simons, J. W., Jaffee, E. M., Weber, C. E., Levitsky, H. I., Nelson, W. G., Carducci, M. A., ... & Marshall, F. F. (1997). Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer research*, *57*(8), 1537-1546.

- Sonderegger, I., Iezzi, G., Maier, R., Schmitz, N., Kurrer, M., & Kopf, M. (2008). GM-CSF mediates autoimmunity by enhancing IL-6–dependent Th17 cell development and survival. *The Journal of experimental medicine*, *205*(10), 2281-2294.

- Spann, N. J., Garmire, L. X., McDonald, J. G., Myers, D. S., Milne, S. B., Shibata, N., ... & Glass, C. K. (2012). Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. *Cell*, *151*(1), 138-152.

- Srivastava, M. K., Sinha, P., Clements, V. K., Rodriguez, P., & Ostrand-Rosenberg, S. (2010). Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. Cancer research, 70(1), 68-77.

- Stoitzner, P., Green, L. K., Jung, J. Y., Price, K. M., Atarea, H., Kivell, B., & Ronchese, F. (2008). Inefficient presentation of tumor-derived antigen by tumor-infiltrating dendritic cells. *Cancer Immunology, Immunotherapy*, *57*(11), 1665-1673.

- Strauss, L., Sangaletti, S., Consonni, F. M., Szebeni, G., Morlacchi, S., Totaro, M. G., ... & Sica, A. (2015). RORC1 regulates tumor-promoting “emergency” granulo-monocytopoiesis. Cancer cell, 28(2), 253-269.

- Su, S., Liu, Q., Chen, J., Chen, J., Chen, F., He, C., ... & Song, E. (2014). A positive feedback loop between mesenchymal-like cancer cells and macrophages is essential to breast cancer metastasis. *Cancer cell*, *25*(5), 605-620.

- Tanaka, A., & Sakaguchi, S. (2017). Regulatory T cells in cancer immunotherapy. *Cell research*, *27*(1), 109-118.

- Tavazoie, M. F., Pollack, I., Tanqueco, R., Ostendorf, B. N., Reis, B. S., Gonsalves, F. C., ... & Tavazoie, S. F. (2018). LXR/ApoE activation restricts innate immune suppression in cancer. *Cell*, *172*(4), 825-840.

- Thorn, M., Guha, P., Cunetta, M., Espat, N. J., Miller, G., Junghans, R. P., & Katz, S. C. (2016). Tumor-associated GM-CSF overexpression induces

immunoinhibitory molecules via STAT3 in myeloid-suppressor cells infiltrating liver metastases. *Cancer gene therapy*, *23*(6), 188-198.

- Töröcsik, D., Baráth, M., Benkő, S., Széles, L., Dezső, B., Póliska, S., ... & Nagy, L. (2010). Activation of liver X receptor sensitizes human dendritic cells to inflammatory stimuli. *The Journal of Immunology*, *184*(10), 5456-5465.

- Tymoszuk, P., Evens, H., Marzola, V., Wachowicz, K., Wasmer, M. H., Datta, S., ... & Doppler, W. (2014). In situ proliferation contributes to accumulation of tumor-associated macrophages in spontaneous mammary tumors. *European journal of immunology*, *44*(8), 2247-2262.

- Upadhyay, S., Sharma, N., Gupta, K. B., & Dhiman, M. (2018). Role of immune system in tumor progression and carcinogenesis. *Journal of cellular biochemistry*, *119*(7), 5028-5042.

- Urdinguio, R. G., Fernandez, A. F., Moncada-Pazos, A., Huidobro, C., Rodriguez, R. M., Ferrero, C., ... & Fraga, M. F. (2013). Immune-dependent and independent antitumor activity of GM-CSF aberrantly expressed by mouse and human colorectal tumors. *Cancer research*, *73*(1), 395-405.

- Valledor, A. F., Hsu, L. C., Ogawa, S., Sawka-Verhelle, D., Karin, M., & Glass, C. K. (2004). Activation of liver X receptors and retinoid X receptors prevents bacterial-induced macrophage apoptosis. *Proceedings of the National Academy of Sciences*, *101*(51), 17813-17818.

- Van Overmeire, E., Stijlemans, B., Heymann, F., Keirsse, J., Morias, Y., Elkrim, Y., ... & Laoui, D. (2016). M-CSF and GM-CSF receptor signaling differentially regulate monocyte maturation and macrophage polarization in the tumor microenvironment. *Cancer research*, *76*(1), 35-42.

- Varin, A., Thomas, C., Ishibashi, M., Ménégaut, L., Gautier, T., Trousson, A., ... & Masson, D. (2015). Liver X receptor activation promotes polyunsaturated fatty acid synthesis in macrophages: relevance in the context of atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*, *35*(6), 1357-1365.

- Vedin, L. L., Gustafsson, J. Å., & Steffensen, K. R. (2013). The oxysterol receptors LXRα and LXRβ suppress proliferation in the colon. *Molecular carcinogenesis*, *52*(11), 835-844.

- Vedin, L. L., Lewandowski, S. A., Parini, P., Gustafsson, J. Å., & Steffensen, K. R. (2009). The oxysterol receptor LXR inhibits proliferation of human breast cancer cells. *Carcinogenesis*, *30*(4), 575-579.

- Ventura, J. J., Tenbaum, S., Perdiguero, E., Huth, M., Guerra, C., Barbacid, M., ... & Nebreda, A. R. (2007). p38α MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation. *Nature genetics*, *39*(6), 750-758.

- Villa, G. R., Hulce, J. J., Zanca, C., Bi, J., Ikegami, S., Cahill, G. L., ... & Mischel, P. S. (2016). An LXR-cholesterol axis creates a metabolic co-dependency for brain cancers. *Cancer cell*, *30*(5), 683-693.

- Villablanca, E. J., Raccosta, L., Zhou, D., Fontana, R., Maggioni, D., Negro, A., ... & Russo, V. (2010). Tumor-mediated liver X receptor-α activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. *Nature medicine*, *16*(1), 98-105.

- Vinnakota, K., Zhang, Y., Selvanesan, B. C., Topi, G., Salim, T., Sand-Dejmek, J., ... & Sjölander, A. (2017). M2-like macrophages induce colon cancer cell invasion via matrix metalloproteinases. *Journal of cellular physiology*, *232*(12), 3468-3480.

- Waghray, M., Yalamanchili, M., Dziubinski, M., Zeinali, M., Erkkinen, M., Yang, H., ... & Simeone, D. M. (2016). GM-CSF mediates mesenchymal–epithelial cross-talk in pancreatic cancer. *Cancer discovery*, *6*(8), 886-899.

- Wagner, B. L., Valledor, A. F., Shao, G., Daige, C. L., Bischoff, E. D., Petrowski, M., ... & Glass, C. K. (2003). Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Molecular and cellular biology*, *23*(16), 5780-5789.

- Wang, B., & Tontonoz, P. (2018). Liver X receptors in lipid signalling and membrane homeostasis. *Nature Reviews Endocrinology*, *14*(8), 452-463.

- Wang, L., Rubinstein, R., Lines, J. L., Wasiuk, A., Ahonen, C., Guo, Y., ... & Noelle, R. J. (2011). VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. *Journal of Experimental Medicine*, *208*(3), 577-592.

- Wang, X., Collins, H. L., Ranalletta, M., Fuki, I. V., Billheimer, J. T., Rothblat, G. H., ... & Rader, D. J. (2007). Macrophage ABCA1 and ABCG1, but not SR-BI, promote macrophage reverse cholesterol transport in vivo. *The Journal of clinical investigation*, *117*(8), 2216-2224.

- Wei, C., Yang, C., Wang, S., Shi, D., Zhang, C., Lin, X., & Xiong, B. (2019). M2 macrophages confer resistance to 5-fluorouracil in colorectal cancer through the activation of CCL22/PI3K/AKT signaling. *OncoTargets and therapy*, *12*, 3051.

- Wicks, I. P., & Roberts, A. W. (2016). Targeting GM-CSF in inflammatory diseases. *Nature Reviews Rheumatology*, *12*(1), 37-48.

- Wiktor-Jedrzejczak, W., Bartocci, A., Ferrante, A. W., Ahmed-Ansari, A., Sell, K. W., Pollard, J. W., & Stanley, E. R. (1990). Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (op/op) mouse. *Proceedings of the National Academy of Sciences*, *87*(12), 4828-4832.

- Wilund, K. R., Yu, L., Xu, F., Hobbs, H. H., & Cohen, J. C. (2004). High-level expression of ABCG5 and ABCG8 attenuates diet-induced hypercholesterolemia and atherosclerosis in Ldlr−/− mice. *Journal of lipid research*, *45*(8), 1429-1436.

- Wimmers, F., Schreibelt, G., Sköld, A. E., Figdor, C. G., & De Vries, I. J. M. (2014). Paradigm shift in dendritic cell-based immunotherapy: from in vitro generated monocyte-derived DCs to naturally circulating DC subsets. *Frontiers in immunology*, *5*, 165.

- Wong, C. K., Lau, K. M., Chan, I. H. S., Hu, S., Lam, Y. Y. O., Choi, A. O. K., & Lam, C. W. K. (2013). MicroRNA-21* regulates the prosurvival effect of GM-CSF on human eosinophils. *Immunobiology*, *218*(2), 255-262.

- Woo, S. R., Corrales, L., & Gajewski, T. F. (2015). The STING pathway and the T cell-inflamed tumor microenvironment. *Trends in immunology*, *36*(4), 250-256.

- Wrzesinski, S. H., Wan, Y. Y., & Flavell, R. A. (2007). Transforming growth factor-β and the immune response: implications for anticancer therapy. *Clinical cancer research*, *13*(18), 5262-5270.

- Wu, C., Ning, H., Liu, M., Lin, J., Luo, S., Zhu, W., ... & Zheng, L. (2018). Spleen mediates a distinct hematopoietic progenitor response supporting tumor-promoting myelopoiesis. *The Journal of clinical investigation*, *128*(8), 3425-3438.

- Wu, L., Diny, N. L., Ong, S., Barin, J. G., Hou, X., Rose, N. R., ... & Čiháková, D. (2016). Pathogenic IL-23 signaling is required to initiate GM-CSF-driven autoimmune myocarditis in mice. European journal of immunology, 46(3), 582-592.

- A) Wu, T. C., Xu, K., Banchereau, R., Marches, F., Chun, I. Y., Martinek, J., ... & Palucka, K. (2014). Reprogramming tumor-infiltrating dendritic cells for CD103+ CD8+ mucosal T-cell differentiation and breast cancer rejection. *Cancer immunology research*, *2*(5), 487-500.

- B) Wu, W. C., Sun, H. W., Chen, H. T., Liang, J., Yu, X. J., Wu, C., ... & Zheng, L. (2014). Circulating hematopoietic stem and progenitor cells are myeloid-biased

in cancer patients. *Proceedings of the National Academy of Sciences*, *111*(11), 4221-4226.

- Wynn, T. A., Chawla, A., & Pollard, J. W. (2013). Macrophage biology in development, homeostasis and disease. *Nature*, *496*(7446), 445-455.

- Xiao, J., Chen, Q., Tang, D., Ou, W., Wang, J., Mo, Z., ... & Wang, D. (2017). Activation of liver X receptors promotes inflammatory cytokine mRNA degradation by upregulation of tristetraprolin. *Acta Biochimica et Biophysica Sinica*, *49*(3), 277-283.

- Xiong, H., Zhang, Y., Chen, S., Ni, Z., He, J., Li, X., ... & He, F. (2017). Induction of SOCS3 by liver X receptor suppresses the proliferation of hepatocellular carcinoma cells. *Oncotarget*, *8*(38), 64083.

- Xu, Y., Zhan, Y., Lew, A. M., Naik, S. H., & Kershaw, M. H. (2007). Differential development of murine dendritic cells by GM-CSF versus Flt3 ligand has implications for inflammation and trafficking. *The Journal of Immunology*, *179*(11), 7577-7584.

- Yamada, M., Mizuguchi, M., Otsuka, N., Ikeda, K., & Takahashi, H. (1997). Ultrastructural localization of CD38 immunoreactivity in rat brain. Brain research, 756(1-2), 52-60.

- Yong, K. L., Rowles, P. M., Patterson, K. G., & Linch, D. C. (1992). Granulocyte-macrophage colony-stimulating factor induces neutrophil adhesion to pulmonary vascular endothelium in vivo: role of beta 2 integrins.

- Youlin, K., Li, Z., Weiyang, H., Jian, K., Siming, L., & Xin, G. (2017). Liver X receptor activation inhibits PC-3 prostate cancer cells via the beta-catenin pathway. *Pathology-Research and Practice*, *213*(3), 267-270.

- Yousefi, S., Mihalache, C., Kozlowski, E., Schmid, I., & Simon, H. U. (2009). Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death & Differentiation*, *16*(11), 1438-1444.

- Yu, J., Du, W., Yan, F., Wang, Y., Li, H., Cao, S., ... & Ren, X. (2013). Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. The Journal of Immunology, 190(7), 3783-3797.

- Yu, L., Li-Hawkins, J., Hammer, R. E., Berge, K. E., Horton, J. D., Cohen, J. C., & Hobbs, H. H. (2002). Overexpression of ABCG5 and ABCG8 promotes biliary

cholesterol secretion and reduces fractional absorption of dietary cholesterol. *The Journal of clinical investigation*, *110*(5), 671-680.

- Zanotti, I., Pedrelli, M., Potì, F., Stomeo, G., Gomaraschi, M., Calabresi, L., & Bernini, F. (2011). Macrophage, but not systemic, apolipoprotein E is necessary for macrophage reverse cholesterol transport in vivo. *Arteriosclerosis, thrombosis, and vascular biology*, *31*(1), 74-80.

- Zelcer, N., Hong, C., Boyadjian, R., & Tontonoz, P. (2009). LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. *Science*, *325*(5936), 100-104.

- Zeng, Y., Peng, Y., Tang, K., Wang, Y. Q., Zhao, Z. Y., Wei, X. Y., & Le Xu, X. (2018). Dihydromyricetin ameliorates foam cell formation via LXRα-ABCA1/ABCG1-dependent cholesterol efflux in macrophages. *Biomedicine & Pharmacotherapy*, *101*, 543-552.

- Zhang, Q. W., Liu, L., Gong, C. Y., Shi, H. S., Zeng, Y. H., Wang, X. Z., ... & Wei, Y. Q. (2012). Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PloS one*, *7*(12), e50946.

- Zhang, W., Jiang, H., Zhang, J., Zhang, Y., Liu, A., Zhao, Y., ... & Yuan, X. (2014). Liver X receptor activation induces apoptosis of melanoma cell through caspase pathway. *Cancer cell international*, *14*(1), 1-6.

- Zhang-Gandhi, C. X., & Drew, P. D. (2007). Liver X receptor and retinoid X receptor agonists inhibit inflammatory responses of microglia and astrocytes. *Journal of neuroimmunology*, *183*(1-2), 50-59.

- Zhao, D. M., Thornton, A. M., DiPaolo, R. J., & Shevach, E. M. (2006). Activated CD4+ CD25+ T cells selectively kill B lymphocytes. *Blood*, *107*(10), 3925-3932.

- Zhao, L., Lei, W., Deng, C., Wu, Z., Sun, M., Jin, Z., ... & Yang, Y. (2021). The roles of liver X receptor α in inflammation and inflammation-associated diseases. *Journal of Cellular Physiology*, *236*(7), 4807-4828.

- Zhao, Y. J., Lam, C. M. C., & Lee, H. C. (2012). The membrane-bound enzyme CD38 exists in two opposing orientations. Science signaling, 5(241), ra67-ra67.

- Zhu, F., Li, X., Chen, S., Zeng, Q., Zhao, Y., & Luo, F. (2016). Tumor-associated macrophage or chemokine ligand CCL17 positively regulates the tumorigenesis of hepatocellular carcinoma. *Medical oncology*, *33*(2), 1-12.

- Zhu, Y., Herndon, J. M., Sojka, D. K., Kim, K. W., Knolhoff, B. L., Zuo, C., ... & DeNardo, D. G. (2017). Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity*, *47*(2), 323-338.

ANNEX

Publication I

Pharmacologic Activation of LXR Alters the Expression Profile of Tumor-Associated Macrophages and the Abundance of Regulatory T Cells in the Tumor Microenvironment

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Pharmacologic Activation of LXR Alters the Expression Profile of Tumor-Associated Macrophages and the Abundance of Regulatory T Cells in the Tumor Microenvironment

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ABSTRACT

Liver X receptors (LXR) are transcription factors from the nuclear receptor family that are activated by oxysterols and synthetic high-affinity agonists. In this study, we assessed the antitumor effects of synthetic LXR agonist TO901317 in a murine model of syngeneic Lewis Lung carcinoma. Treatment with TO901317 inhibited tumor growth in wild-type, but not in LXR-deficient mice, indicating that the antitumor effects of the agonist depends on functional LXR activity in host cells. Pharmacologic activation of the LXR pathway reduced the intratumoral abundance of regulatory T cells (Treg) and the expression of the Treg-attracting chemokine *Ccl17* by MHCII^{high} tumor-associated macrophages (TAM). Moreover, gene expression profiling indicated a broad negative impact of the LXR agonist on other mechanisms used by TAM for the maintenance of an immunosuppressive environment. In studies exploring the macrophage response to GM-CSF or IL4, activated LXR repressed IRF4 expression, resulting in subsequent downregulation of IRF4-dependent genes including *Ccl17*. Taken together, this work reveals the combined actions of the LXR pathway in the control of TAM responses that contribute to the antitumoral effects of pharmacologic LXR activation. Moreover, these data provide new insights for the development of novel therapeutic options for the treatment of cancer.

Significance: This study reveals unrecognized roles of LXR in the transcriptional control of the tumor microenvironment and suggests use of a synthetic LXR agonist as a novel therapeutic strategy to stimulate antitumor activity.

Introduction

Nuclear receptors are a family of transcription factors with key functions in health and disease. Many members within this family are activated in a ligand-dependent manner. In particular, liver X receptors (LXR) are activated by cholesterol derivatives, including specific oxysterols, and by synthetic high-affinity agonists. Two LXR subtypes have been identified, LXRα (NR1H3) and LXRβ (NR1H2), which are expressed in tissues in an overlapping but not identical manner. Both LXRs bind to DNA as heterodimers with another subgroup of the nuclear receptor family, the retinoid X receptors (RXR), to regulate positively the transcription of a variety of target genes involved in lipid and glucose metabolism (revised in ref. 1) and in immune cell function (2–6). Moreover, upon ligand binding, LXRs repress inflammatory gene expression (revised in ref. 7).

Synthetic LXR agonists activate different mechanisms that translate into antiproliferative effects in a wide variety of cancer cell types (revised in ref. 8). *In vivo* studies, however, have produced contradictory results on the role of the LXR pathway in controlling tumor growth. In several mouse models of cancer, LXR agonists efficiently reduced primary tumor growth (9–11). Interestingly, while inhibiting the metastasis of melanoma cells in an apolipoprotein E (APOE)-dependent manner (9), the agonist GW3965 exacerbated the dissemination of breast cancer cells to the lung (10). Another study showed

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that endogenous LXR ligands can be secreted by tumor cells as a strategy for immune evasion (12). In that setting, the activation of LXRα repressed the expression of CC chemokine receptor (CCR)7 on dendritic cells, thus impairing their migration to lymphoid organs and compromising the establishment of an antitumor adaptive immune response. Conversely, recent work has shown that LXR agonism, and subsequent APOE production, reduces the levels of myeloid-derived suppressor cells (MDSC), thus enhancing CTL activity (11). These contrasting observations support the need to dissect the roles of the LXR pathway in the tumor microenvironment in different contexts of cancer.

Solid tumors are infiltrated by heterogeneous populations of leukocytes. Among the immune cells within the tumor site, tumor-associated macrophages (TAM) are particularly abundant and present at all stages of tumor progression. Interestingly, TAMs have been associated with poor prognosis in a variety of cancers (13, 14). In most solid tumors, TAMs exhibit protumoral functions by promoting cancer cell survival and proliferation, extracellular matrix remodeling, and angiogenesis that benefit tumor cell migration and dissemination to secondary locations (15). TAMs also adopt immune-suppressive roles within the tumor microenvironment. Through the surface expression of a number of regulatory molecules, TAMs are able to directly suppress immune responses against tumor cells. For instance, TAMs express human leucocyte antigen (HLA)-C, HLA-G, and HLA-E, which inhibit the activation of NK cells, and ligands for programmed cell death protein 1 (PD-1) or CTL antigen 4 (CTLA4; PD-L1 and B7–1, respectively), which inhibit T-cell proliferation and activation as well as the cytolytic activity of $CD8^+$ T cells. TAMs can also influence the antitumoral immune response indirectly through the induction of L-arginine–consuming enzymes, namely nitric oxide synthase (NOS)2 and arginase 1 (ARG1), and the secretion of an array of cytokines and chemokines (revised in ref. 16). For example, TAMs secrete CC chemokine ligand (CCL)17 and CCL22 upon stimulation by granulocyte/monocyte-colony stimulating factor (GM-CSF) produced by tumor cells (17, 18). Through their binding to surface, CCR4, CCL17, and CCL22 promote the migration of regulatory T cells (Treg) to the tumor microenvironment (19, 20), thus facilitating the establishment of an immunosuppressive environment.

In mice, different TAM populations have been identified within solid tumors, displaying markers that partially fit with the classical versus alternative macrophage activation paradigm (21). TAMs exhibiting a more proinflammatory gene signature are enriched in normoxic areas of the tumor and express high levels of MHCII, whereas TAMs displaying a more alternative phenotype are located mostly within hypoxic tumor areas, have a superior proangiogenic activity and express low MHCII levels (22). Nevertheless, both TAM subsets are poor antigen-presenting cells, express *Ccl17* and *Ccl22* (although $MHCII^{high}$ TAMs produce higher levels of these chemokines), and are able to suppress T-cell activation.

In this work, we demonstrate unrecognized roles of LXRs in the control of TAM gene expression. The synthetic LXR agonist TO901317 (T1317) inhibited the growth of syngeneic Lewis Lung carcinoma in wild-type (WT) but not in LXR-deficient ($LXR\alpha/\beta^{-/-}$) mice, despite the fact that injected cancer cells express LXR isoforms in both settings and that these cells are sensitive to growth inhibition by high doses of LXR agonists *in vitro*. This indicates that LXR activity in host cells is essential for the antitumor effects of the synthetic LXR agonist. In this context, several mechanisms used by TAMs for the maintenance of an immunosuppressive environment were downregulated upon pharmacologic LXR activation, including the expression of the chemokine CCL17, which correlated with a decrease in the abundance of intratumoral Tregs *in vivo*. In addition, LXR activity repressed other genes that are part of the protumoral program of TAMs and reduced partially the capability of these cells to suppress T-cell proliferation *in vitro*. Moreover, repression of IRF4 expression emerged as a mechanism linking LXR activation with the downregulation of selective genes, such as *Ccl17*, in different macrophage populations. Taken together, this work provides novel insights about the biological actions of LXR agonists and supports their pharmacologic use as antitumoral drugs.

Materials and Methods

Reagents

The synthetic high-affinity LXR agonists T1317 and GW3965 were purchased from Cayman Europe and Tocris, respectively. Recombinant murine GM-CSF and IL4 and human IL4 and macrophage-colony stimulating factor (M-CSF) were purchased from PeproTech.

Animals

C57BL/6 mice were purchased from Harlan and raised as a colony in our animal facility. LXR-deficient mice were initially donated by Dr. David Mangelsdorf (UT Southwestern Medical Center, Dallas, TX) and backcrossed into C57BL/6 background for more than ten generations. Foxp3EGFP mice were generated by crossing the NOD. Foxp3EGFP strain (23) with C57BL/6 mice for five generations. PyMT mice (24) with an FVB/N background were obtained from The Mouse Models of Human Cancers Consortium Repository (National Cancer Institute, Frederick, MD) and backcrossed into the C57BL/6 background for nine generations. Three-week-old *JunB* f/f; MxCre mice (25) and Cre-negative control littermates (C57BL/6 background) were administered three intraperitoneal injections of poly I:C at weekly intervals to induce *JunB* deletion systemically (including the hematopoietic lineage/bone marrow). JUND-deficient mice (26) and WT control littermates were generated by heterozygote intercrosses (C57BL/6 background). IRF4-deficient mice and control C57BL/6 mice were bred at the animal facility of the Biomedical Research Center at the University of Marburg (Marburg, Germany). Unless otherwise stated, the mice were fed a regular chow diet. All the protocols requiring animal manipulation have been approved by the Institutional Animal Care and Use Committees from Parc Científic de Barcelona (#9672), Universitat de Barcelona (#7088), Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau (#7281), and the University of Marburg (R.P. Giessen, Germany).

Cells

Bone marrow–derived macrophages were obtained from six to ten-week-old mice as described previously (27). Bone marrow precursors were differentiated to macrophages in DMEM supplemented with 20% heat-inactivated FBS (Sigma-Aldrich) and 30% L929 conditioned media as a source of M-CSF.

Human macrophages were differentiated *in vitro* from peripheral blood mononuclear cells (PBMC) from healthy donors. See more details in Supplementary Methods. The protocol has been approved by the Bioethics Commission of the University of Barcelona and the blood samples were obtained from the Blood and Tissue Bank from Generalitat de Catalunya.

The 3LL-R cell line (used in our lab since 2016; ref. 28) was maintained in RPMI media with L-glutamine (L-Gln, 0.3 g/L; BioWest) supplemented with 10% FBS. Raw264.7 macrophages (ATCC, RRID:

CVCL_0493, obtained in 2011) were cultured in DMEM-10% FBS. All cell lines were used within 15 passages after thawing.

Tumor progression studies

3LL-R cells (3×10^6) were subcutaneously injected in eight- to ten-week-old WT or LXRα/β-deficient male mice. The tumors were allowed to grow for two weeks. From day 7 to day 15, length (D) and width (d) measures were taken with a digital caliper and tumor volume was calculated using the formula $V = \pi \times (d2 \times D)/6$ (29). In some experiments, at day 7, once the tumor was established and for the next 8 days, the animals received a daily dose of T1317 (15 mg/kg) through an intraperitoneal injection. Control animals received an equivalent dose of vehicle (DMSO) diluted in PBS. At day 15, the mice were euthanized and the tumors excised and processed.

Alternatively, tumor development was evaluated in PyMT transgenic mice. After weaning, PyMT female mice were administered either a regular chow diet (A04; Scientific Animal Food & Engineering) or the same diet supplemented with 50 mg/kg of T1317. The mice were monitored every three days for palpable tumors starting at 6 weeks of age. Tumor latency was defined as the time to the development of the first palpable tumor in each mouse. The mice were euthanized at 22 weeks of age. Total tumor burden was determined after all the mammary glands were excised and weighed, and the mass of the tumor-bearing mammary glands was measured. Each mammary gland was numerically labeled as in ref. 30.

Identification of immune cell populations

3LL-R cells (3×10^6) were injected subcutaneously in recipient mice as described above. At day 15 postinjection, the tumors were dissected and processed as indicated in Supplementary Methods. The final cell suspension was diluted to a concentration of 10^7 cells/mL in PBS and incubated first with Fc block (rat anti-mouse CD16/CD32, BD Biosciences; 1:50 dilution, 30 minutes, 4°C). For myeloid cell determination, the cells were incubated with specific antibodies against CD11b, Ly6G, Ly6C, and IA/IE (MHCII; see more details in Supplementary Methods; ref. 21). Cell populations were analyzed through flow cytometry using a FACSaria Fusion cell sorter (BD Biosciences; see gating strategy in Supplementary Fig. S1).

For lymphoid cell determination, the cells were incubated with specific antibodies against CD4 and CD8 (see more details in Supplementary Methods; Supplementary Fig. S1) The cells were then permeabilized and fixed using the Foxp3/Transcription factor staining buffer set (Invitrogen) following the manufacturer's specifications. The cells were analyzed by flow cytometry.

Alternatively, for lymphocyte cell determination, 3LL-R cells were injected subcutaneously in Foxp3EGFP reporter mice. The tumors were collected at day 10 post cancer cell injection. Cell suspensions were blocked with Fc block and incubated with specific antibodies against CD4 and CD8 (Supplementary Methods). The cells were analyzed by flow cytometry. Tregs were identified via EGFP expression analysis (Supplementary Fig. S1).

In some experiments, the spleens were also harvested. See details in Supplementary Methods. Lymphocyte populations were analyzed as described above.

Treg depletion

To downregulate the frequency of Tregs within tumors, male Foxp3EGFP reporter mice were administered antibodies anti-CD25 (InVivoMab anti-mouse CD25 (IL2Ra), clone PC-61.5.3, BioXCell (#BE0012); 200 μg per animal diluted in PBS; i.p. injection) at days 2, 5, and 8 post tumor cell injection. Control mice were administered the isotype control [InVivoMAb rat IgG isotype control anti-horseradish peroxidase, clone HRPN, BioXCell (#BE0088)]. At day 5, and until day 9, the mice received a daily dose of T1317 (15 mg/kg) or vehicle (DMSO) through intraperitoneal injection. From day 5 to day 10, tumor progression was evaluated. At day 10, the mice were euthanized, and the spleens and tumors were recovered and processed as described above.

Isolation of TAMs

3LL-R cells were injected subcutaneously in recipient C57BL/6 mice and, at day 15 postinjection, the tumors were excised and processed as described above. Cells suspensions were generated from pooled tumors (five tumors per sample) and incubated with the antibodies described for myeloid cell determination. $MHCII^{low}$ TAM and $MHCII^{high}$ TAM populations were isolated using a FACSaria Fusion Cell Sorter (BD Biosciences). For *ex vivo* experiments, TAMs were cultured in RPMI-10% FBS, supplemented with L-glutamine, HEPES, 10 mmol/L sodium pyruvate, nonessential amino acids (BioWest), and 3.7 nmol/L 2-mercaptoethanol (Sigma-Aldrich).

Isolation of Tregs

3LL-R cells were injected subcutaneously in Foxp3EGFP transgenic mice. At day 10 postinjection, cell suspensions were generated from pooled tumors and Tregs were sorted as $EGFP^+$ cells using a FACSaria Fusion cell sorter. The cells were maintained in RPMI-10% FBS for subsequent analysis. Alternatively, Tregs were isolated from the spleens of Foxp3EGFP mice.

Proliferation assays

3LL-R cells were plated in 24-well plates (10^5 cells/well) and starved in RPMI without FBS during 24 hours in the presence of LXR ligands or vehicle (DMSO). After starvation, the cells were incubated with 10% FBS and 0.3 g/L L-glutamine for 24 hours. Finally, the cells were pulsed with ^{3}H-thymidine (1 μCi/mL; ICN Pharmaceuticals) for 6 hours. The cells were fixed in 70% methanol, washed in 10% TCA, and lysed in 1% SDS/0.3 mol/L NaOH. Radioactivity was counted by liquid scintillation using a 1400 Tri-Carb Packard counter (GMI). Each experimental condition was performed in triplicates.

Purified Tregs (10^5 cells/well) were stained with CellTrace CFSE Cell Proliferation Kit (Invitrogen) following the manufacturer's recommendations and then stimulated with T1317 (1 μmol/L) or DMSO for 18 hours at 37°C. Unstained cells were grown separately as a negative control for cytometry. Treg proliferation was induced during 48 hours using the mouse T Cell Activation/Expansion Kit (Miltenyi Biotec), which consists of anti-biotin MACSiBead particles and biotinylated antibodies against mouse CD3ε and CD28, in the presence of IL2 (100 U/mL). Cell proliferation was analyzed by flow cytometry as the percentage of cells with CFSE dispersion compared with nonactivated Tregs. Murine IL2 was expressed in and purified from *E. coli* as described previously (31).

Suppression of T-cell proliferation

TAMs or Tregs were seeded in 96-well plates (200,000 TAMs/well or 100,000 Tregs/well in RPMI-10% FBS) and stimulated with T1317 (1 μmol/L) or DMSO for 18 hours at 37°C. In experiments using TAMs, the medium was then replaced by fresh medium (without LXR agonist). Total splenocytes were obtained from the spleens of C57BL/6 mice as a cell suspension and stained with the CellTrace CFSE Cell Proliferation Kit. Unstained splenocytes were grown separately as a negative control for cytometry. CFSE-stained splenocytes were either

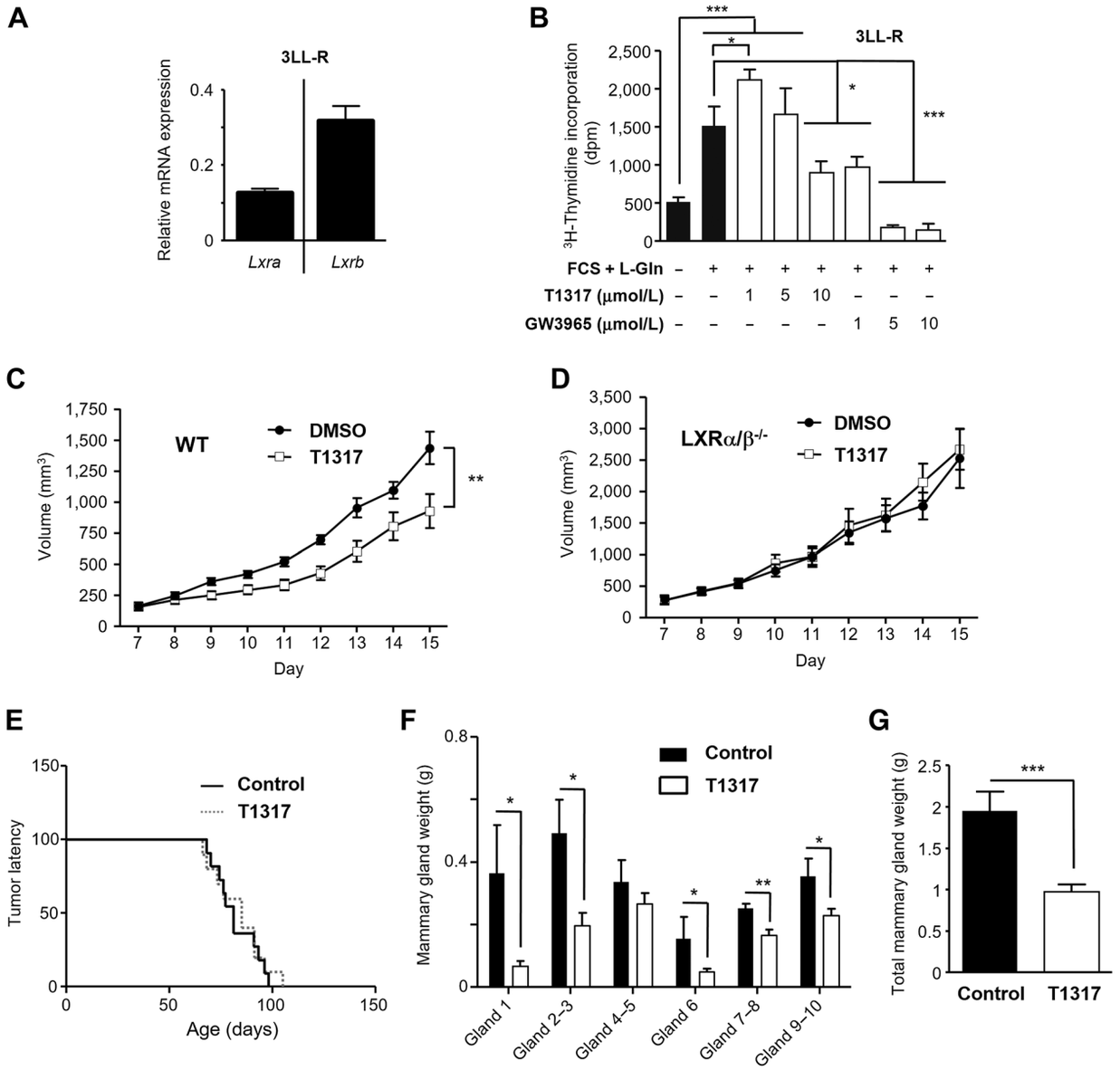


Figure 1.

Functional LXR expression in host cells is essential for the antitumoral actions of the LXR agonist T1317. **A,** 3LL-R cells express *Lxr*α and β, as measured by qPCR. Expression values normalized to the expression levels of *L14*. Data represent mean ± SEM; $n = 4$ independent experiments. **B,** LXR agonists inhibit 3LL-R cell proliferation *in vitro*. 3LL-R cells were starved for 24 hours in RPMI without FBS in the presence of either vehicle (DMSO) or the indicated concentrations of LXR agonists, T1317 or GW3965. Cell proliferation was induced during 24 hours in the presence of 10% FBS and 0.3 g/L L-Gln, whereas control cells were kept in RPMI alone. In all samples, ^{3}H-thymidine was added for 6 hours. After cell lysis, ^{3}H-thymidine incorporation was measured as an indication of DNA synthesis. Data represent mean ± SD from a representative experiment performed in triplicates. ANOVA-Bonferroni. *, $P < 0.05$; ***, $P < 0.001$. Similar results were obtained in $n = 3$ independent experiments. **C** and **D,** WT (**C**) or LXRα/β$^{-/-}$ (**D**) male mice were subjected to the subcutaneous injection of 3×10^6 3LL-R cells. From day 7 after tumor cell injection, the mice were treated daily (intraperitoneal injection) with either vehicle (DMSO) or the LXR agonist T1317 (15 mg/kg). Tumor volume was measured daily up to day 15. Mean ± SEM; $n = 14$ (**C**), $n = 9$ (**D**). Two-way ANOVA with repeated measures. In **E–G**, PyMT female mice were administered a chow diet with or without supplementation with T1317 (50 mg/kg) right after weaning. Tumor latency was evaluated daily; log-rank–Wilcoxon test (**E**). At 22 weeks of age, the mice were sacrificed and the weight was measured for each mammary gland (**F**). Total mammary gland weight is represented in **G**. Mean ± SEM; $n = 8$ (Control), $n = 10$ (T1317). Mann–Whitney *U* test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

grown alone in RPMI-10% FBS or incubated with TAMs or Treg cells at a 1:1 ratio. T-cell proliferation was induced using the Mouse T Cell Activation/Expansion Kit during 48 hours at 37°C and analyzed by flow cytometry.

Phagocytosis assay

MHCII^{low} TAMs and MHCII^{high} TAMs were seeded in 24-well plates (500,000 cells/well) and stimulated with T1317 1 μmol/L or DMSO for 18 hours at 37°C. The cells were incubated with 3-μm

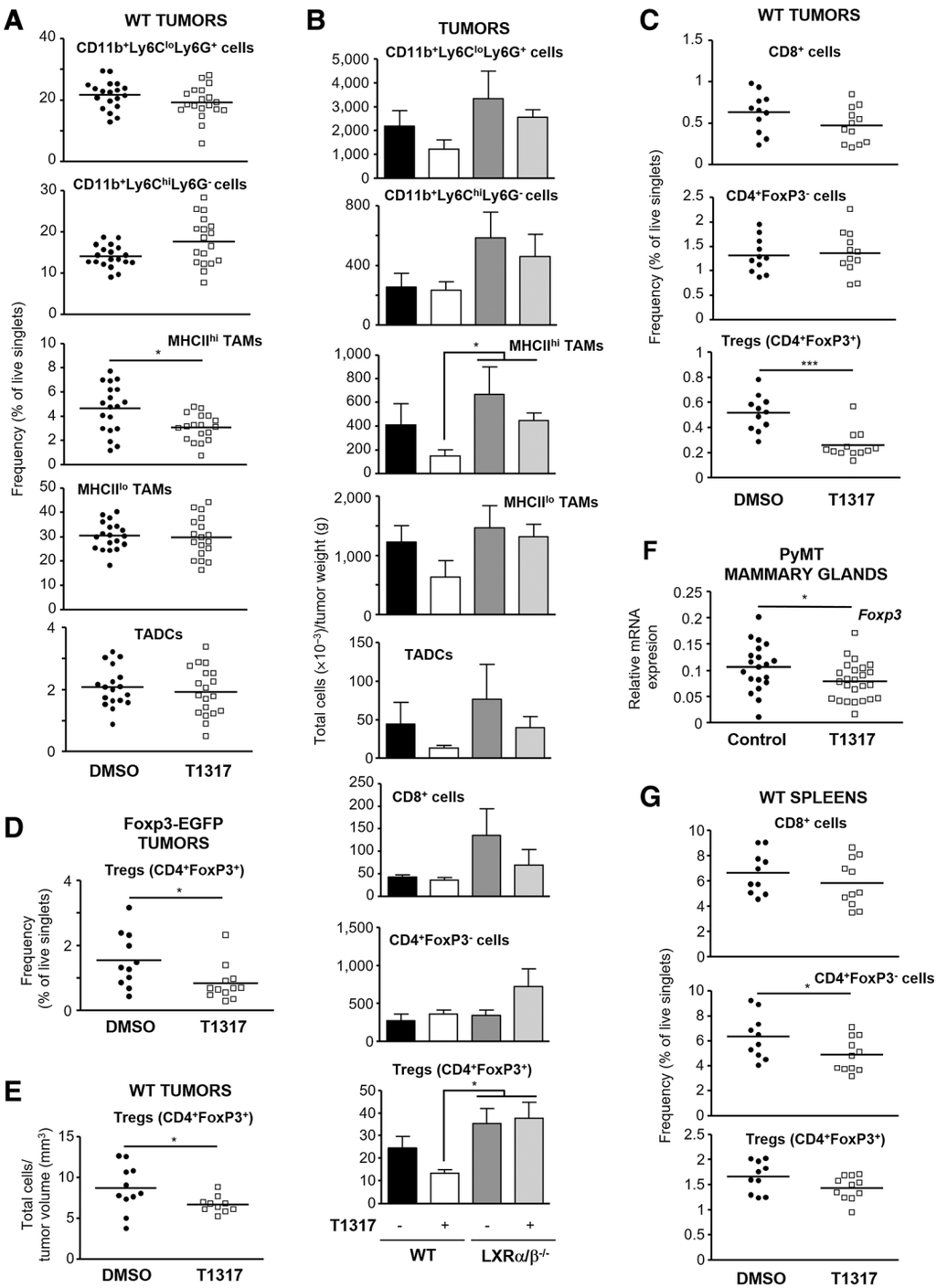


Figure 2.

Pharmacologic stimulation of the LXR pathway reduces the abundance of Tregs in the tumor. WT C57BL/6 mice (**A–C**, **E**, and **G**), Foxp3EGFP transgenic mice (**D**) or LXR-deficient mice (**B**) were subjected to subcutaneous injection of 3LL-R cells. The mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 (**A–C**, **E**, and **G**) or day 5 (**D**) of tumor establishment. The tumors were collected at day 15 (**A–C** and **E**) or day 10 (**D**) after tumor inoculation. The spleens were collected at day 15 (**G**). The abundance of different immune cell populations was measured by flow cytometry. In **A**, **C**, **D**, and **G**, the graphics represent the frequencies of immune cell populations (percentage of gated live singlets) in the tumors (**A**, **C**, and **D**) or spleens (**G**). Horizontal bars indicate mean values from each experimental group. Pooled data from two (**C** and **G**) or three (**A** and **D**) independent experiments; $n = 18–19$ (**A**), $n = 11–12$ (**C** and **D**), $n = 10–11$ (**G**) mice/group (Mann–Whitney test). In **B**, the absolute numbers of intratumoral immune cells normalized to the tumor weight was determined in WT and LXR-deficient mice. $n = 5$ mice/group. One-way ANOVA test followed by Newman–Keuls *post hoc*. In **E**, the absolute amount of Tregs was determined in each tumor and normalized to the tumor volume. Pooled data from two independent experiments; $n = 10–11$ mice/group (*t* test). In **F**, PyMT female mice were administered a chow diet with or without supplementation with T1317 (50 mg/kg). Relative expression levels of *Foxp3* mRNA (normalized to *L14*) in mammary glands at 22 weeks of age (qPCR). $n = 8–10$ mice/group. *t* test; *, $P < 0.05$; ***, $P < 0.001$.

fluorescent microspheres (Fluoresbrite YG microspheres, Polysciences) at a ratio of 20 beads/cell for 30 minutes at 37 °C. After this time, the cells were placed on ice, washed three times with ice-cold PBS, and fixed in PBS-2% PFA. The phagocytosis of microspheres was analyzed by flow cytometry.

RNA extraction, cDNA synthesis, and quantitative real-time PCR analysis

Total RNA was extracted from cells or tissues using TRIzol (Invitrogen) as recommended by the manufacturer. For cDNA synthesis, 1 μg of RNA was subjected to reverse transcription using M-MLV Reverse transcriptase RNase H Minus, Point Mutant, oligo $(dT)_{15}$ primer and PCR nucleotide mix (Promega). Quantitative real-time PCR (qPCR) was performed using the Power SYBR Green Reagent Kit (Applied Biosystems) following the manufacturer's recommendations. See more details in Supplementary Methods. The data were expressed as mRNA levels relative to ribosomal *L14* or to *Gapdh* expression in murine and human samples, respectively.

Gene expression profiling

Total RNA was purified using the RNAeasy Kit (Qiagen) following the manufacturer's instructions. The samples were processed as described in Supplementary Methods and hybridized to GeneChip Mouse Clariom S Array (Affymetrix). For each sample, expression estimates were calculated from probe intensities and represented as $\log_2$ values. Heatmaps were produced with Heatmapper (Wishart Research Group, University of Alberta,

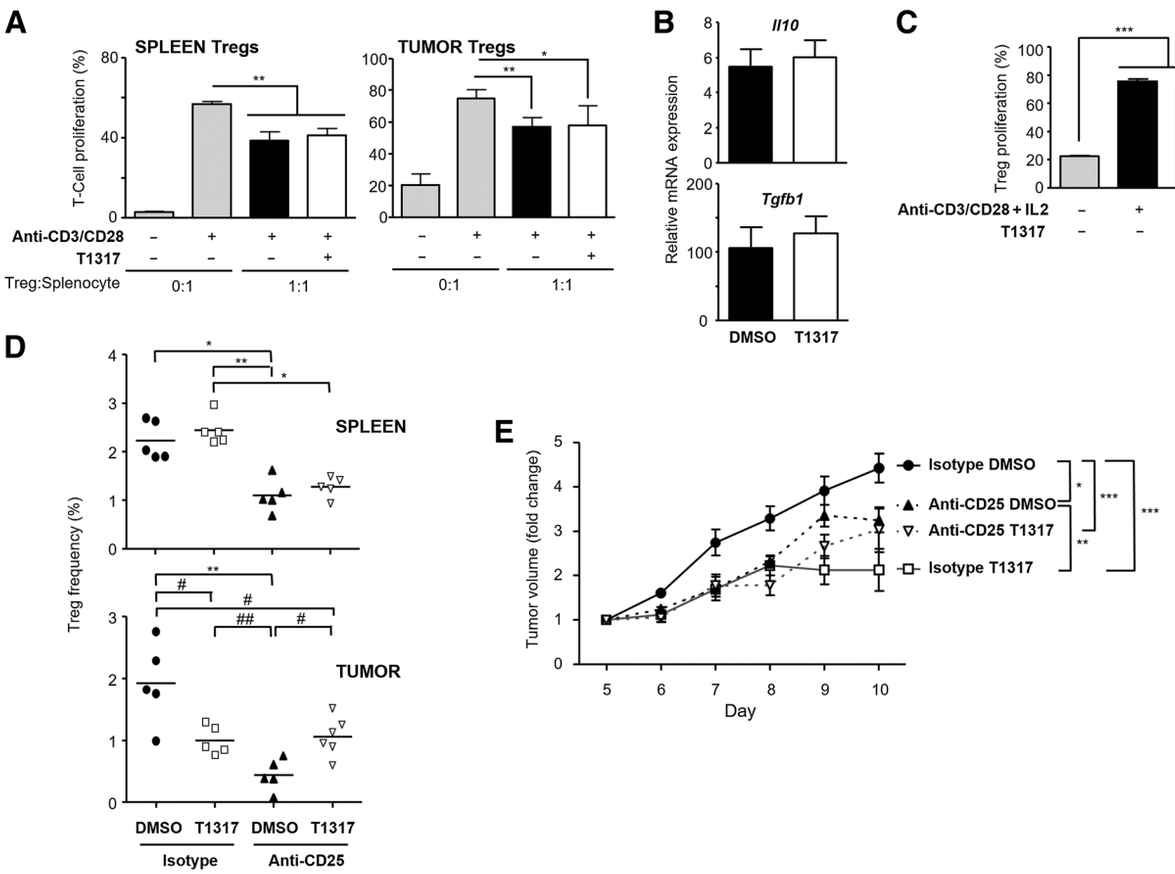
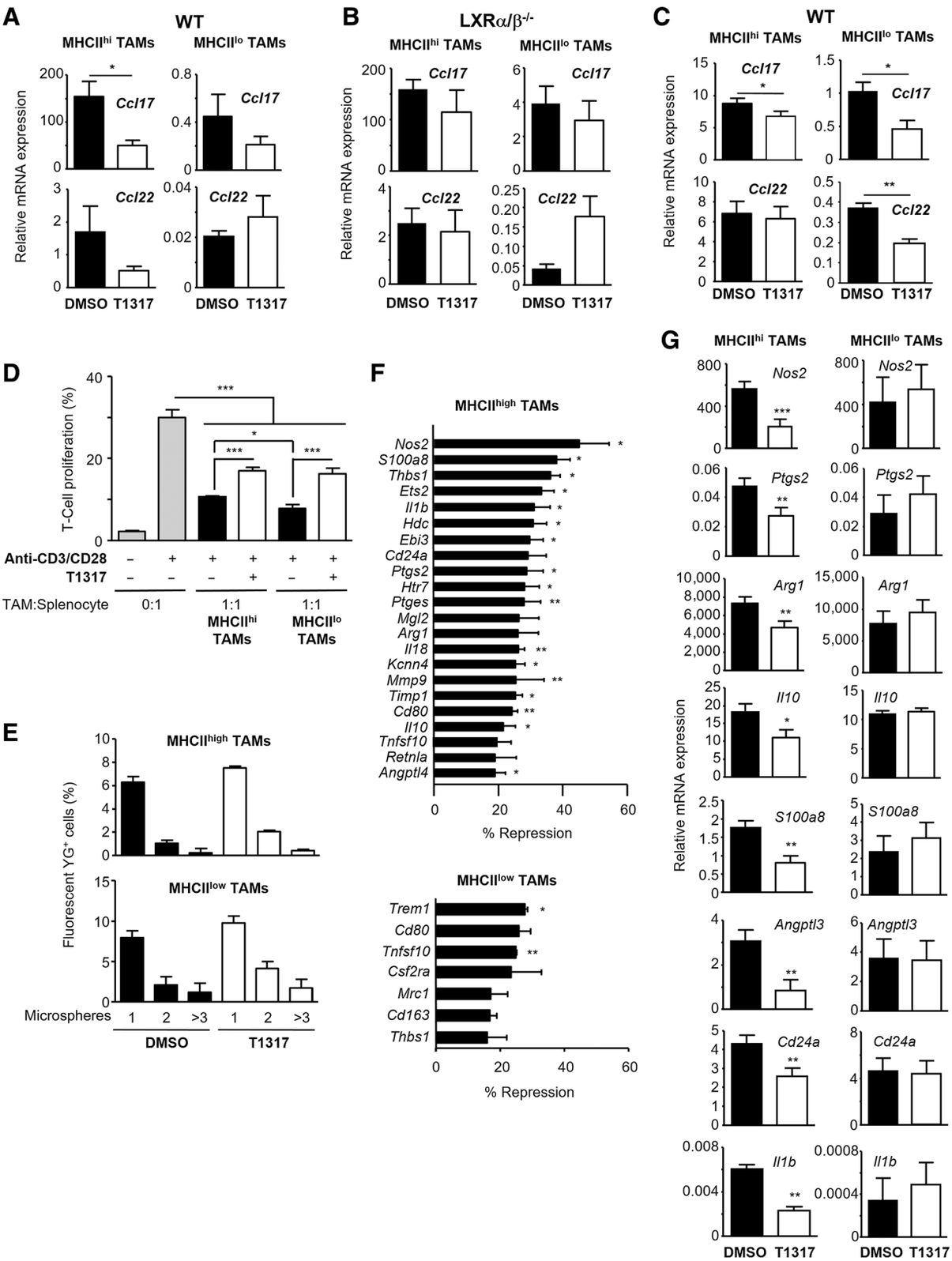


Figure 3.

The antitumoral action of T1317 is not effective in mice undergoing Treg depletion. **A,** T1317 does not impair the capability of Tregs to suppress T-cell proliferation. Tregs were isolated from the spleens (left) or tumors (right) of Foxp3EGFP mice. Total splenocytes were isolated from the spleens of WT mice, stained with CFSE, and incubated with Tregs at a 1:1 (Treg:splenocyte) ratio. Control cells were grown in the absence of Tregs. T-cell proliferation was induced *in vitro* for 48 hours using antibodies against CD3ε and CD28 and analyzed by flow cytometry. The graphics represent the percentage of cells with CFSE dispersion compared with nonactivated splenocytes. Mean ± SD. Left, $n = 3$ biological replicates. Right, $n = 5$ biological replicates (pooled data from two independent experiments; *t* test; *, $P < 0.05$; **, $P < 0.01$). **B** and **C,** Tregs were isolated from the spleen of Foxp3EGFP mice. In **B**, purified Tregs were incubated with DMSO or T1317 *in vitro* and the expression of *Il10* and *Tgfb* was analyzed by qPCR; mean ± SD ($n = 3$). In **C**, Tregs were stained with CFSE, incubated with DMSO or T1317, and induced to proliferate in the presence of a combination of anti-CD3/CD28 antibodies and murine recombinant IL2 (100 U/mL). Mean ± SD, $n = 3$ (one-way ANOVA). **D** and **E,** Male Foxp3EGFP reporter mice were subjected to the subcutaneous injection of 3×10^6 3LL-R cells. At days 2, 5, and 8 post tumor cell injection, the mice were administered either antibodies anti-CD25 or control isotype antibodies (200 μg per animal in PBS by intraperitoneal injection). At day 5, and until day 9, the mice received a daily dose of T1317 (15 mg/kg) or vehicle (DMSO). $n = 5$–6 animals/group. **D,** At day 10, the mice were euthanized. The frequency of FOXP3-GFP$^+$ Treg in spleens (top) and tumors (bottom) was evaluated by flow cytometry. Horizontal bars represent mean values. Kruskal–Wallis test followed by Dunn multiple comparison test; *, $P < 0.05$; **, $P < 0.01$. Selected experimental conditions were also compared using a Mann–Whitney test (#, $P < 0.05$; ##, $P < 0.01$). **E,** Tumor volumes were measured from day 5 to day 10 and represented as fold change. Two-way ANOVA with repeated measures; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



Canada). Gene ontology (GO) analysis was carried out with the PANTHER Classification System (32). Microarray data have been deposited at the ArrayExpress database with accession number E-MTAB-9707.

Identification and cloning of potential enhancer regions with IRF4-binding sites

Public data from chromatin immunoprecipitation (ChIP-seq) experiments (GSE40918; ref. 33) were mapped with Bowtie2 to the mm9 assembly of the mouse genome. The resulting SAM files were subsequently analyzed using HOMER. Each sequencing experiment was normalized to 107 uniquely mapped tags. Sequencing experiments were visualized at UCSC genome browser from tracks generated with HOMER. IRF4-binding sites were identified and annotated using HOMER. Regions of interest showing IRF4 peaks were scanned for IRF4-binding motifs using public JASPAR motif matrix with DMINDA (34).

Potential enhancer regions containing IRF4-binding sites upstream and downstream of the *Ccl17* gene were amplified from mouse tail genomic DNA using REDExtract N-Amp PCR Ready Mix (Sigma-Aldrich). See more details in Supplementary Methods. Amplified regions were subsequently cloned between the *Kpn*I and *Xho*I restriction sites of a pGL3-promoter vector (Promega).

Reporter activity assays

To evaluate the potential activity of *Ccl17* enhancers containing IRF4-binding sites, Raw264.7 macrophages (10^5 cells/well in 12-well plates) were cotransfected with 100 ng of the enhancer-containing pGL3 plasmid and either 100 ng of a pMIG-IRF4 plasmid that constitutively expresses IRF4 (gift from David Baltimore, California Institute of Technology, Pasadena, CA; Addgene plasmid # 58987; ref. 35) or an empty vector. All cells were cotransfected with 500 ng of pCDNA3-LXRα plasmid that constitutively expresses LXRα to ensure an optimal response to LXR ligands and 10 ng of a *Renilla* expression plasmid (pBOS-Renilla) as a control of the transfection efficiency. Transfections were carried out using Superfect (Qiagen) following the manufacturer's instructions. Luciferase activity was assessed using the Dual Luciferase Reporter Assay (Promega) in an Infinite M200 luminometer (Tecan).

Protein extraction and Western blot analysis

The cells were washed twice in cold PBS and lysed on ice with lysis solution (1% Triton X-100, 10% glycerol, 50 mmol/L HEPES, pH 7.5, 250 mmol/L NaCl, protease inhibitors, 1 mmol/L sodium orthovanadate). Insoluble material was removed by centrifugation at 13,000 × *g* for 8 minutes at 4°C. Cell lysates were processed for Western blot analysis as described in Supplementary Methods.

ELISA

The supernatants from macrophage cultures were recovered and stored at −80°C. ELISA kits from Thermo Scientific [Mouse MDC (CCL22) ELISA kit and Mouse TARC (CCL17) ELISA kit] were used for quantitative measurement of secreted mouse CCL22 and CCL17, respectively, using the manufacturer's recommendations. See a more detailed protocol in Supplementary Methods.

Statistical analysis

The GraphPad Prism 6.0 software was used to perform all statistical analyses. Differences in tumor volume in the 3LL-R model were analyzed by a two-way repeated-measure ANOVA with a Bonferroni *post hoc* test. The log-rank and Gehan–Wilcoxon tests were used to compare tumor latency curves in the PyMT model. The rest of the data was analyzed using either one-way ANOVA, or two-tailed Student *t* test for data with normal distribution, or the nonparametric Kruskal–Wallis–Dunn test or Mann–Whitney *U* test for data not following normal distribution.

To make different experiments comparable in **Figs. 4C**, **5A–D**, **F**, **6A**, **C**, **D**, **7C–E** and **K**, and Supplementary Figs. S2C and S2D; S5A–S5D, the data were normalized using the following procedure. The intensity of each experiment (ie) was calculated by determining the mean value of gene expression between the negative and positive controls. The intensities of separate experiments were normalized by the mean intensity value of all the experiments (im) and, for each experiment, the resulting normalization factor (im/ie) was multiplied by the expression levels of all the samples in that experiment. In **Fig. 2A** and **C**, the data were normalized using the mean percentage of cells in the DMSO group in each experiment.

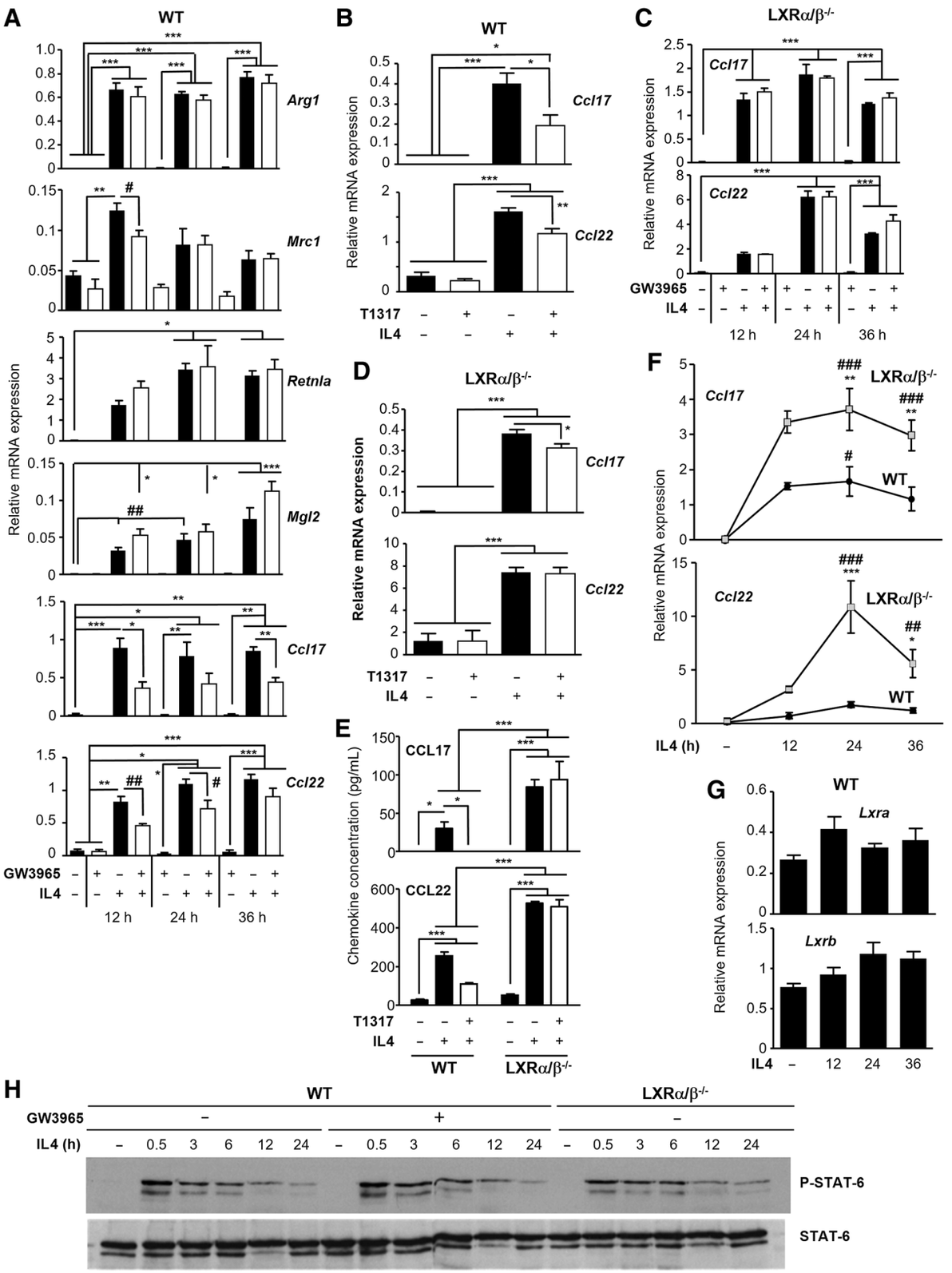
Results

Functional LXR expression in host cells is required for the antitumoral actions of the LXR agonist T1317

We and others had previously reported antiproliferative actions of LXR agonists in primary immune cells (36, 37) and in tumor cell lines *in vitro* (revised in ref. 8). These observations were confirmed in this study using the Lewis lung carcinoma cell line 3LL-R, which expresses both LXRα and β (**Fig. 1A** and **B**). Interestingly, however, tumor progression studies based on the subcutaneous injection of 3LL-R cells into syngeneic mice (C57BL/6 background) revealed that the administration of the LXR agonist T1317 was able to inhibit the growth of established tumors in WT mice, but not in mice lacking functional LXRs (**Fig. 1C** and **D**) despite the fact that the injected cancer cells express LXRs. Altogether, these observations suggest that LXR expression in host cells is essential for the inhibitory actions of the synthetic LXR agonist on tumor growth.

Figure 4.

Pharmacologic LXR activation downregulates the expression of genes that are part of the protumoral program of TAMs. **A** and **B,** WT (**A**) or LXR-deficient (**B**) male mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 of tumor establishment. MHCII^{high} and MHCII^{low} TAMs were isolated at day 15 after tumor cell inoculation. **C–G,** MHCII^{high} and MHCII^{low} TAMs were isolated from 15-day tumors and stimulated *ex vivo* with T1317 (1 μmol/L) or DMSO for 18 hours (**D** and **E**) or 24 hours (**C**, **F**, and **G**). In **A–C**, the expression of *Ccl17* and *Ccl22* was analyzed by qPCR (mean ± SEM). **A,** $n = 5$ mice/group. **B,** $n = 7$–8 mice/group; pooled data from two independent experiments. **C,** $n = 3$–5 experiments (each using 4–5 pooled male mice). **A–C,** *t* test; *, $P < 0.05$; **, $P < 0.01$. **D,** LXR activation inhibits the capability of TAMs to suppress T-cell proliferation. Freshly isolated splenocytes were stained with CFSE and incubated with TAMs at a 1:1 (TAM:splenocyte) ratio. Control cells were maintained in the absence of TAMs. T-cell proliferation was induced for 48 hours using antibodies against CD3ε and CD28 and analyzed by flow cytometry. The graphic represents the percentage of cells with CFSE dispersion using nonactivated splenocytes as reference. Mean ± SD; $n = 3$ biological replicates. One-way ANOVA. *, $P < 0.05$; ***, $P < 0.001$. **E,** TAMs were incubated with 3-μm fluorescent microspheres (20 beads/cell) for 30 minutes. The phagocytosis of microspheres was analyzed by flow cytometry. Mean ± SD; $n = 2$ biological replicates. **F,** Changes in gene expression were analyzed by gene profiling. The results from three (MHCII^{high} TAMs) or two (MHCII^{low} TAMs) independent experiments are included. The graphic shows the percentage of repression by T1317 of genes that have been selected because of their reported involvement in immunosuppressive functions in the tumor microenvironment or in macrophage alternative activation. Paired *t* test. **G,** The selective repression of several genes in MHCII^{high} TAMs was validated through qPCR. Mean ± SEM, $n = 3$ independent experiments (*t* test). **F** and **G,** *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (T1317-treated vs. control cells).



The LXR agonist was also effective in preventing tumor growth in PyMT female mice, a model for spontaneous breast adenocarcinoma development and progression (**Fig. 1E–G**). The oral administration of the agonist through the diet did not affect tumor latency (**Fig. 1E**), but resulted in a significant decrease in mammary gland weight at 22 weeks of age (**Fig. 1F** and **G**). This finding argues for the importance of LXR activity in the microenvironment once tumors are established, rather than affecting cancer cell proliferation and early-stage carcinogenesis.

Pharmacologic LXR activation reduces the abundance of Tregs within the tumor

To dissect the actions of the LXR agonist in the tumor microenvironment, we next used flow cytometry to assess, in 3LL-R tumors, the abundance of different intratumoral immune cell populations that have prognostic values. At the level of myeloid cells, several populations were distinguished in 3LL-R tumors: $CD11b^{+}/Ly6C^{high}/Ly6G^{-}$ cells (compatible with $Ly6C^{high}$ monocytes and monocytic MDSCs), $CD11b^{+}/Ly6C^{low}/Ly6G^{+}$ cells (compatible with neutrophils and polymorphonuclear MDSCs), tumor-associated dendritic cells (TADCs; $CD11b^{+}/Ly6C^{-}/Ly6G^{-}/MHCII^{bright}$), and two TAM subsets ($CD11b^{+}/Ly6C^{-}/Ly6G^{-}$) expressing different levels of MHCII and termed $MHCII^{low}$ TAMs and $MHCII^{high}$ TAMs (gating strategy shown in Supplementary Fig. S1). Pharmacologic LXR activation specifically decreased the frequency of $MHCII^{high}$ TAMs without significantly affecting the frequency of other myeloid cell populations analyzed here (**Fig. 2A**). However, the total numbers of $MHCII^{high}$ TAMs differed considerably between tumors and only a tendency toward a decreased amount of these cells (when normalized to the weight or volume of the tumor) was observed in the tumors from T1317-treated WT mice (**Fig. 2B**). These observations suggest that the decrease in the relative frequency of $MHCII^{high}$ TAMs in response to LXR activation does not consistently reflect reduced absolute numbers of these cells in the tumor.

At the level of T lymphocytes, the administration of T1317 significantly decreased the frequency and the total numbers of $CD4^{+}FOXP3^{+}$ Tregs within the tumor (**Fig. 2C–E**). In addition, decreased levels of *Foxp3* expression were observed in the mammary glands from T1317-treated PyMT mice in comparison with mice fed a regular diet (**Fig. 2F**), which suggests that T1317 may also downregulate Treg infiltration in spontaneous breast adenocarcinoma. In LXR-deficient mice, T1317 did not inhibit the total numbers of the immune cell populations tested, including Tregs (**Fig. 2B**), which is in line with the LXR-specific effects of the agonist in the control of tumor growth (**Fig. 1C** and **D**).

Because a major goal of this study was to characterize the mechanisms underlying the anti-tumoral actions of synthetic LXR agonists, we further explored the effects of LXR activation in Tregs. First, the treatment of tumor-bearing mice with T1317 did not impact the frequency of Tregs in the spleen (**Fig. 2G**), suggesting that the decrease in Treg abundance is specific to the tumor. Of note, the frequency of splenic $CD4^{+}$ $FOXP3^{-}$ cells was downregulated upon pharmacologic LXR activation (**Fig. 2G**), in line with previous work showing a role for LXR in the negative control of central T-cell proliferation (36). Next, we evaluated whether the immunosuppressive capacity of Tregs is affected by T1317. Tregs were isolated from either the spleens or tumors of Foxp3EGFP reporter mice and treated with the LXR agonist or vehicle. Their capability to inhibit T-cell proliferation induced by anti-CD3/CD28 antibodies was tested *in vitro*. Interestingly, the LXR agonist did not impair the suppressive capacity of Tregs on T-cell proliferation (**Fig. 3A**). Moreover, activation of LXRs did not reduce the expression of the anti-inflammatory cytokines *Il10* and *Tgfb* in Tregs from the spleen (**Fig. 3B**). Taken together, these results suggest that pharmacologic LXR activation does not inhibit the immunosuppressive capacity of the Treg itself, but rather results in a decrease in the amount of Tregs within the tumor. In addition, the LXR agonist did not inhibit the polyclonal proliferation of Tregs (**Fig. 3C**).

Importantly, higher infiltration of Tregs often correlates with less favorable outcomes in different types of tumors and accumulated evidence indicates that the removal of Tregs is able to enhance antitumor immune responses (reviewed in ref. 38). To assess the relevance of the Treg population in the antitumoral actions of T1317, the levels of Tregs were reduced by the intraperitoneal injection of mAbs against CD25 in Foxp3EGFP reporter mice (**Fig. 3D**). In comparison with control mice (injected with isotype control antibodies), Treg depletion resulted in reduced tumor progression (**Fig. 3E**). In line with the data in **Fig. 2C** and **D**, treatment with T1317 reduced the frequency of intratumoral $FOXP3\text{-}GFP^{+}$ Tregs (**Fig. 3D**) and inhibited tumor progression in control mice (**Fig. 3E**). Interestingly, the LXR agonist was not effective in reducing tumor volumes in mice undergoing anti-CD25–mediated Treg depletion (**Fig. 3E**), which suggests that the actions of T1317 in tumor progression are Treg-dependent.

Pharmacologic LXR activation downregulates *Ccl17* expression in TAMs

Treg abundance in the tumor has been shown to correlate with the local production of the chemokines CCL17 and CCL22 (19, 20, 39). Interestingly, TAMs, predominantly $MHCII^{high}$ TAMs in mice, highly

Figure 5.

Pharmacologic LXR activation inhibits the IL4-mediated induction of *Ccl17* and *Ccl22*. **A–E,** Bone marrow–derived macrophages were obtained from WT mice (**A, B,** and **E**) or LXRα/β$^{-/-}$ mice (**C–E**). The cells were stimulated with either vehicle (DMSO), GW3965 (1 μmol/L; **A** and **C**), or T1317 (1 μmol/L; **B** and **D**) for 6 hours and then treated with IL4 (**A** and **C:** 10 ng/mL during 12, 24, or 36 hours; **B** and **D:** 20 ng/mL for 12 hours). In **A** and **C,** similar results were obtained in experiments using more prolonged preincubation times with LXR agonists (up to 18 hours) and higher doses of IL4 (up to 20 ng/mL). **A–D,** Mean ± SEM; pooled data from $n = 3$–6 experiments using 1–3 biological replicates/experiment. ANOVA-Bonferroni. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. **A,** a *t* test was also used for selected comparisons; #, $P < 0.05$; ##, $P < 0.01$. **E,** Determination of the secreted levels of CCL17 and CCL22 in the supernatants of WT and LXRα/β$^{-/-}$ macrophages stimulated with IL4 (10 ng/mL; 24 hours) in the presence or absence of T1317 (1 μmol/L; pretreatment 16 hours). ELISA. Mean ± SD; $n = 3$ biological replicates. Two-way ANOVA–Bonferroni. *, $P < 0.05$; ***, $P < 0.001$. **F,** The expression curves of *Ccl17* and *Ccl22* in response to IL4 was compared between WT and LXRα/β$^{-/-}$ macrophages in the absence of LXR agonists (qPCR). Mean ± SEM; $n = 4$ independent experiments. Two-way ANOVA-Bonferroni. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus WT; ##, $P < 0.01$; ###, $P < 0.001$ versus unstimulated cells from each genotype. **G,** The stimulation of WT macrophages with IL4 did not affect negatively *Lxra* or *Lxrb* mRNA expression, as determined by qPCR. Mean ± SEM, $n = 4$ independent experiments using 1–3 biological replicates/experiment. ANOVA-Bonferroni. **H,** The activation of LXRs did not interfere with STAT-6 phosphorylation in response to IL4. Macrophages were incubated with GW3965 (1 μmol/L; 18 hours) or vehicle and then stimulated with IL4 (20 ng/mL) for the indicated periods of time. Phosphorylated STAT-6 (P-STAT-6) and total STAT-6 were analyzed in whole cell extracts (50 μg/lane) by Western blotting using specific antibodies. The same blot was stripped and probed repeatedly.

contribute to the secretion of these chemokines in the tumor microenvironment (21). Given our observations on Treg abundance, we next sought to investigate the impact of LXR activation on chemokine expression by TAMs from 3LL-R tumor-bearing mice. Interestingly, the treatment of WT mice with T1317 resulted in the reduced expression of *Ccl17* in MHCII^{high} TAMs, but not in TAMs from LXR-deficient mice (**Fig. 4A** and **B**). Culturing these cells *in vitro* led to a drastic drop in *Ccl17* mRNA levels (compare **Fig. 4A** and **C**), which suggests that the expression of this chemokine in MHCII^{high} TAMs is highly dependent on signals present in the tumor microenvironment. *Ex vivo* administration of the LXR agonist had little impact on *Ccl17* expression under these conditions (**Fig. 4C**). A tendency for the downregulation of *Ccl17* was also observed in MHCII^{low} TAMs from T1317-treated WT mice (**Fig. 4A**). When WT MHCII^{low} TAMs were treated *ex vivo* with the LXR agonist, clear inhibitory effects were observed on *Ccl17* and *Ccl22* expression (**Fig. 4C**), suggesting that the production of such chemokines by this TAM population may also be susceptible to downregulation by LXRs provided that the agonist can reach the (hypoxic) areas within the tumor where these cells reside.

We further explored whether the pharmacologic activation of LXRs affects other pathways that may be involved in the maintenance of an immunosuppressed tumor microenvironment. Both MHCII^{high} and MHCII^{low} TAMs were isolated from established tumors and treated with the LXR agonist *ex vivo*. When incubated with splenocytes, both TAM subsets were able to suppress T-cell proliferation (**Fig. 4D**). Interestingly, the pretreatment with the LXR agonist counteracted partially the suppressive capacity of TAMs. In contrast, their capability to phagocytose latex microspheres was not downregulated by the agonist (**Fig. 4E**).

Microarray studies were performed to evaluate global effects on gene expression and some of the actions were validated in independent experiments through qPCR analysis (**Fig. 4F** and **G**; Supplementary Figs. S2–S4). The expression of the isoforms LXRα and LXRβ and of their heterodimeric partners RXRα and RXRβ was confirmed in both TAM subpopulations and stimulation with the LXR agonist did not change their expression levels (Supplementary Fig. S2A), as is the case in many other cellular systems. Low levels of RXRγ were detected in these cells. As expected, several genes previously recognized as direct targets of the LXR-RXR heterodimer were induced in both TAM subsets (Supplementary Fig. S2B). In contrast to observations in other cell types, however, no induction of *Apoe* expression was observed in TAMs treated with T1317 (Supplementary Figs. S2B and S2C). Similar results were obtained from whole tumors exposed to the LXR agonist *in vivo* (Supplementary Fig. S2D).

We used the gene expression profiling data to further characterize the effects of the LXR agonist on selected markers of macrophage activation, including surface markers, enzymes, cytokines, and chemokines other than *Ccl17*/*Ccl22* (Supplementary Fig. S3). As demonstrated in previous work (21), MHCII^{high} and MHCII^{low} TAMs differ in the levels of expression of a number of activation markers. The expression of most of the genes selected for this analysis was unaffected by the LXR agonist. However, significant downregulation was observed in the expression of several markers of activation (*Cd80, Nos2, Il1b, Il10, Il4ra, Mmp9*, and *Ptges*) in T1317-treated MHCII^{high} TAMs.

In parallel, whole profiling analysis revealed that different subsets of genes were repressed (>25% repression) by T1317 in MHCII^{high} and MHCII^{low} TAMs (Supplementary Fig. S4A). An unbiased GO analysis of genes repressed by T1317 in MHCII^{high} TAMs showed the enrichment of several biological processes, including the positive regulation of nitric oxide and reactive oxygen species (ROS) biosynthesis (Supplementary Fig. S4B), which are important biological mechanisms contributing to the maintenance of an immunosuppressive state by tumor-infiltrated myeloid cells. No significant enrichment of specific biological processes was observed among the genes repressed by T1317 in MHCII^{low} TAMs. In addition, we selected a list of genes with reported involvement in immunosuppression in tumors and/or in the acquisition of a macrophage alternative activation phenotype. T1317 repressed the expression of several genes within this category in MHCII^{high} TAMs and MHCII^{low} TAMs (**Fig. 4F**). The fact that the agonist was able to repress a larger set of genes in MHCII^{high} TAMs (**Fig. 4F** and **G**) suggests differences between both cell subpopulations in the repertoire of genes susceptible to LXR-mediated repression.

Synthetic LXR agonists inhibit the induction of *Ccl17* and *Ccl22* by IL4 or GM-CSF

Ccl17 and *Ccl22* expression in cells from the monocyte-macrophage lineage is regulated by GM-CSF and IL4 (40–42). Moreover, GM-CSF has been shown to induce *Ccl17* expression in MHCII^{high} TAMs *in vivo* (18). Considering the repressive effect of LXR agonism on *Ccl17* expression in TAMs, we further explored whether synthetic LXR agonists modulate the actions of GM-CSF or IL4 in macrophages. In these experiments, bone marrow–derived macrophages, obtained from either WT or LXRα/β$^{-/-}$ mice, were preincubated with the LXR agonists GW3965 or T1317 and then stimulated with IL4 or GM-CSF for different periods of time. Pharmacologic activation of the LXR pathway inhibited the expression of *Ccl17* and *Ccl22* induced by IL4 (**Fig. 5A** and **B**), whereas other key marker genes of the macrophage response to IL4 were not affected (**Fig. 5A**). The effects of the agonists on chemokine expression were drastically reduced or abolished in LXR-deficient macrophages (**Fig. 5C** and **D**), indicating that these effects largely depend on functional LXR activity. In line with the changes in gene expression, pharmacologic activation of LXRs inhibited the secretion of CCL17 and CCL22 in WT macrophages (**Fig. 5E**). Interestingly, the induction of these chemokines at different time points after IL4 treatment was significantly higher in LXR-deficient macrophages than in WT cells (**Fig. 5E** and **F**), suggesting that LXRs can perform basal repression of these chemokines in the absence of synthetic high-affinity agonists. Of note, the expression of LXRs was not reciprocally inhibited by IL4 (**Fig. 5G**) and the effects of the LXR agonist could not be attributed to changes in the pattern of STAT-6 phosphorylation in response to IL4 (**Fig. 5H**).

Stimulation with T1317 or GW3965 also resulted in lower production of *Ccl17* and *Ccl22* in response to GM-CSF (**Fig. 6A** and **B**). Notably, and contrary to the effects on the IL4 response, LXR agonists negatively impacted other GM-CSF target genes such as *Arg1, Retnla*, and *Mgl2* (**Fig. 6A**) and GM-CSF itself downregulated *Lxra* expression (**Fig. 6C**), suggesting a more general reciprocal negative interaction between GM-CSF signaling and the LXR pathway. Nevertheless, the effects of the agonists were abolished or severely reduced in LXR-deficient cells (**Fig. 6B** and **D**).

Pharmacologic LXR activation inhibits IRF4 expression

Despite the use of different signaling modules, macrophage responses to IL4 and GM-CSF share the induction of the transcription factor IRF4 (41, 43). The use of IRF4-deficient (IRF4$^{-/-}$) macrophages showed that the functional expression of IRF4 is required for the induction of a subset of the genes evaluated, namely *Ccl17, Ccl22, Retnla*, and *Il1b*, but not for *Arg1, Mrc1*, and *Mgl2* (**Fig. 7A** and **B**). Of note, *Ccl17* and *Ccl22*, which are inhibited by LXR agonists during the

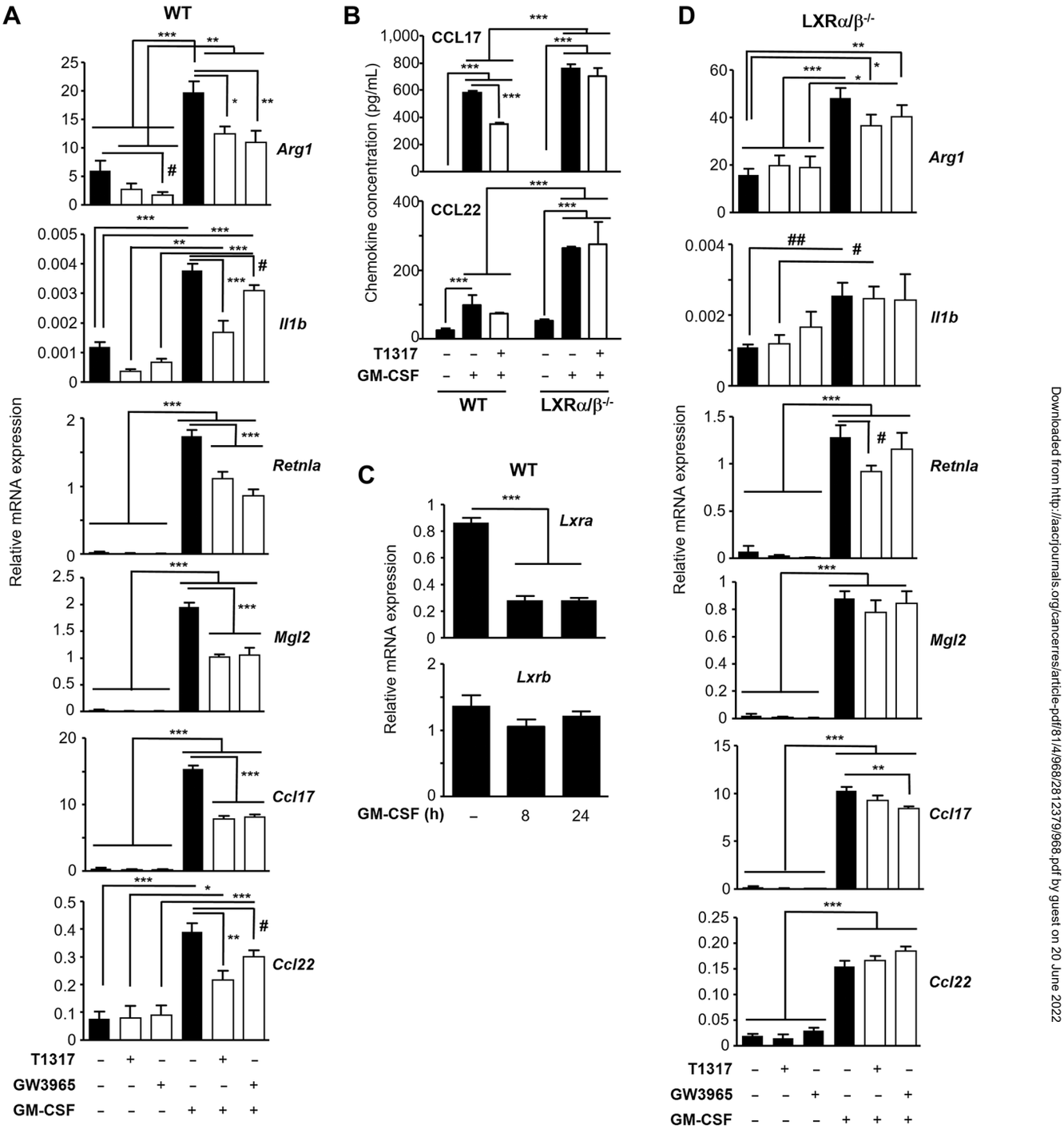
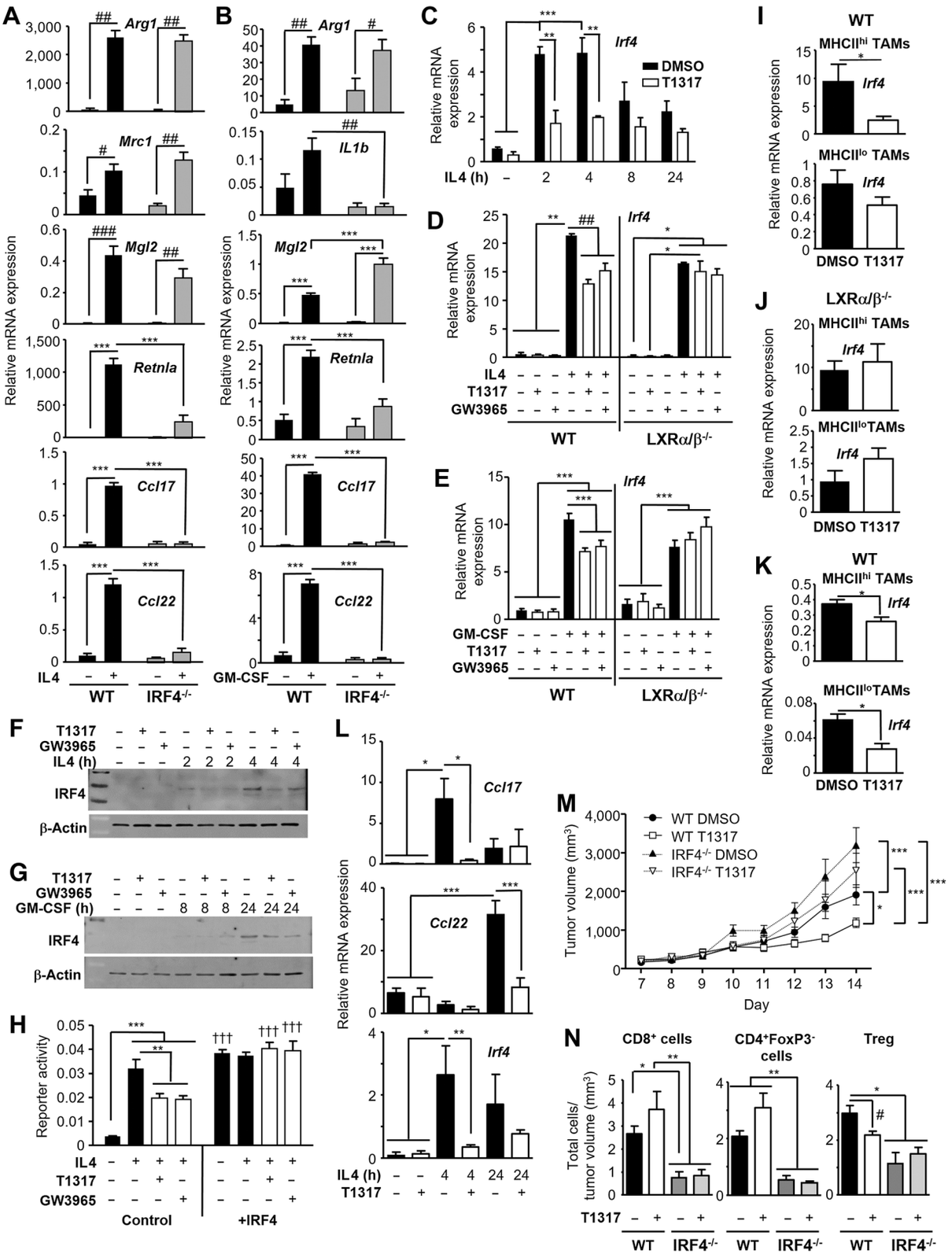


Figure 6.

LXR agonists inhibit the induction of *Ccl17* and *Ccl22* in response to GM-CSF. Bone marrow–derived macrophages from WT (**A**–**C**) or LXR$\alpha/\beta^{-/-}$ (**B** and **D**) mice were treated with vehicle, T1317 or GW3965 (1 μmol/L) for 16 hours and then stimulated with GM-CSF (5 ng/mL) for 24 hours. In **C**, WT macrophages were treated with GM-CSF for the indicated periods of time. In **A**, **C**, and **D**, gene expression levels were determined by qPCR. Mean ± SEM. $n = 3$–8 (**A**), $n = 3$–4 (**C**), $n = 4$ (**D**) independent experiments using 1–2 biological replicates/experiment (ANOVA–Bonferroni). **B,** Determination of the secreted levels of CCL17 and CCL22 by ELISA. Mean ± SD; $n = 3$ biological replicates. Two-way ANOVA–Bonferroni. **A–D,** **, $P < 0.01$; ***, $P < 0.001$. Selected experimental conditions were also compared using a *t* test (#, $P < 0.05$; ##, $P < 0.01$).



macrophage response to both IL4 and GM-CSF, were the most drastically impaired genes in IRF4$^{-/-}$ macrophages.

IRF4 has been shown to cooperatively bind activator protein-1 (AP1) complexes in T cells to regulate gene transcription from AP1-IRF composite elements (44). We therefore evaluated whether the induction of IRF4-dependent genes required cooperation with JUNB or JUND. Although we cannot discard redundant roles from these proteins, the upregulation of *Ccl17, Ccl22, Retnla*, and *Il1b* in response to IL4 or GM-CSF was not impaired in macrophages deficient for either JUNB or JUND (Supplementary Figs. S5A–S5D).

Interestingly, the expression of IRF4 itself during the macrophage response to IL4 or GM-CSF was inhibited by LXR agonists both at the mRNA and protein level (**Fig. 7C–G**). It has been recently reported that both cytokines induce the upregulation of the expression of the demethylase jumonji domain-containing protein 3 (JMJD3) upstream of the transcriptional activation of IRF4 (45, 46). However, LXR activity did not repress the expression of this enzyme in response to IL4 or GM-CSF (Supplementary Fig. S6A and S6B), which suggests that the inhibitory effects on IRF4 expression reported here are not related to upstream alterations in JMJD3.

Previous work had analyzed the binding of IRF4 across the murine genome in T cells through ChIP-seq analysis (33). By reanalyzing these data, we identified three sites with enriched binding of IRF4 in the proximity of the *Ccl17* gene both in naïve CD4^{+} T cells and during Th17 cell differentiation (Supplementary Fig. S7A–S7C). To translate this finding to macrophages, we transfected Raw264.7 cells with reporter plasmids containing various IRF4-binding regions. Peak 2, located approximately 6.5 Kb upstream of the *Ccl17* transcription start site, displayed strong enhancer activity in response to IL4, which was downregulated upon pharmacologic activation of the LXR pathway (**Fig. 7H**). The region containing peak 3 was also cloned but did not respond to IL4 in transfection studies. To know whether the inhibitory action on IRF4 could help explain the effects of LXR agonists on *Ccl17* expression, we assessed the consequences of IRF4 overexpression in transfected cells. Interestingly, the overexpression of IRF4 upregulated the activity of *Ccl17* enhancer 2 in the absence of IL4 and counteracted the inhibitory action of LXR agonists (**Fig. 7H**), thus supporting the crosstalk between LXRs and IRF4 as a mechanism mediating the control of *Ccl17* expression.

Provided the inhibitory effects of LXR agonists on *Ccl17* expression in TAMs (**Fig. 4A–C**), we further explored whether LXR activation resulted in decreased IRF4 expression in these cells. Interestingly, a decline was observed in the levels of *Irf4* in MHCII^{high} TAMs from T1317-treated tumors (**Fig. 7I**) in WT mice but not in LXR-deficient mice (**Fig. 7J**). In addition, *Irf4* expression was significantly inhibited in MHCII^{high} TAMs and MHCII^{low} TAMs treated *ex vivo* with T1317 (**Fig. 7K**). Of note, as is the case for *Ccl17*, the levels of *Irf4* expression dropped down drastically when MHCII^{high} TAMs were cultured *in vitro*. Taken together, these results suggest that the inhibitory action of the LXR agonist on IRF4 expression may contribute to reduce the levels of CCL17 in the tumor microenvironment. Importantly, pharmacologic activation of the LXR pathway also inhibited the induction of *Ccl17, Ccl22*, and *Irf4* by IL4 in human macrophages (**Fig. 7L**), suggesting that this crosstalk is evolutionary conserved and may also be relevant in humans.

To determine whether functional IRF4 expression is important for the antitumoral actions of the LXR agonist *in vivo*, tumor progression studies were carried out in WT and IRF4$^{-/-}$ mice treated with either DMSO or T1317. Tumors acquired larger volumes in IRF4$^{-/-}$ mice as compared with WT mice (**Fig. 7M**). Notably, there was lower infiltration of lymphocytes in general (not only Tregs) in the tumors in IRF4$^{-/-}$ mice (**Fig. 7N**), which may explain the increased tumor growth in these mice. Nevertheless, the tumors in the IRF4$^{-/-}$ background were not as responsive to T1317 as the tumors grown in WT mice, which supports the importance of IRF4 as a mediator of the antitumoral actions of pharmacologic LXR activation.

Discussion

In this study, we have identified novel roles of activated LXRs in the control of tumor growth. Despite the fact that the synthetic LXR agonist T1317 is able to directly inhibit cancer cell proliferation *in vitro*, its antitumoral potential is compromised in LXR-deficient mice carrying WT tumors, thus highlighting the importance of LXR functional activity in the tumor microenvironment over direct antiproliferative effects in a cancer cell-autonomous manner. Indeed, most studies, including ours, have only demonstrated significant effects of LXR agonists at directly inhibiting the proliferation of transformed cells when used at relatively high doses (5–10 μmol/L; refs. 47–49), in

Figure 7.

Pharmacologic LXR activation inhibits the induction of *Irf4* by IL4 or GM-CSF. **A** and **B,** IRF4 is required for specific gene subsets during the macrophage response to IL4 or GM-CSF. WT and IRF4-deficient (IRF4$^{-/-}$) macrophages were stimulated with IL4 (10 ng/mL; 24 hours; **A**) or GM-CSF (5 ng/mL; 24 hours; **B**). Gene expression was analyzed by qPCR. Mean ± SEM, $n = 4$ independent experiments. Two-way ANOVA–Bonferroni. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. In datasets without factor interaction, selected conditions were compared by *t* test (#, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$). **C–G,** LXR inhibits *Irf4* expression. WT (**C–G**) or LXRα/β$^{-/-}$ (**D** and **E**) macrophages were preincubated with T1317 (**C–G**) or GW3965 (**D–G**; each at 1 μmol/L; 18 hours) and stimulated with IL4 (10 ng/mL; indicated periods of time in **C** and **F**; 4 hours in **D**) or GM-CSF (5 ng/mL; 24 hours in **E**; indicated times in **G**). In **C–E**, *Irf4* expression levels were analyzed by qPCR. Mean ± SEM; $n = 3$ (**C**); $n = 6$ (**D** and **E**). ANOVA-Bonferroni (**C** and **E**), Kruskal–Wallis (**D**). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. In **D**, a Mann–Whitney test was also used for selected comparisons (##, $P < 0.01$). In **F** and **G**, IRF4 expression was analyzed by Western blotting (7 μg whole cell extract/lane). **F** and **G,** Representative experiment. **F,** Two blots were generated in parallel from the same set of samples. **G,** The same blot was stripped and reprobed. **H,** Raw264.7 macrophages were cotransfected with pGL3-Ccl17 enhancer 2, a plasmid overexpressing LXRα, a plasmid expressing *Renilla*, and either empty pcDNA3 or a plasmid overexpressing IRF-4. After transfection, the cells were incubated in DMEM-10% FBS in the presence or absence of LXR agonists T1317 or GW3965 (1 μmol/L; 18 hours). Next, the cells were stimulated with IL4 (10 ng/mL; 24 hours). Luciferase activity was measured and normalized to *Renilla* activity. Mean ± SEM; $n = 3$ independent experiments. Two-way ANOVA-Bonferroni. **, $P < 0.01$; ***, $P < 0.001$ (*vs.* control unstimulated cells); †††, $P < 0.01$ (vs. the same treatment in the control group). **I–K,** Effects of LXR activation on *Irf4* expression in TAMs. WT (**I**) or LXRα/β$^{-/-}$ (**J**) male mice were treated daily with DMSO or T1317 (15 mg/kg) from day 7 of tumor establishment. MHCII^{high} and MHCII^{low} TAMs were isolated at day 15 after tumor cell inoculation. **K,** MHCII^{high} and MHCII^{low} TAMs were isolated from 15-day tumors and stimulated *ex vivo* with T1317 (1 μmol/L) or DMSO for 24 hours. In **I–K**, the expression of *Irf4* was analyzed by qPCR. Mean ± SEM, $n = 5$ mice/group (**I**); $n = 6$–8 mice/group (pooled data from two independent experiments; **J**); $n = 3$ independent experiments (each using 4–5 pooled mice; **K**). *t* test. *, $P < 0.05$. **L,** The treatment of human macrophages with T1317 inhibited the IL4-induced expression of *Ccl17, Ccl22*, and *Irf4*. qPCR. $n = 3$ independent experiments. One-way ANOVA. **M** and **N,** WT or IRF4$^{-/-}$ mice were subjected to subcutaneous injection of 3LL-R cells and treated daily with DMSO or T1317 (15 mg/kg) from day 7 of tumor establishment. **M,** Tumor volume progression curve. **N,** Absolute numbers of intratumoral lymphocytes were measured by flow cytometry and normalized to tumor volume. Mean ± SEM; $n = 6$–7 mice/group. **M,** Two-way ANOVA-repeated measures. **N,** One-way ANOVA – Bonferroni (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). In addition, *t* test, #, $P < 0.05$.

contrast to lower doses required for the activation of metabolic pathways or for the repression of inflammatory gene expression in primary cells.

Previous work by Tavazoie and colleagues also placed the focus on the role of LXRs in the tumor microenvironment by showing the downregulation of the frequency of MDSCs and an increased CTL activity within tumors in response to high doses of LXR agonists (11). Mechanistic studies using melanoma B16F10 cells indicated that LXR activation, in an *ApoE*-dependent manner, resulted in increased apoptosis of MDSCs. In the work presented here, the characterization of infiltrated myeloid cells from lung carcinoma tumors indicated that LXR agonism slightly reduced the relative frequency of MHCII^{high} TAMs without affecting other, more abundant populations in the tumor, including those populations theoretically enriched for MDSCs. It is possible that differences in the type of tumor, in the type of agonist, or in the stage of tumor progression at the time of agonist administration help explain why MDSC frequencies were not decreased in our model. In addition, the dose of agonist used during development of LLC tumors in our study was considerably lower (15 mg/kg/day) than the doses at which LXR agonists inhibited intratumoral MDSC frequencies (80–100 mg/kg/day) in the work mentioned above. In this sense, *ApoE* mRNA levels were not upregulated in whole tumors from mice treated with the dose of T1317 used in our study nor in TAMs exposed to the agonist *in vitro*, in contrast to the increase in other well-established LXR targets genes.

TAMs may locally expand in response to M-CSF (50). Although it is not clear which signals specifically modulate the proliferation or survival of MHCII^{high} TAMs, we have previously shown that LXR agonists inhibit macrophage proliferation induced by several growth factors, including M-CSF and GM-CSF (37). For these reasons, we cannot discard some contribution of the LXR pathway to the control of local TAM proliferation. However, the decreased proportion of MHCII^{high} TAMs was not consistently accompanied by reduced absolute numbers of these cells within the tumor, which suggests that the changes in their frequency are mostly influenced by the relative distribution of other intratumoral cells.

The results from this work strongly suggest that combined actions that result from the modulation of TAM responses contribute to the antitumoral effects of pharmacologic LXR activation. The LXR agonist is able to repress several genes that play key roles in the protumoral program of TAMs. In fact, the capability of TAMs to suppress T-cell proliferation *in vitro* was reduced by the LXR agonist. Notably, different subsets of genes were repressed by T1317 in MHCII^{high} TAMs and MHCII^{low} TAMs. For example, *Nos2* expression was selectively repressed in MHCII^{high} TAMs, whereas *Trem1* expression was inhibited in MHCII^{low} TAMs. We do not have a clear explanation for these differences, but we cannot discard that hypoxic conditions may result in intracellular changes increasing the resistance of some genes to LXR-mediated repression. In addition, previous work has reported differences in the mechanisms used by these two subpopulations to suppress T-cell activation, with MHCII^{high} TAMs being more dependent on NOS2 activity (21). Therefore, further understanding of the relative contribution of some of these genes in the suppressive activity of TAM subpopulations is still warranted.

In addition to direct suppressive mechanisms, MHCII^{high} TAMs are major contributors to CCL17 and CCL22 production (21), which are important signals for Treg recruitment to the tumor. The analysis of the actions of the LXR agonist on TAM subpopulations *in vivo* showed the significant repression of *Ccl17* in MHCII^{high} TAMs and a tendency for such repression in MHCII^{low} TAMs. The effects of the LXR agonist on the latter population were more evident when the cells were exposed to the agonist *ex vivo*, suggesting that the availability of the LXR ligand might be lower in hypoxic areas of the tumor. Treatment with the LXR agonist did not impact the frequency of Tregs in the spleen or their capacity to proliferate *in vitro*, which supports the notion that the repression of CCL17 production in TAMs has an important role in the reduction of the abundance of intratumoral Tregs upon LXR activation. Despite the fact that Tregs exist in low numbers in LLC tumors, depletion of these cells *in vivo* diminished tumor progression in mice. Importantly, the pharmacologic activation of LXRs did not efficiently reduce tumor growth under these conditions, suggesting that the inhibitory effects on Treg abundance are an important mechanism mediating the antitumoral actions of the LXR agonist. Because LXR activation did not inhibit functional aspects on isolated Tregs, such as the expression of anti-inflammatory cytokines or their capacity to suppress T-cell proliferation *ex vivo*, we conclude that the importance of this mechanism lies in the ability of the LXR agonist to downregulate the abundance of Tregs within the tumor, in correlation with reduced chemokine expression by TAMs, rather than in compromising the immunosuppressive potential of the Treg itself.

Our *in vitro* studies indicate that, in the absence of a synthetic LXR agonist, saturating doses of recombinant IL4 or GM-CSF are able to induce higher production of CCL17/22 in LXR-deficient macrophages than in the WT counterparts. However, within a more complex entity, the tumor microenvironment, WT and LXR-deficient MHCII^{high} TAMs express similar levels of these chemokines in the absence of pharmacologic LXR activation, in line with the fact that tumors grown in LXR-deficient mice do not display a drastic increase in Treg abundance. Therefore, whether or not LXR-deficient cells produce higher levels of the chemokine might depend on additional signals that are present in each setting. Similar observations in other cellular/physiologic scenarios (5, 11, 51, 52) support the notions that LXR biology is complex and that genetic ablation and pharmacologic activation of these nuclear receptors do not necessarily result in opposite biological effects.

At the molecular level, the results from this work propose IRF4 targeting as a relevant mechanism that links pharmacologic LXR activation with the downregulation of *Ccl17*. On one hand, the induction of this chemokine by IL4 or GM-CSF is fully dependent on IRF4 functionality, and LXR activity is able to downregulate IRF4 expression in both settings. On the other hand, IRF4 overexpression blocked the capability of LXR agonists to negatively modulate the transcriptional enhancing activity of a region containing IRF4-binding sites upstream of the *Ccl17* promoter (and downstream of *Ccl22*). It is plausible that the downregulation of IRF4 expression may also contribute to the repressive actions of LXR agonists on other genes induced by GM-CSF.

Within tumors, in correlation with the inhibitory effects on *Ccl17* expression, the levels of *Irf4* were downregulated in MHCII^{high} TAMs in response to the administration of the LXR agonist *in vivo*. However, interpretation of the data from the systemic deficiency in IRF4 should be done with caution. IRF4 is involved in the development and function of different subsets of CD4$^+$ T cells, not only Tregs, and in the generation of Th1 responses (reviewed in ref. 53). The reduced infiltration of CD8$^+$ and CD4$^+$ lymphocytes in LLC tumors most probably compromises the development of antitumoral immune responses, which would explain the increased tumor growth in the IRF4-deficient model despite Treg numbers being downregulated. In line with this notion, systemic IRF4

deficiency promoted an immunosuppressed tumor microenvironment in other models of cancer (54). Importantly, these mice did not respond to pharmacologic LXR activation as efficiently as WT mice, which suggests that IRF4 plays a relevant role in the antitumoral actions of the LXR agonist. However, we are aware that a deficiency of IRF4 specifically in macrophages would help to better answer this question. Future studies will be addressed in this direction.

In this work we have validated that pharmacologic activation of LXRs also exerts inhibitory effects on the induction of the *Irf4*/*Ccl17* axis in human macrophages. However, whether or not the expression levels of *Irf4* specifically in TAMs have prognostic value in human tumors remains elusive. Importantly, the accumulation of CCL17-expressing macrophages has been described in lung adenocarcinoma, favoring the recruitment of effector Tregs (55). In addition, high expression levels of intratumoral CCL17 have been associated with poorer overall survival rates in hepatocellular carcinoma (56). Moreover, Kaplan–Meier analysis using the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl) to assess the probability of overall survival in patient cohorts of kidney renal clear cell carcinoma from The Cancer Genome Atlas project, as well as in non–small cell lung cancer (57), indicates that high expression of CCL17 also associates with poorer overall survival probability in these types of cancer (Supplementary Fig. S8A and S8B). On the other side, in a mouse model of subcutaneous tumor development, the downregulation of CCL17 upon transduction of short hairpin RNA in CT26 cancer cells resulted in decreased Treg infiltration within tumors and suppressed tumor growth (58), suggesting that targeting the intratumoral levels of CCL17 may represent a promising strategy against cancer.

Taken together, this work reveals unappreciated roles for pharmacologic LXR activation in the control of several macrophage-mediated mechanisms contributing to the maintenance of an immunosuppressive microenvironment (Supplementary Fig. S9), thus providing novel insights about the mechanisms of action of LXR agonists as therapeutic drugs against cancer.

Authors' Disclosures

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Authors' Contributions

J. Carbó: Conceptualization, formal analysis, validation, investigation, visualization, methodology, writing-original draft, writing-review and editing. **T.E. Leon:** Conceptualization, formal analysis, validation, investigation, visualization, methodology, writing-original draft, writing-review and editing. **J. Font-Díaz:** Conceptualization, formal analysis, validation, investigation, visualization, methodology, writing-original draft, writing-review and editing. **J.V. De la Rosa:** Formal analysis, visualization, methodology. **A. Castrillo:** Resources, formal analysis, funding acquisition. **F.R. Picard:** Formal analysis, investigation. **D. Staudenraus:** Formal analysis, investigation. **M. Huber:** Funding acquisition, investigation, methodology. **L. Cedó:** Formal analysis, investigation, visualization, methodology. **J.C. Escolà-Gil:** Formal analysis, funding acquisition, investigation, visualization, methodology. **L. Campos:** Resources. **L. Bakiri:** Resources. **E.F. Wagner:** Resources. **C. Caelles:** Supervision, funding acquisition, writing-review and editing. **T. Stratmann:** Resources, investigation, methodology. **J.A. Van Ginderachter:** Conceptualization, methodology, writing-review and editing. **A.F. Valledor:** Conceptualization, resources, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing.

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References

1. Schulman IG. Liver X receptors link lipid metabolism and inflammation. FEBS Lett 2017;591:2978–91.
2. A-Gonzalez N, Bensinger SJ, Hong C, Beceiro S, Bradley MN, Zelcer N, et al. Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. Immunity 2009;31:245–58.
3. Feig JE, Pineda-Torra I, Sanson M, Bradley MN, Vengrenyuk Y, Bogunovic D, et al. LXR promotes the maximal egress of monocyte-derived cells from mouse aortic plaques during atherosclerosis regression. J Clin Invest 2010;120:4415–24.
4. Joseph SB, Bradley MN, Castrillo A, Bruhn KW, Mak PA, Pei L, et al. LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell 2004;119:299–309.
5. Matalonga J, Glaria E, Bresque M, Escande C, Carbó JM, Kiefer K, et al. The nuclear receptor LXR limits bacterial infection of host macrophages through a mechanism that impacts cellular NAD metabolism. Cell Rep 2017;18:1241–55.
6. Valledor AF, Hsu LC, Ogawa S, Sawka-Verhelle D, Karin M, Glass CK. Activation of liver X receptors and retinoid X receptors prevents bacterial-induced macrophage apoptosis. Proc Natl Acad Sci U S A 2004;101:17813–8.
7. Steffensen KR, Jakobsson T, Gustafsson J-Å. Targeting liver X receptors in inflammation. Expert Opin Ther Targets 2013;17:977–90.
8. Lin C-Y, Gustafsson J-Å. Targeting liver X receptors in cancer therapeutics. Nat Rev Cancer 2015;15:216–24.

9. Pencheva N, Buss CG, Posada J, Merghoub T, Tavazoie SF. Broad-spectrum therapeutic suppression of metastatic melanoma through nuclear hormone receptor activation. Cell 2014;156:986–1001.
10. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. Science 2013;342:1094–8.
11. Tavazoie MF, Pollack I, Tanqueco R, Ostendorf BN, Reis BS, Gonsalves FC, et al. LXR/ApoE activation restricts innate immune suppression in cancer. Cell 2018; 172:825–840.
12. Villablanca EJ, Raccosta L, Zhou D, Fontana R, Maggioni D, Negro A, et al. Tumor - mediated liver X receptor - α activation inhibits CC chemokine receptor - 7 expression on dendritic cells and dampens antitumor responses. Nat Med 2009;16:98–105.
13. Zhang C, Lei G-S, Shao S, Jung H-W, Durant PJ, Lee C-H. Accumulation of myeloid-derived suppressor cells in the lungs during Pneumocystis pneumonia. Infect Immun 2012;80:3634–41.
14. Campbell MJ, Tonlaar NY, Garwood ER, Huo D, Moore DH, Khramtsov AI, et al. Proliferating macrophages associated with high grade, hormone receptor negative breast cancer and poor clinical outcome. Breast Cancer Res Treat 2011; 128:703–11.
15. Mantovani A, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol 2010;22:231–7.
16. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. Immunity 2014;41:49–61.
17. Su S, Liu Q, Chen J, Chen J, Chen F, He C, et al. A positive feedback loop between mesenchymal-like cancer cells and macrophages is essential to breast cancer metastasis. Cancer Cell 2014;25:605–20.
18. Van Overmeire E, Stijlemans B, Heymann F, Keirsse J, Morias Y, Elkrim Y, et al. M-CSF and GM-CSF receptor signaling differentially regulate monocyte maturation and macrophage polarization in the tumor microenvironment. Cancer Res 2016;76:35–42.
19. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004;10:942–9.
20. Ishida T, Ueda R. CCR4 as a novel molecular target for immunotherapy of cancer. Cancer Sci 2006;97:1139–46.
21. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, Van den Bossche J, et al. Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. Cancer Res 2010;70:5728–39.
22. Laoui D, Van Overmeire E, Di Conza G, Aldeni C, Keirsse J, Morias Y, et al. Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like macrophage population. Cancer Res 2014;74: 24–30.
23. Presa M, Ortiz AZ, Garabatos N, Izquierdo C, Rivas EI, Teyton L, et al. Cholera toxin subunit B peptide fusion proteins reveal impaired oral tolerance induction in diabetes-prone but not in diabetes-resistant mice. Eur J Immunol 2013;43: 2969–79.
24. Guy CT, Cardiff RD, Muller WJ. Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. Mol Cell Biol 1992;12:954–61.
25. Thomsen MK, Bakiri L, Hasenfuss SC, Hamacher R, Martinez L, Wagner EF. JUNB/AP-1 controls IFN-γ during inflammatory liver disease. J Clin Invest 2013;123:5258–68.
26. Thépot D, Weitzman JB, Barra J, Segretain D, Stinnakre MG, Babinet C, et al. Targeted disruption of the murine junD gene results in multiple defects in male reproductive function. Development 2000;127:143–53.
27. Valledor AF, Comalada M, Xaus J, Celada A. The differential time-course of extracellular-regulated kinase activity correlates with the macrophage response toward proliferation or activation. J Biol Chem 2000;275:7403–9.
28. Remels LM, De Baetselier PC. Characterization of 3LL-tumor variants generated by *in vitro* macrophage-mediated selection. Int J Cancer 1987; 39:343–52.
29. Casazza A, Laoui D, Wenes M, Rizzolio S, Bassani N, Mambretti M, et al. Impeding macrophage entry into hypoxic tumor areas by sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. Cancer Cell 2013;24:695–709.
30. Cedó L, García-León A, Baila-Rueda L, Santos D, Grijalva V, Martínez-Cignoni MR, et al. ApoA-I mimetic administration, but not increased apoA-I-containing HDL, inhibits tumour growth in a mouse model of inherited breast cancer. Sci Rep 2016;6:36387.
31. Izquierdo C, Ortiz AZ, Presa M, Malo S, Montoya A, Garabatos N, et al. Treatment of T1D via optimized expansion of antigen-specific Tregs induced by IL-2/anti-IL-2 monoclonal antibody complexes and peptide/MHC tetramers. Sci Rep 2018;8:1–14.
32. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, et al. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res 2017;45: D183–9.
33. Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F, et al. A validated regulatory network for Th17 cell specification. Cell 2012;151: 289–303.
34. Yang J, Chen X, McDermaid A, Ma Q. DMINDA 2.0: integrated and systematic views of regulatory DNA motif identification and analyses. Bioinformatics 2017; 33:2586–8.
35. So AYL, Sookram R, Chaudhuri AA, Minisandram A, Cheng D, Xie C, et al. Dual mechanisms by which miR-125b represses IRF4 to induce myeloid and B-cell leukemias. Blood 2014;124:1502–12.
36. Bensinger SJ, Bradley MN, Joseph SB, Zelcer N, Janssen EM, Hausner MA, et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. Cell 2008;134:97–111.
37. Pascual-García M, Carbó JM, León T, Matalonga J, Out R, Berkel TV, et al. Liver X receptors inhibit macrophage proliferation through downregulation of cyclins D1 and B1 and cyclin-dependent kinases 2 and 4. J Immunol 2011; 186:4656–67.
38. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. Cell Res 2017;27:109–18.
39. Mizukami Y, Kono K, Kawaguchi Y, Akaike H, Kamimura K, Sugai H, et al. CCL17 and CCL22 chemokines within tumor microenvironment are related to accumulation of Foxp3+ regulatory T cells in gastric cancer. Int J Cancer 2008; 122:2286–93.
40. Andrew DP, Chang MS, McNinch J, Wathen ST, Rihanek M, Tseng J, et al. STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. J Immunol 1998;161:5027–38.
41. Lacey DC, Achuthan A, Fleetwood AJ, Dinh H, Roiniotis J, Scholz GM, et al. Defining GM-CSF- and macrophage-CSF-dependent macrophage responses by in vitro models. J Immunol 2012;188:5752–65.
42. Pechkovsky DV, Prasse A, Kollert F, Engel KMY, Dentler J, Luttmann W, et al. Alternatively activated alveolar macrophages in pulmonary fibrosis—mediator production and intracellular signal transduction. Clin Immunol 2010;137:89–101.
43. El Chartouni C, Schwarzfischer L, Rehli M. Interleukin-4 induced interferon regulatory factor (Irf) 4 participates in the regulation of alternative macrophage priming. Immunobiology 2010;215:821–5.
44. Li P, Spolski R, Liao W, Wang L, Murphy TL, Murphy KM, et al. BATF–JUN is critical for IRF4-mediated transcription in T cells. Nature 2012;490: 543–6.
45. Achuthan A, Cook AD, Lee MC, Saleh R, Khiew HW, Chang MWN, et al. Granulocyte macrophage colony-stimulating factor induces CCL17 production via IRF4 to mediate inflammation. J Clin Invest 2016;126: 3453–66.
46. Hsu AT, Lupancu TJ, Lee M-C, Fleetwood AJ, Cook AD, Hamilton JA, et al. Epigenetic and transcriptional regulation of IL4-induced CCL17 production in human monocytes and murine macrophages. J Biol Chem 2018;293: 11415–23.
47. Fukuchi J, Hiipakka RA, Kokontis JM, Hsu S, Ko AL, Fitzgerald ML, et al. Androgenic suppression of ATP-binding cassette transporter A1 expression in LNCaP human prostate cancer cells. Cancer Res 2004;64: 7682–5.
48. Rough JJ, Monroy MA, Yerrum S, Daly JM. Anti-proliferative effect of LXR agonist T0901317 in ovarian carcinoma cells. J Ovarian Res 2010;3:13.
49. Vedin L-L, Lewandowski SA, Parini P, Gustafsson J-A, Steffensen KR. The oxysterol receptor LXR inhibits proliferation of human breast cancer cells. Carcinogenesis 2009;30:575–9.
50. Tymoszuk P, Evens H, Marzola V, Wachowicz K, Wasmer M-H, Datta S, et al. In situ proliferation contributes to accumulation of tumor-associated macrophages in spontaneous mammary tumors. Eur J Immunol 2014;44: 2247–62.
51. Saini SPS, Zhang B, Niu Y, Jiang M, Gao J, Zhai Y, et al. Activation of liver X receptor increases acetaminophen clearance and prevents its toxicity in mice. Hepatology 2011;54:2208–17.

52. Pourcet B, Gage MC, Leon TE, Waddington KE, Pello OM, Steffensen KR, et al. The nuclear receptor LXR modulates interleukin-18 levels in macrophages through multiple mechanisms. Sci Rep 2016;6:25481.
53. Crepeau RL, Ford ML. Programmed T cell differentiation: Implications for transplantation. Cell Immunol 2020;351:104099.
54. Metzger P, Kirchleitner S V., Boehmer DFR, Hörth C, Eisele A, Ormanns S, et al. Systemic but not MDSC-specific IRF4 deficiency promotes an immunosuppressed tumor microenvironment in a murine pancreatic cancer model. Cancer Immunol Immunother 2020;69:2101–12.
55. Kinoshita T, Kudo-Saito C, Muramatsu R, Fujita T, Saito M, Nagumo H, et al. Determination of poor prognostic immune features of tumour microenvironment in non-smoking patients with lung adenocarcinoma. Eur J Cancer 2017;86:15–27.
56. Zhu F, Li X, Chen S, Zeng Q, Zhao Y, Luo F. Tumor-associated macrophage or chemokine ligand CCL17 positively regulates the tumorigenesis of hepatocellular carcinoma. Med Oncol 2016;33:17.
57. Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 2006;439:353–7.
58. Hirata A, Hashimoto H, Shibasaki C, Narumi K, Aoki K. Intratumoral IFN-α gene delivery reduces tumor-infiltrating regulatory T cells through the down-regulation of tumor CCL17 expression. Cancer Gene Ther 2019;26:334–43.

Publication II

Nuclear receptors: Lipid and hormone sensors with essential roles in the control of cancer development

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Nuclear receptors: Lipid and hormone sensors with essential roles in the control of cancer development

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ABSTRACT

Nuclear receptors (NRs) are a superfamily of ligand-activated transcription factors that act as biological sensors and use a combination of mechanisms to modulate positively and negatively gene expression in a spatial and temporal manner. The highly orchestrated biological actions of several NRs influence the proliferation, differentiation, and apoptosis of many different cell types. Synthetic ligands for several NRs have been the focus of extensive drug discovery efforts for cancer intervention. This review summarizes the roles in tumour growth and metastasis of several relevant NR family members, namely androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), thyroid hormone receptor (TR), retinoic acid receptors (RARs), retinoid X receptors (RXRs), peroxisome proliferator-activated receptors (PPARs), and liver X receptors (LXRs). These studies are key to develop improved therapeutic agents based on novel modes of action with reduced side effects and overcoming resistance.

1. Introduction

Nuclear receptors (NRs) are a superfamily of ligand-activated transcription factors that play important roles in the physiology and pathology of many biological processes, including development, metabolism, reproduction, ageing and cancer [1]. NRs constitute an integral platform that connects environmental and hormonal signals to genomic responses, which govern all kinds of cell fate decisions at the level of gene expression. As a consequence of this central role, NRs represent the target for nearly 15 % of all pharmacologic drugs [2].

Progress in genomic sequencing over the years led to the identification of 48 and 49 NRs encoded by the human and mouse genomes, respectively. Members of the superfamily present a common modular structure with four main functional and structural domains, that yield a diversity of quaternary structures [3]. The NR-composing modules are a long, disordered N-terminal domain, a highly conserved DNA-binding domain (DBD), a hinge region, and a C-terminal ligand-binding domain (LBD) (Fig. 1).

The superfamily can be sub-divided in subfamilies depending on their cellular location and ligand genomic response. A group of NRs include high-affinity receptors for steroid hormones, and are typically cytoplasmic. This subfamily contains several receptors, including the androgen receptor (AR), the estrogen receptor (ER), and the glucocorticoid receptor (GR). Ligand binding allows chaperone-release and homodimerization followed by translocation into the nucleus. Once in the nucleus, the liganded receptor associates with transcriptional

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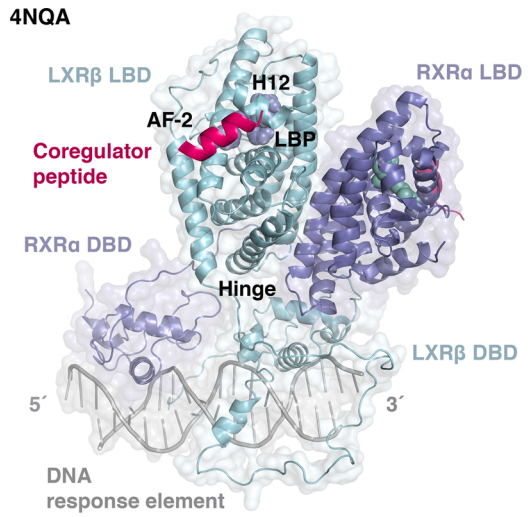

Fig. 1. NRs present a common modular structure with four main functional domains. The N-terminal domain is highly variable and includes several distinct regulatory regions (also known as AF1 for activation function 1) (not shown). A central DBD, composed of two highly conserved zinc fingers, directs the receptor to specific consensus DNA motifs that serve as response elements. An intermediate domain known as the hinge region exhibits domain flexibility for simultaneous receptor dimerization and binding to DNA, and contains a nuclear localization signal. The C-terminal half of the receptor harbours a large LBD, which confers the crucial property of ligand recognition and shifts the receptor to a transcriptionally active state in a specific and selective manner. The LBD also contributes to various protein-protein interactions in the subset of NRs that form heterodimers. There is also a C-terminal domain that contains an important pocket, the activating function 2 (AF-2). Within this pocket, H12 allows many NRs to interact with coactivators in a ligand-dependent manner. The image displays the quaternary organization of the RXRα–LXRβ heterodimer. Ribbon-diagram overview of the complex including LXRβ (cyan)–RXRα (lila), ligands as sphere representation (cyan and lila) and coactivator peptide (fucsia) shown as an helix docked on the AF-2 pocket. The image is shown on the direction along the DNA response element (5′ to 3′; grey). The hinge, LBP and H12 are also labelled. The Protein Data Bank coordinates of the 4NQA structure have been used and the rendered figure has been made with Pymol (https://pymol.org/2/).

coregulators that facilitate binding to the transcriptional machinery and the modulation of target gene expression [4].

A second subfamily is composed of receptors that normally reside in the nucleus and are bound to their cognate DNA sequences even in the absence of ligand. These members are exemplified by NRs that bind diet-derived ligands or intermediates of metabolic pathways, such as vitamin D receptor (VDR), retinoic acid receptor (RAR), peroxisome-proliferator activated receptors (PPARs) or liver X Receptors (LXRs). Thyroid hormone receptors (TR) are also part of this subfamily. They generally form heterodimers with the retinoid X receptor (RXR) (Fig. 1) and, in the absence of ligand, interact with NR corepressor (NCoR) and/or silencing mediator for retinoid and thyroid receptors (SMRT/NCoR2), which are part of corepressor complexes associated with histone deacetylases (HDACs), thereby repressing transcription. Conformational changes upon ligand binding lead to the dissociation of corepressors and the association of the NR with coactivator complexes, which normally include proteins with histone acetyl-transferase activity that allow an open chromatin conformation to facilitate the activation of target gene expression [1].

NRs regulate transcription by several mechanisms. As mentioned above, NRs can activate target genes by binding with their DBD directly to response elements, either as homodimers or heterodimers. Ligands allosterically control the interactions of the NR with coregulator proteins (coactivators and corepressors) by influencing either the folding or the dislodging of the C-terminal helix (helix 12, H12) as part of the AF-2 pocket (Fig. 1). Interaction with coactivators promotes the recruitment of the transcriptional machinery and the remodelling of chromatin. Nevertheless, the recently solved structure of the full-length AR has provided evidence that a coregulator can bind independently of the AF-2 pocket [5]. Whether this is an exception or the rule for other NRs remains to be further investigated.

In addition, several NRs can actively repress gene expression in the presence or absence of ligand, and some of them have been reported to inhibit transcription in a ligand-dependent manner by tethering and antagonizing the activity of other transcription factors (mechanisms collectively named as transrepression). The functions of NRs can also be modulated by posttranslational modifications that include phosphorylation, ubiquitylation, and SUMOylation, among others [4].

The pivotal role of several NRs in cancer development and progression has long been acknowledged [6]. The biological actions of several NRs in the control of proliferation, differentiation, and apoptosis are indicative of the potential influence for NRs and their ligands on tumour progression (Fig. 2). Indeed, several drugs targeting NRs have been the focus of extensive drug discovery efforts for efficacious cancer interventions (Table 1). In this review, we present a summary of the roles of a selected subset of druggable NR family members with established importance in the regulation of tumour growth. This review does not include VDR, which has been extensively covered in a separate review from this series [7].

2. Androgen receptor as a therapeutic target in prostate cancer

Androgens are steroid hormones required for the development of the male reproductive system and secondary sexual characteristics. AR (NR3C4) is the main transcription factor that mediates the biological effects of androgens. In addition to the male reproductive system, AR is expressed in several other tissues such as bone, muscle, adipose tissue, brain and hematopoietic cells. The endogenous ligands that bind AR are 5α-dihydrotestosterone (DHT) and testosterone [8]. In prostate cells, testosterone may act directly on AR or be irreversibly converted to DHT by 5α-reductase.

Several pathological situations associated with AR and androgens have been described, including androgen insensitivity syndrome and prostate cancer, among others [8]. In this section, we will focus on the roles of AR in prostate cancer.

The androgen signalling axis plays a pivotal role in the pathogenesis of prostate cancer. As AR regulates multiple cellular events, including proliferation, apoptosis, migration, invasion, and differentiation, this receptor has been associated to all stages of this type of cancer [8]. In this regard, AR is both expressed in primary prostate cancer and in tumour metastasis, and DHT promotes the growth and survival of prostate cells. The heterogeneous nature of prostate cancer suggests that the predisposition to this disease may involve multiple genes and variable phenotypic expression of AR-regulated genes. Expression profiling identified indeed over 200 androgen-responsive genes involved in prostate cell proliferation, communication, differentiation, and cancer progression [9]. Of note, chromosomal rearrangements fusing the androgen-regulated gene coding for transmembrane protease serine 2 (TMPRSS2) to the ETS transcription factor ERG occur in approximately 50 % of prostate cancers. The up-regulation of the TMPRSS2:ERG fusion by AR plays an early role in prostate cancer development and progression [10,11]. In addition, several growth-promoting and survival pathways interact with AR signalling during the development of prostate cancer. These include the PI3K/AKT/mTOR pathway and DNA repair pathways [12].

The gonadal depletion of androgens has been widely used to treat the disease; however, the progression towards more aggressive forms of the disease due to the restoration of AR signalling or intratumoral steroidogenesis sparked the development of AR protein-targeted therapies to inhibit its hormone binding. In concrete, a battery of drugs that block androgen production to inhibit the AR axis or inhibitors that directly

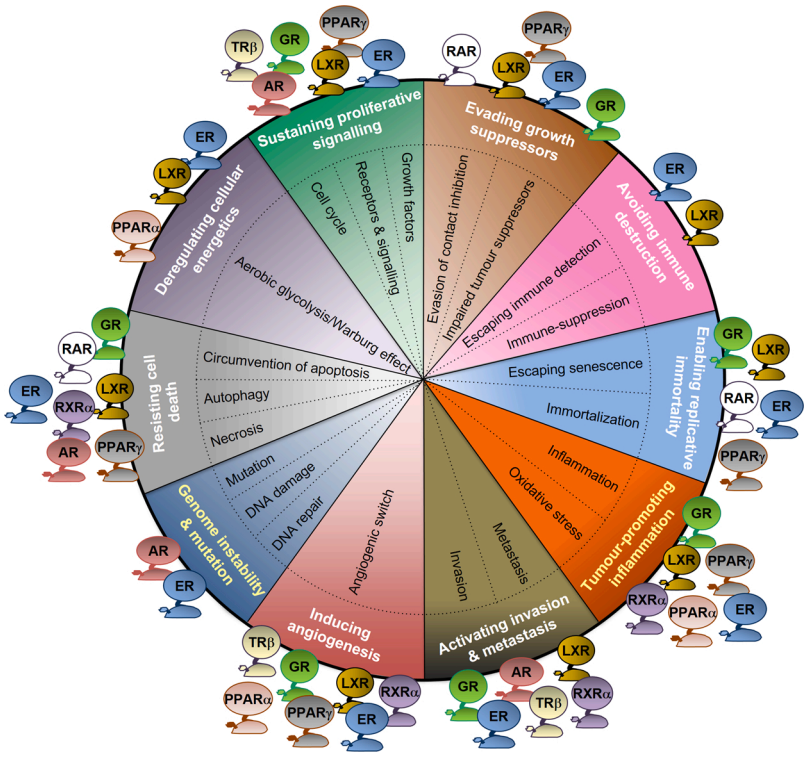

Fig. 2. Ligands that modulate NR function have the ability to impact several hallmarks and enabling characteristics of cancer. In an attempt to rationalize the complexity of biological processes leading to cancer, Hanahan and Weinberg [259] proposed a framework depicting several biological capabilities (hallmarks) acquired during tumour development (white titles): sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism and evading immune destruction. Underlying these hallmarks are two enabling characteristics (yellow titles): genome instability and tumour-promoting inflammation, which are crucial to the acquisition of the hallmark capabilities. Ligands for several NRs exert anti-tumoral effects by antagonizing the acquisition of hallmarks or the presence of enabling characteristics; the implicated NRs are indicated in each section. Image adapted from https://chat.lionproject.net/hallmarks.

compete for DHT binding result in the inhibition of AR activity and have proven to be effective to delay prostate cancer growth and progression [12].

Several anti-androgens have been developed that differ in their chemical structure and exhibit different efficacy and safety profiles. For instance, flutamide, nilutamide and bicalutamide were primarily developed to be used in combination with chemical or surgical castration to provide combined androgen blockade. Later on, clinical trials in patients with metastatic, castration resistant prostate cancer showed significant survival benefits by the usage of enzalutamide. Improved antiandrogens such as apalutamide and darolutamide have proven to be efficacious as well to overcome resistance caused by other anti-androgens (i.e. bicalutamide) (Table 1) [12].

However, targeting the AR ligand-binding-pocket (LBP) remains to be prone for the drug-acquired appearance of mutations that render these compounds ineffective within 2–5 years. Thus, the effect of this type of LBP-centred treatment remains transient, as, almost universally, patients relapse after developing a castration-resistant form of the disease (metastatic castration-resistant prostate cancer, mCRPC) that is usually due to increased levels of AR expression or point-mutations [13,14]. Most of these molecular mechanisms of resistance cause the AR to recognize anti-androgens as if they were agonists, which is associated with cancer progression to lethal stages of the disease. Several mutations inside the LBP (e.g. T878A, W742 L, F877 L) have been found in patients that contribute to the acquisition of agonistic properties of anti-androgens [13,14]. Moreover, the interplay between AR and the PI3K-AKT-mTOR pathway contributes to enhance drug-resistance, raising the interest for testing the efficacy of PI3K inhibitors to overcome mCRPC [15].

The synthesis of truncated AR splice variants devoid of the receptor LBD has emerged as an additional mechanism in mCRPC [16]. The discovery of AR variants without the canonical druggable hormone-binding domain has both posed a challenge but most importantly contributed in exploring non-LBD centred pharmacological strategies. However, the exact role of these variants in cancer is still not fully understood. It is not clear whether the loss of different C-terminal parts of the AR protein, including the bipartite nuclear localization signal in the hinge region, deeply affect their nuclear localization and ligand-independent functions or whether the full length AR is still the driving transcription factor in prostate cancer. Indeed, studies still point to the fact that AR truncated variants require the full length AR as partner of heterodimerization to exert their functions in cancer. The molecular profiling of AR variants, mainly AR-V7, in liquid biopsies seems to be an emerging field to monitor the treatment response [17].

A new class of compounds has emerged over the last years with the capacity to induce AR protein degradation (AR degraders). In particular, ARV-110, which has been developed using the Proteolysis-Targeting Chimera (PROTAC) technology [18], is a selective AR degrader capable of potently reducing AR signaling. Using patient-derived xenograft models, ARV-110 inhibited the growth of tumors that are resistant to enzalutamide [19]. In addition, data from an ongoing clinical trial shows some efficacy of ARV-110 in patients with mCRPC (Table 1) [20].

A potent steroidal multi-target agent named galeterone has been developed as well, which exhibits three different activities, it inhibits cytochrome P450 family member 17A1 (CYP17A1), it antagonizes AR, and it acts as an AR degrader [21]. The compound is currently under investigation and it has been proposed to potentially control full length AR as well as truncated variants such as AR-V7 [22]. Recent studies have shown its anti-tumor activity in CRPC patients (Table 1) [23]. It is important to point out that galeterone may be also effective in patients with the point mutation T878A in the AR LBD [24].

Recently, GR has also received significant traction as the NR driving prostate cancer resistance. Indeed, enhanced GR expression was identified as a common feature in the development of resistance to anti-androgens [25]. As GR and AR can recognize identical regulatory sequences, the functional substitution of GR over AR in prostate cancer cells resulted in the regulation of a subset of AR targets, which was sufficient to preserve the resistant phenotype [25,26]. Treatment with the GR ligand dexamethasone was sufficient to induce enzaludamide-resistance whereas GR blockade restored sensitivity. Also, AR blockade led to high levels of GR in a subset of prostate cancer cells due to the lack of AR-mediated feedback repression of GR. These

Table 1
Several drugs targeting NRs are used to treat different types of cancer. Examples of compounds that target NR activity/stability and are either currently used in the clinics or being tested in clinical trials for the therapeutic treatment of cancer. CLL, chronic lymphocytic leukemia; HER2, erb-b2 receptor tyrosine kinase 2; HL, Hodgkin's lymphoma; HR^+, hormone-receptor-positive; MM, multiple myeloma; MR, mineralocorticoid receptor; NHL, non-Hodgkin's lymphoma.

Ligand	NR	Action	To treat
Enzalutamide	AR	Antagonist	Metastatic and non-metastatic CRPC
Darolutamide	AR	Antagonist	Non-metastatic CRPC
Apalutamide	AR	Antagonist	Non-metastatic CRPC
Abiraterone	AR	Antagonist	mCRPC
Galeterone	AR	Antagonist	Clinical trial: mCRPC
ARV-110	AR	AR degrader	Clinical trial: mCRPC
Tamoxifen	ER	SERM	Early- and advanced-stage HR^+ breast cancer, neoadjuvant therapy; to lower the risk of breast cancer in women at high risk
Toremifene	ER	SERM	Postmenopausal women with metastatic, HR^+ breast cancer
Raloxifene	ER	SERM	To lower the risk of breast cancer in high-risk postmenopausal women
Fulvestrant	ER	SERD	Postmenopausal women, advanced-stage breast cancer or after tamoxifen failure
Dexamethasone	GR	Agonist	Lymphoid cancers (ALL, CLL, MM, HL and NHL); palliative use in several cancers (breast, lung, bladder)
Prednisolone	GR/ MR	Agonist	Lymphoid cancers (ALL, CLL, MM, HL, and NHL; palliative use in several cancers
Methylprednisolone	GR	Agonist	Coadjuvant for hematopoietic and non-hematopoietic cancers
Hydrocortisone	GR	Agonist	Palliative use in several cancers
Recalorilant/ CORT125134	GR	SEGRAM	Cinical trials: solid tumors
ORIC-101	GR	SEGRAM	Clinical trials: solid tumors/ prostate cancer
ATRA	RAR	Agonist	APL; Clinical trials: NSCLC, HR^+/ $HER2^-$ early breast carcinoma, metastatic kidney cancer, melanoma, neuroblastoma, advanced adenoid cystic carcinoma
Tamibarotene	RAR	Agonist	Recurrent (ATRA-resistant) APL; Clinical trial: AML
NRX195183	RAR	Agonist	Clinical trial: relapsed or refractory APL
Fenretinide	RAR	Agonist	Clinical trial: adult giant cell glioblastoma
Bexarotene/ Targretin	RXR	Agonist	CTCL, NSCLC; Clinical trial: thyroid cancer, lymphoma, MM
Alitretinoin/panretin	RXR	Agonist	Cutaneous lesions of AIDS-related Kaposi's sarcoma
Peretinoin	RXR	Agonist	HCC
9cUAB30	RXR	Agonist	Clinical trials: breast cancer, non-melanoma skin cancer
IRX-4204	RXR	Agonist	Clinical trials: prostate cancer, NSCLC
TPST-1120	PPARα	Antagonist	Clinical trials: hepatocellular carcinoma, renal cell carcinoma, mCRPC
Troglitazone	PPARγ	Agonist	Clinical trial: liposarcoma
Pioglitazone	PPARγ	Agonist	Clinical trials: Head and neck cancer, bladder cancer, NSCLC, chronic myeloid leukemia
Rosiglitazone	PPARγ	Agonist	Clinical trials: pituitary tumors, liposarcoma, oral pre-malignant lesions
Efatutazonne	PPARγ	Agonist	Clinical trial: anaplastic thyroid cancer, liposarcoma
CS-7017	PPARγ	Agonist	Clinical trials: NSCLC, lymphoma, MM
RGX-104	LXR	Agonist	Clinical trials: lymphoma, advanced solid malignancies

results support the use of combined AR/GR targeting therapies for the treatment of resistant prostate cancer [17].

For these reasons, the mechanisms implicated in the development of resistance to AR inhibition in prostate cancer are multiple and complex (Fig. 3), and still poses major challenges to develop drugs with fewer side effects. A major problem is also the fact that there is no agreement as to whether prostate cancer is just one complex disease or whether there are different molecular subtypes. Multiple genomic alterations that result in distinct gene patterns and clinical implications have been also recently described [27], which will impact the design of future therapeutic drugs.

3. Estrogen receptor: from biomarker to therapeutic marker in breast cancer

Estrogens are steroid hormones that are important in the estrous cycle of humans and other animals, being the primary female sex hormones. The biological effects of estrogens are primarily mediated through the binding and activation of ERα (NR3A1) and ERβ (NR3A2). The expression and activity of ERs are important for normal development and function in various tissues, including endometrium, ovary and breast, and are also implicated in tumorigenesis [28]. In this section, we will focus on the roles of ERα, the major ER in breast cancer, although some activities of ERβ have also been reported [29,30].

The classical definitions of breast cancer subtypes based on histopathological analysis, included both ER and progesterone receptor (PR) expression, which are prognostic factors. This has been further refined by the identification of the molecular subtypes of breast tumours based on their molecular profiles and their consequent association with clinical outcomes [31,32], providing new opportunities for tumour classification and prognostic tools. Exposure to ovarian hormones, including estrogen, has been associated with increased risk of developing breast cancer [33]. Approximately 75 % of primary breast cancers express ER and these tumours show good overall patient survival. Patients with ER-positive tumours are treated with endocrine therapy, commonly in the form of tamoxifen (an ER-antagonist) or aromatase inhibitors (which lead to estrogen deprivation). Tamoxifen belongs to a group of ligands called selective ER modulators (SERMs) (Table 1), which have been developed to achieve improved safety profiles because they differentially modulate the activity of ER in a context specific manner. Tamoxifen competes with estrogen for ER binding and prevents LBD-mediated coregulator recruitment. In addition, tamoxifen can work as a partial agonist in other tissues, such as endometrium, since it promotes the activation of the AF1 domain and thus it poses an increased risk of endometrial cancer [34]. Another strategy is to use drugs that degrade ER, selective ER degraders or downregulators (SERDs), designed to overcome the agonistic effects of SERMs. Among SERDs, fulvestrant is an effective treatment for advanced breast cancer in postmenopausal women [35] (Table 1) and has enormous potential in combination therapy with other inhibitors [36]. Aromatase inhibitors were developed to stop estrogen production by inactivating aromatase (the enzyme responsible for estrogen synthesis) and they do not present partial agonist activity [37]. Three aromatase inhibitors are mostly used in the clinic, anastrozole, letrozole and exemestane. However, as it is the case for other types of therapy (including radiation and chemotherapy), therapy failure inevitably occurs in many cases [38]. Indeed, the development of resistance to different types of aromatase inhibitors has been well documented [39]. Futhermore, a recent meta-analysis showed

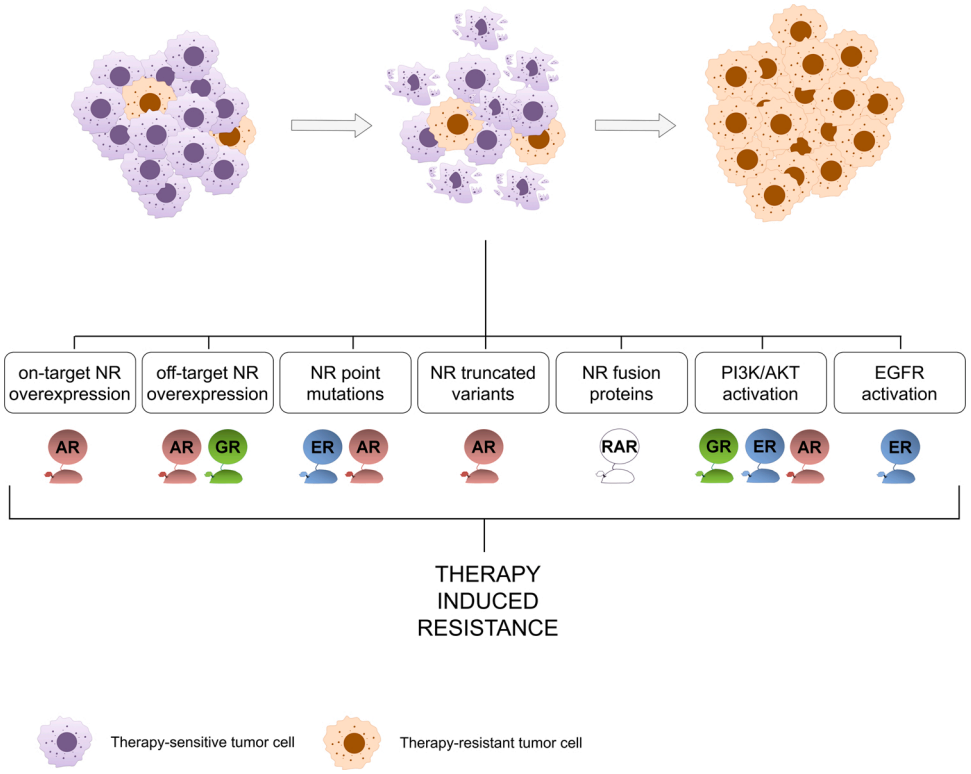

Fig. 3. Mechanisms underlying therapeutic-induced resistance involving NRs. Tumour regression by therapies targeting NR function is bypassed by different adaptation events. More than one molecular mechanisms can be found in different NR-driven tumours, meaning structural changes in the on-target NR, participation of a second off-target NR or the activation of parallel signalling pathways such as PI3K/AKT and EGFR.

that even after 20 years of tamoxifen usage, the risk of recurrence continues [40].

Given the high incidence rates of the disease, development of resistance to endocrine therapy and progression to metastasis remains a critical clinical problem, since the major cause of death in breast cancer is metastasis to distant organs. The etiology of endocrine therapy resistance is complex and diverse molecular mechanisms have been revealed over the years to be implicated in this process. Analysis of the genomic landscape of metastatic breast cancers has identified nine driver genes that were the most frequently mutated in metastasis from ER-positive tumours, including those encoding for AKT1 and estrogen receptor 1 (ESR1) [41]. Targeting components of the PI3K-AKT-mTOR pathway has been widely tested to treat endocrine refractory disease [42]. Dysregulation of the epidermal growth factor receptor (EGFR) family and their ligands has been reported [43], although results from clinical trials testing EGFR inhibitors in endocrine treatment-resistant breast cancer have been modest [44,45]. Variations of cell cycle components are also common in ER-positive breast cancer, including amplification of cyclin D1, gene copy gain of CDK4 and loss of negative regulators such as p16 and p18. Development of CDK4/6 inhibitors are now approved by the Food and Drug Administration (FDA) for use in combination with endocrine therapy to treat metastatic breast cancer, although acquired resistance quickly develops [46]. It is now well established that alterations in *ESR1* itself, such as amplifications or chromosomal aberrations, have been detected in a subset of primary tumours and have been shown to be further enriched in recurrent and metastatic disease [41,47,48]. In addition, point mutations have been identified in endocrine resistant metastatic breast tumours, although those are particularly enriched in tumours pre-treated with aromatase inhibitors [49,50].

Breast cancers, like most tumours, are hypoxic and high levels of hypoxia-inducible transcription factors (HIF) correlate with poor prognosis in breast cancer patients. Hypoxia induces ER phosphorylation in the absence of estrogen [51] and alterations in ER phosphorylation can have a profound impact on ER function in breast cancer [52]. In addition, hypoxia reduces ER expression and transcriptional activity in breast cancer cells [53] and, conversely, adjuvant tamoxifen treatment has been shown to increase HIF1α-positivity, which is related to tamoxifen resistance and poor prognosis [54]. These findings support the notion that the loss of ER activity is associated with worse prognosis. Indeed, although ER expression is often maintained during development of resistance to endocrine therapy, its activity is frequently lost, as indicated by reduced levels of PR [55], a marker of functional ER. In addition, the loss of PR may be due to increased growth factor signalling activity, as a mechanism of resistance, leading to hyperactive crosstalk with ER signalling [56]. The analysis of ER signalling in normal breast epithelial cells and primary tumours has shown that tumour-specific ER signalling is gained during breast tumorigenesis [57], suggesting that further understanding of ER role in normal and cancer cells is still warranted.

In addition to estrogens, ER can be activated by the action of the oxysterol 27-hydroxycholesterol (HC) [58,59]. This compound is a primary metabolite of cholesterol and is considered an endogenously occurring SERM. Depending on the cellular context, 27-HC can act as an ER antagonist or as a partial ER agonist, which is the case in ER + breast cancer cells, where it promotes proliferation. Indeed, evidence from murine models and breast cancer cells suggests that the promoting effects of cholesterol on breast cancer progression may be mediated by its conversion to 27-HC (reviewed in [60]).

Cancer heterogeneity is now well appreciated, both among patients and within each tumour at molecular, phenotypic and functional levels, complicating diagnosis and presenting challenges for cancer therapy. Molecular and cellular complexity allows the tumours to evolve and evade currently used therapeutics. Accumulated evidence supports the presence of a small fraction of cells with characteristics of stem cells, cancer stem cells (CSCs), which have been implicated in tumour

initiation and resistance to current forms of therapy. The identification of stem cells in the normal breast [61] and in cancer [62,63] has originated a new vision of the disease and new hopes for its prevention, prognosis and treatment. Estrogen reduces the pool of stem cells in healthy breast epithelial cells [64], which could provide an explanation for the better prognosis of ER-positive breast tumours, since it has been found that poorly differentiated breast cancers contain more CSCs than well-differentiated tumours with good prognosis [65]. Interestingly, breast stem cells have low or absent ER expression [55,66], which allows breast CSCs to ignore the anti-proliferative effects of tamoxifen. In contrast, treatment with tamoxifen leads to increased CSC content and, as a consequence, tamoxifen resistant tumours are enriched in CSCs, with enhanced expression of the embryonic stem cell factor SRY-box transcription factor (SOX)2 [55,67]. Furthermore, a regulatory axis has been identified between SOX2 and SOX9, which maintains human breast luminal progenitor and breast CSCs, suggesting common signalling pathways in normal and cancer stem cells [68]. Importantly, the expression of Sox2 and Sox9 stem cell markers is down-regulated by estrogen and induced by tamoxifen, supporting the notion that ER-positive tumours are more differentiated than resistant tumours or those that lack ER. Furthermore, enhanced CSC mitochondrial metabolism has been linked to resistance and inhibitors that target the large mitochondrial ribosome are able to prevent tamoxifen resistance and cancer metastasis [69]. An important clinical implication of the existence of CSCs within the tumour is that they become novel therapeutic targets and, therefore, combinatorial treatment strategies should be considered to address tumour heterogeneity.

To summarize, resistance to hormone therapy presents multiple mechanisms, including activation of the PI3K/AKT or EGFR signalling pathways, ER phosphorylation and mutations, epigenetics, cell cycle deregulation and CSCs and tumour heterogeneity (Fig. 3). Moreover, the implication of other steroid hormone receptors, beyond ER and PR, in breast cancer has also been studied, including RAR, VDR, AR and GR, among others [70]. For example, AR is expressed in the majority of ER-positive breast tumours, however, increased AR levels reduce the response to endocrine therapy, which has paved the way for a number of clinical trials using AR antagonists to treat advanced breast cancer [71].

In conclusion, in addition to ER, several NRs present opportunities to introduce combinatorial treatments to target this complex disease and reduce the development of tumour recurrence and metastasis.

4. Glucocorticoid receptor: a dual role as tumour promoter or suppressor

Glucocorticoids (GCs) are steroid hormones that are primarily produced in the adrenal gland in response to stress and are necessary to regulate numerous physiological processes. The biological actions of GCs are mediated by GR (NR3C1), which is ubiquitously expressed. GCs are very effective anti-proliferative and anti-inflammatory agents currently used in the clinics. In general, GR-mediated transactivation contributes to the efficacy of GCs by inducing the transcription of anti-inflammatory mediators such as dual specificity phosphatase (DUSP)1, NFκB inhibitor alpha (NFKBIA), and GC-induced leucine zipper (GILZ) and of anti-proliferative mediators such as p21 (CDKN1A), while GR-mediated transrepression antagonizes the activation of proliferative and pro-inflammatory transcription factors such as AP-1 and NFκB [72–75].

GCs are the standard therapy for treating malignancies of the lymphoid lineage including leukemia, lymphomas, and multiple myeloma due to their effects promoting apoptosis and arresting cell growth in cells of the immune system [76,77] (Table 1). Although GCs alone are not curative, the initial response to GCs in children with acute lymphoblastic leukemia (ALL) is the best predictor of the overall outcome to full treatment. GCs (dexamethasone or prednisone) are routinely administered in combination with other agents (such as vincristine, mercaptopurine, asparaginase, and methotrexate), leading to complete remission in approximately 90 % of children with both B and T-cell ALL [76]. In this context, GR induces apoptosis in a cell-autonomous manner by modulating the balance between pro- and anti-apoptotic mediators through the transcriptional regulation of apoptosis-related genes (B-cell lymphoma 2 (Bcl2) family) and the inhibition of survival factors (Myc). Unfortunately, some patients with ALL are resistant to GCs due to several factors that prevent GC-induced cell death including the imbalanced expression of Bcl2 family members. GCs are also successfully used in combination with other drugs (thalidomide, cyclophosphamide, and proteasome inhibitors) for treating multiple myeloma [76].

In non-hematopoietic cancers, the overall efficacy of GCs is controversial and at most, their use (as monotherapy or adjuvants) only provides partial benefits in certain hormone-driven cancers [78]. Recent findings indicate that in breast and prostate cancers, whether GCs promote or inhibit tumour progression depends on the functional crosstalk between GR and other NRs of the steroid subfamily, namely ER or AR [78]. Indeed, high levels of GR correlated with poor prognosis in ER negative breast cancer and ovarian cancer, and also contributed to the progression of CRPC or the resistance to the AR antagonist enzalutamide. The fact that GR and AR are structural and functionally similar, in particular in their DBD, theoretically allows their binding to identical DNA sequences [79]. Indeed, GR binds to more than 50 % of AR binding sites in enzalutamide-resistant cells. Therefore, GR activation may represent an escape mechanism for tumour cells in an attempt to compensate for the loss of ER or AR (Fig. 3). Consistent with this, the use of GR antagonists restored the sensitivity of mCRPC to AR blockade supporting the usefulness of combination therapies [26].

GCs are not prescribed for the treatment of other epithelial cancers such as non-melanoma skin cancer. However, experimental data from mouse models demonstrated that GR acted as tumour suppressor in chemically induced skin tumours [80–82]. Mice with gain- and loss-of-function of GR showed reduced and increased susceptibility, respectively, to the onset, development, and malignization of skin tumours. In these epithelial tumours, GR decreased epidermal cell proliferation and inflammation by antagonizing PI3K/AKT/NFκB activities [80,81].

Finally, GCs are commonly used in non-hematopoietic cancers as adjuvant in chemo- or radio-therapy due to their ability to ameliorate several associated side effects. However, the long-term use of GCs is restricted by the GC accompanying adverse effects including metabolic effects, muscle wasting and osteoporosis [83]. This highlights the need of developing strategies that improve the beneficial/risk ratio of GCs.

The precise molecular mechanisms underlying the multiple actions of GR in the context of cancer remain unclear [77]. In epithelial cancers, GR may affect cell survival by inhibiting p53, activating p38 and AKT signalling, or stimulating the secretion of pro-inflammatory cytokines such as interleukin (IL)-6 [84–86]. In breast cancer, GC anti-apoptotic effects are due to the induction of DUSP1 and NFKBIA, as they inhibit MAPK and NFκB pathways, respectively [87–90]. GILZ is a major mediator of GC anti-inflammatory effects in many cell types by inhibiting Ras, AP-1 and NFκB signalling. These actions should contribute to alleviate inflammation-related tumour growth, metastasis, and conversion. However, as GILZ also mediates immunosuppression, it could enhance tumour development. Therefore, GILZ can either promote or inhibit tumour growth depending on the context [91]. It seems thus necessary to identify the cell-type specific genes regulated by GR in each tissue as well as the specific coregulators that would allow selectively enhancing beneficial GR functions.

GR loss occurs in many cancers, and may cause malignant transformation [92,93]. However, the role of GC signalling in tumour progression and metastasis is greatly unknown. Overall, GCs seem to inhibit cell migration, invasion, and angiogenesis as well as down-regulate pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and IL-8. GCs are also common palliatives in metastatic cancers (lung, bladder) used to prevent hypersensitivity reaction and skin rashes

[76]. However, stress- and aging-related cancers have been associated with increased GC levels concomitant with the dysregulation of the hypothalamus-pituitary-adrenal axis. The seemingly controversial actions of GCs are particularly relevant in the immune system, which becomes dysregulated at some point in all cancers. In this regard, GCs constitute a double sword-edge as their intended immunosuppressive actions can simultaneously reduce the capacity of the immune system to mount a response against cancer.

In addition, several oxysterols have been reported to behave as GR ligands, thus modifying the action of classical GC agonists on GR. For example, 6-oxo-cholestan-3β,5α-diol (OCDO) can bind to GR, inducing its nuclear translocation, and regulating gene expression [94]. However, the resulting transcriptional outcome is different from that elicited by cortisol or dexamethasone. Given that OCDO levels are increased in breast cancer samples relative to healthy tissue, and that elevated GR expression/function correlates with poor therapeutic response or prognosis in these tumours [94,95], novel therapeutic strategies could be aimed to targeting this metabolite in breast cancer or other epithelial cancers.

5. Anti-tumorigenic and anti-metastatic actions of the thyroid hormone receptor β

Thyroid hormones (thyroxine (T4) and triiodothyronine (T3)) and thyroid hormone receptors (TRs) are crucial regulators of growth, development and metabolism, affecting virtually all cells in the organism [96]. Two different genes encode TRα (NR1A1) and TRβ (NR1A2) proteins, respectively, which are widely expressed. In general, TRs can bind response elements on their target genes either as monomers or, preferentially, as heterodimers with RXR [1].

Besides their classical actions, thyroid hormones also play important roles in cell proliferation and cancer [97,98]. Since these hormones are highly pleiotropic, the different TR isotypes might have opposing effects depending on the cell type, the cellular context or the transformation status [96]. In addition, it has been proposed that thyroid hormones exert non-genomic actions that are initiated at the cell membrane, which could be mediated by a fraction of membrane-associated NRs, or by the occupancy of putative membrane receptors, such as integrin αVβ3 [99], adding to the complexity of the effects of thyroid hormones in normal and neoplastic cells.

The first evidence connecting TRs with cancer was the discovery that TRα is the cellular counterpart of the v-erbA oncogene of the erythroblastosis virus, which causes avian erythroleukemia and sarcomas. This oncogene has a constitutive dominant-negative activity that inhibits the actions of endogenous TRs. Moreover, mice transgenic for v-erbA develop hepatocellular carcinomas (HCC) [100], reinforcing the idea that TRs could have tumour suppressor activity and that the loss of their function could result in a selective advantage for cellular transformation and tumour growth. In addition, reduced expression, anomalous cellular localization and/or alterations of the TRβ gene occur in many types of human tumours [98]. These alterations include point mutations, deletions, loss of heterozygosity or biallelic inactivation by promoter methylation, and most of the TRβ mutants found in tumours also have a dominant-negative activity, acting as TR inhibitors. The fact that TRβ could function as a tumour suppressor was also shown in mouse models of metastatic follicular thyroid carcinoma [101], mammary tumours [102] and chemical skin carcinogenesis [103].

Interestingly, TR deficiency inhibits benign tumour formation at early stages of skin carcinogenesis, whereas it increases malignization at later stages, indicating that TRs could mediate divergent effects on cell proliferation and malignant transformation. These diverging effects are very clear in the case of the liver. Thyroid hormone administration to mice causes liver hyperplasia [104] and TRβ, the main liver TR, mediates the effect of the hormone in promoting hepatocyte proliferation in response to hepatectomy [105]. However, thyroid hormones induce a rapid regression of carcinogen-induced hepatic nodules in rodents, reducing the incidence of HCC and lung metastasis [106]. These findings are in agreement with the fact that hypothyroidism is considered a risk factor for the development of hepatocarcinoma in humans [107] and that the down-regulation of TRβ is an early event in human and rat hepatocarcinoma development [108].

Contrary to the well-accepted role of TRβ as a tumour suppressor, TRα can have oncogenic effects by cooperating with the WNT pathway in the induction of intestinal tumorigenesis in mice [109]. This contrasts with the selective loss of TRβ in human colon carcinoma [110] and with its expression being associated with a benign phenotype [111]. Both receptors may also have an opposite role in breast cancer, where TRα expression is high and TRβ expression is lost [112,113]. In fact, hypothyroid patients have been reported to present both a higher and a reduced incidence of breast carcinomas [114]. These confounding effects could be secondary not only to thyroid hormone binding to different receptor isotypes, but also to the profound metabolic changes associated with hypothyroidism. In this sense, tumour growth is retarded in hypothyroid immunodeficient mice inoculated with both parental and TRβ-expressing mammary tumour cells, but hypothyroidism also increases the number of invasion fronts of the tumours, the infiltration to neighbouring tissues and metastatic growth [115]. Such divergent effects may contribute to explain the contradictory reports on the influence of hypothyroidism in human breast cancer.

The re-expression of TRβ in human liver and breast cancer cell lines that have lost receptor expression results in the suppression of proliferation, migration and invasion in cultured cells, and in tumour growth retardation, partial mesenchymal to epithelial cell transition and suppression of metastasis in nude mice [103]. Several mechanisms involved in the anti-tumorigenic and anti-metastatic effects of TRβ have been identified. Among them, thyroid hormones can induce cellular senescence in the liver, a mechanism that is believed to act as a first barrier against cellular transformation and tumour development [116]. This action is mediated by binding to TRβ, and not to TRα [117], and may be an important component of the initial tumour suppressor activity of the receptor. In addition, TRβ induces the transcription of microRNAs (miRNAs) miR-424 and miR-503, which belong to the miR-16 family, known for their capacity to down-regulate cell proliferation, migration and invasion. Induction of these miRNAs mediates some of the anti-proliferative and anti-metastatic actions of T3 in cancer cells [118]. Furthermore, TRs can antagonize the actions of the transforming growth factor beta (TGFβ) pathway, thus inhibiting tumour cell proliferation and migration, and alleviating fibrosis [119,120]. TRβ also blocks the mitogenic action of other growth factors suppressing the activation of MAPK and PI3K signalling pathways, which are critical for cell proliferation and invasion [103]. Finally, tumour lymphangiogenesis, which is a main event in the metastatic spread of breast cancer tumours, and sentinel lymph node invasion is also inhibited by TRβ [121].

Genes that are relevant for metastatic progression have been identified [122]. Strikingly, TRβ coordinately down-regulates the expression of many pro-metastatic genes both in cultured cells and in tumours [103], and represses the expression of genes encoding for VEGF-C and VEGF-D [121], which are crucial regulators of lymphangiogenesis. NCoR, which is inactivated in various solid human tumours [123], plays a key role in the anti-tumorigenic and anti-metastatic actions of TRβ. In the absence of NCoR, tumour xenografts were bigger, metastatic growth was enhanced and the inhibitory effect of TRβ in tumour growth, metastasis and lymphangiogenesis was significantly attenuated [120]. The relevance of these results is supported by the fact that NCoR and TRβ transcripts are significantly reduced in human hepatocarcinoma tumours when compared with normal tissue, with a strong correlation between the levels of both transcripts. These genes are also down-regulated in the more aggressive ER-negative human breast tumours in comparison to ER-positive tumours with better prognosis [120]. In addition, NCoR and TRβ transcripts correlate negatively with those derived from lymphangiogenic genes [121], suggesting that NCoR is important for the silencing of pro-metastatic and lymphangiogenic

genes by TRβ.

A subpopulation of CSCs with capacity for self-renewal, which can grow in culture as mammospheres, is believed to drive initiation, progression, and relapse of breast tumours [124]. The tumour suppressor actions of TRβ may be also related to the regulation of the CSC population. Indeed, the treatment of TRβ-expressing breast cancer cells with thyroid hormone decreases significantly the self-renewal capacity of CSCs, the efficiency of mammosphere formation, the expression of pluripotency factors within the mammospheres, and the tumour initiating capacity in immunodeficient mice, indicating that TRβ limits the breast CSC population [125].

6. RAR: a paradigm of cancer cell differentiation therapeutic strategy

RARs regulate the expression of a vast array of genes that control cell proliferation, differentiation and survival as well as full body homeostasis [126]. RARs consist of three subtypes, RARα (NR1B1), RARβ (NR1B2) and RARγ (NR1B3), encoded by separate genes. Abundant levels of RARα are detected in most tissues, whereas the other two isotypes are more abundant in specific tissues, e.g. RARβ in retina and RARγ in esophagus and skin. RARs are activated by retinoids, endogenously-derived from vitamin A metabolites, such as all-trans retinoid acid (ATRA) [127].

RARs inhibit cell cycle progression by the direct transcriptional activation of the p21 cell-cycle inhibitor, which correlates with cell differentiation [128]. In addition, RAR activation may also cause cell death by inducing the tumour necrosis factor (TNF)-related apoptosis-inducing ligand pathway [129]. In adulthood, RARs are key players in the regulation of cell renewal, with essential roles in tissue stem cells [126].

Alterations in the functionality of RARs, caused by dysregulated gene expression, mutations, fusions to other proteins, or anomalous post-translational modifications, are associated to neoplasia and malignant cell transformation [126]. The most representative example is found in acute promyelocytic leukemia (APL), a subtype of acute myeloid leukemia (AML), in which oncogenic RARα fusion proteins are generated by diverse chromosomal translocation events. The chromosomal translocation (t15;17)(q22:q11), which renders the promyelocytic leukemia (PML)-RARα fusion protein, is found in 95 % of all APL cases. Despite the fact that PML-RAR retains the LBD of RAR, this fusion protein is refractory to the physiological levels of ATRA. Under these conditions, PML-RAR binds more strongly to HDAC-corepressor complexes than the RAR-RXR heterodimer, thus leading to a constitutively silenced gene expression program. However, the administration of pharmacological doses of ATRA (in combination with arsenic trioxide) is very effective as cancer cell differentiation therapy because it favours the dissociation of PML-RAR from HDAC-corepressor complexes, thus reverting the differentiation blockade at the promyelocytic stage [130] (Table 1). Nonetheless, some leukemic stem cells and other less common RAR fusion proteins are unresponsive to ATRA but, upon treatment with HDAC inhibitors, the sensitivity to ATRA is restored [131].

In particular for the RARβ2 isoform, compelling evidence indicates that it has tumour suppressive activity. In this regard, the loss of RARβ2 expression due to chromosome rearrangements or epigenetic mechanisms has been documented in a variety of cancer types, including hepatocarcinoma, breast, lung, prostate, and head and neck cancers, and it positively correlates with tumour grade [132]. Moreover, the expression of the RARβ4 isoform is increased in some types of cancer and inversely correlates with RARβ2 abundance. Notably, RARβ4 almost lacks the N-terminal domain and seems to function as a dominant-negative of RARβ2 because of its ability to heterodimerize with RXR. The recovery of RARβ2 expression in patients treated with RAR agonists is associated to a positive clinical response. In the same line, beneficial effects of retinoid administration in APL, head/neck and skin cancers correlated with the induction of RARβ expression [133].

Retinoids also inhibit UV-induced skin cancer development in patients of xeroderma pigmentosum. In this regard, in experimental models for chemically-induced skin carcinogenesis, retinoid treatment showed effectiveness in inhibiting the appearance of squamous papilloma, in correlation with the ability of RAR to block AP-1 activity [134].

Despite the beneficial effects of retinoid chemotherapy in some types of cancer, the use of ATRA is limited due to its teratogenic effects and remains controversial in solid tumours because of mechanisms conferring retinoic acid resistance and toxicity syndrome [135] (Fig. 3), which implies that further studies are required to test the efficacy of isoform-selective RAR ligands and/or alternative combinatorial therapies overcoming such resistance.

7. Anti-carcinogenic activities of RXRα

RXRs play critical roles in a plethora of physiological processes, including embryo development, cell differentiation, metabolism and organ homeostasis. The receptor has three isotypes, RXRα (NR2B1), RXRβ (NR2B2), and RXRγ (NR2B3). RXRα shows abundant expression in the liver, kidney, epidermis and spleen; RXRβ is ubiquitously expressed; and the most restricted of the three receptors is RXRγ, which is abundantly expressed in muscle and brain. RXR has a unique cooperative function because of its ability to form homodimers as well as heterodimers with several other NRs, such as RARs, TRs, PPARs and LXRs. RXR binds the vitamin A derivatives 9-cis-retinoic acid and 9-cis-13,14-dihydroretinoic acid, some fatty acids, such as docosahexanoic acid and phytanic acid, and selective RXR ligands known as rexinoids [127].

Ligands that activate RXRα display potent anti-carcinogenic activities by inhibiting cancer cell growth and promoting apoptosis [136] and by repressing inflammatory pathways critical for carcinogenesis [137,138]. Alterations in the expression and function of RXRα are indeed implicated in the development of a number of cancers. The targeted disruption of the RXRα gene, for example, leads to pre-neoplastic lesions in the prostate and to cervical metaplasia [139,140]. Consistently, the diminished expression of RXRα [141–143] or its malfunction due to phosphorylation by the Ras-MAPK pathway [144,145] are associated with the development of several forms of cancer. In addition, hotspot mutations (S427 F/Y) in the *RXRA* gene are present in approximately 5% of bladder cancer samples [146,147]. An oncogenic role for the S427 F/Y mutation has been proposed in luminal bladder cancer, potentially through the aberrant activation of other dimerization partners (e.g. PPARγ) in the absence of ligand [148].

Interestingly, a cytosolic truncated form of RXRα exists in several tumours and cancer cell lines as a consequence of proteolytic cleavage [149]. Aberrant RXRα function due to limited proteolysis may cause resistance to the anti-proliferative effects of retinoids [150] and promote cancer cell survival by activating the PI3K/AKT pathway [151]. The overexpression of this form accelerated the development of colitis-associated colon cancer in mice through increased IL-6 signalling in myeloid cells [152].

Because of all these considerations RXRα represents an important target for the pharmacologic intervention of cancer. The administration of selective RXR agonists have a well-established beneficial effect in solid tumours because of their capacity to induce the differentiation and/or apoptosis of cancer cells [136]. The endogenous RXR ligand 9-cis-retinoic acid (also known as alitretinoin or panretin) is indicated in the topical treatment of cutaneous lesions of acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma [153], whereas 13-cis-retinoic acid (isotretinoin) has therapeutic potential in the treatment of several other types of cancer [154,155].

The synthetic RXRα ligand bexarotene (also known as targretin) is used currently for treating refractory cutaneous T cell lymphoma (CTCL) [156] (Table 1). Bexarotene acted synergistically with standard first-line cytotoxic chemotherapy [157] and overcame the acquired resistance to paclitaxel in non-small cell lung cancer (NSCLC) [158]. Interestingly, bexarotene also appeared to increase the survival of a segment of NSCLC

patients developing high-grade hypertriglyceridemia [159,160].

RXR agonists, in combination with drugs that increase the levels of cyclic AMP, can induce the terminal granulocytic differentiation and apoptosis of AML cells that are unresponsive or resistant to ATRA therapy [161]. Consistently, clinical trials suggest that bexarotene may also be useful as combined therapy for AML [162]. The mechanism by which RXR agonists exert cell differentiation has been proposed to be independent of heterodimerization with RAR [163]. In contrast, the combined use of bexarotene with LXR agonists induced potent differentiation and cytotoxicity in AML cell lines and primary human AML cells [163], which raises the possibility that the combined activation of RXR-LXR heterodimers might have a potential role in the differentiation therapy of AML.

Apart from agonistic ligands, some compounds target specific properties of the RXRα molecule. This is the case of acyclic retinoid (also known as peretinoin), a synthetic retinoid that binds to both RXR and RAR and prevents the phosphorylation of RXRα by the Ras/MAPK pathway through an undefined mechanism [164]. Clinical studies have shown that it is effective in suppressing HCC recurrence and improving patient survival rates following curative therapy [164]. Peretinoin not only enhances the expression of retinoid target genes in the liver but also modulates various signal transduction pathways involved in hepatocarcinogenesis [165]. On the other hand, the naturally occurring xanthone CF31 is able to inhibit the interaction between the truncated form of RXRα and PI3K, thus facilitating the induction of cell death in response to TNFα [166].

In contrast to the beneficial effects of rexinoids in a number of malignancies, RXRα has been demonstrated to be a binding partner of the PML-RAR fusion, which suggests a contributing role for RXRα in APL [167,168]. Genome-wide epigenetic studies suggested that the PML-RARα-RXR complex acts as a local chromatin modulator that is crucial for oncogenic transformation and for the development of APL in transgenic mice [169], thus raising the possibility that RXRα may be a relevant therapeutic target also in APL.

The role of RXR expression specifically in the myeloid compartment and in the context of cancer has been recently addressed. In line with negative actions of RXRs in inflammatory responses and angiogenesis, RXR expression in myeloid cells has been shown to play a protective role against cancer cell migration and invasion [170]. RXR deletion in myeloid cells resulted in the increased expression of important determinants of premetastatic niche formation in the lung. Mechanistically, RXR mediated the repression of these genes through corepressor recruitment to DNA elements in a ligand-independent manner [170]. However, recent studies have shown that the expression of RXRs in tissue resident macrophages contributes to their accumulation in tumours and to ovarian tumour progression in mice [171], which raises the need to further explore the specific contribution of RXRs in the different cell compartments of the tumour microenvironment.

8. PPARs display multiple functions that are pro and anti-tumoral

The PPAR subfamily includes three members, PPARα (NR1C1), PPARδ (NR1C2), and PPARγ (NR1C3), which are activated by fatty acids, eicosanoids and numerous xenobiotics. Whereas the homology between the DBD domains of the three subtypes reaches up to 80 % identity, their LBDs exhibit a lower degree of sequence homology, thereby allowing the binding of structurally different ligands that may account for the specific biological activities of the three PPAR isotypes.

The expression pattern of each PPAR in adult animals is tissue-specific. PPARα is mainly expressed in the liver, where it activates fatty acid catabolism. PPARδ is expressed ubiquitously and is implicated in fatty acid oxidation and keratinocyte differentiation. PPARγ is mainly expressed in adipose tissue, colon, kidney and immune cells, including lymphocytes and macrophages. PPARγ is an essential modulator of adipocyte differentiation, lipid storage and glucose metabolism, and plays important anti-inflammatory roles in macrophages [172]. In addition, PPARs are also involved in the control of central cellular processes including cell differentiation, proliferation, survival, and apoptosis [173]. Because of their roles at the crosstalk between metabolism and central cellular processes, PPARs have received interest as potential therapeutic targets for a variety of malignancies, including solid tumours, such as liver, lung, gastrointestinal, breast and prostate cancer, as well as leukemia [174,175].

The role of the PPARα and PPARδ isotypes in cancer is controversial. The chronic administration of PPARα agonists in rodents results in a short-term pleiotropic response leading to liver hypertrophy and hyperplasia that is followed by the formation of HCC [176,177]. The development of such tumours occurs via an increase in oxidative stress, the induction of cell proliferation and the inhibition of apoptosis [176,178]. Mice lacking PPARα are resistant to clofibrate- and WY-14,643-induced hepatic neoplasia [177,179]. In contrast, epidemiological studies suggest that similar effects are unlikely to occur in humans [180,181]. A number of experimental observations suggest major differences between rodents and humans in the response to PPARα agonists [182–184], potentially due to the significantly lower expression of PPARα in human hepatocytes, the inefficient ligand activation of human PPARα [185], and the differential expression of coactivators and/or PPARα variants. Another possible explanation suggests that human PPARα does not exert carcinogenic effects, as the activation of a humanized PPARα in transgenic mice results in the increased expression of genes that modulate lipid catabolism but does not induce hepatic tumours [178,185]. This finding raises the question of whether there are structural variations in the regulatory regions of genes involved in hepatocyte growth between humans and rodents that might be responsible for the differential response to PPARα agonists [182,185,186]. In addition, murine, but not humanized, PPARα down-regulated the let-7c miRNA cluster, which targets Myc. Therefore, the increased stability of Myc mRNA might contribute to enhance mitogenic signalling and hepatocyte proliferation in response to murine PPARα activation [187].

Paradoxically, several other studies suggest that activating PPARα could be useful for the prevention or treatment of different cancers. The administration of PPARα agonists inhibited the growth of tumours derived from melanoma, glioblastoma, Lewis lung carcinoma, and fibrosarcoma cell lines, and of xenografts from A549 human lung cancer cells [188–190]. These inhibitory effects were mediated by the PPARα-dependent inhibition of endothelial cell proliferation and angiogenesis, via the suppression of epoxyeicosatrienoic acid biosynthesis [191,192]. In addition, PPARα inhibited inflammatory signalling through repressive interaction with the p65 subunit of NFκB [193] and negatively influenced aerobic glycolysis (the Warburg effect) [194]. In this sense, the activation of PPARα increased the mitochondrial oxidation of fatty acids, and inhibited the expression of glutaminase, thus reducing glutamine levels and limiting cancer cell growth [194,195]. Recently, intestinal PPARα was also shown to protect against colon carcinogenesis via the regulation of DNA methyltransferase 1 and protein arginine methyltransferase 6 [196]. In the same line, the administration of fenofibrate protected transgenic mice expressing human PPARα from chemical-induced colon tumorigenesis [196].

Pro- and anti-tumorigenic roles have also been proposed for PPARδ [177,178]. PPARδ expression is up-regulated in various human cancers, including colorectal cancer [197–199], pancreatic cancer [200], and lung cancer [201]. In general, high PPARδ expression in human cancers is associated with higher pathological grade and negative survival outcomes [197,202]. PPARδ overexpression in intestinal epithelial cells in mice, which mimics the up-regulation of this protein in human colon cancer tissues, promoted chemical-induced colorectal tumorigenesis [203]. Moreover, a recently published study using unbiased global transcriptome analysis identified PPARδ activation as a driver of intestinal stem cell transformation and tumour promotion in APCMin mice maintained on a high-fat diet, suggesting that PPARδ may play a mechanistic role in obesity-driven cancers [204,205].

In contrast with the general pro-tumorigenic roles of PPARδ, a recent retrospective clinical study associated high PPARδ expression in colorectal tumours with an increased survival rate following radiation treatment [206]. In addition, its oncosuppressive activity was also proposed in prostate cancer [207] and in chemically induced skin carcinogenesis [208,209].

The controversy regarding the role of PPARδ in tumorigenesis is also evident in *in vivo* deletion studies in mice, in which the absence of this protein in the germline has either positive [210] or negative [211,212] effects on colon tumour development. Interestingly, in the PPARδ knockout model in which a pro-tumorigenic effect was observed in the colon, tumour development after the implantation of melanoma or Lewis lung carcinoma cells was inhibited [213]. Likewise, backcrossing this model with MMTV-cyclooxygenase (COX)-2 transgenic mice resulted in the suppression of mammary gland tumour formation [214]. Such contradictory findings has prompted the interpretation that PPARδ may play different roles depending on where it is specifically expressed; PPARδ expression in stromal cells within the tumour microenvironment may promote tumorigenesis, whereas PPARδ expression in cancer cells may suppress tumour growth [213].

Apart from the effects on tumour growth, a pro-metastatic role for PPARδ has been suggested in different studies. The down-regulation of PPARδ expression in human cancer cells strongly suppressed metastasis after their orthotopic injection in immunodeficient mouse models. These effects occurred via the suppression of the expression of important pro-metastatic genes in cancer cells and of critical metastatic events including angiogenesis, epithelial-mesenchymal transition, and cancer cell invasion and migration [197]. In addition, PPARδ-deficiency in stromal cells also contributed to inhibit metastasis [197]. The activation of PPARδ promoted the migration and invasion of highly metastatic melanoma cells by up-regulating the Snail Family transcriptional repressor 1 [215]. Furthermore, PPARδ up-regulation in human colon, lung, and breast cancers correlated with reduced metastasis-free survival [197].

In contrast to the other members of the PPAR subfamily, PPARγ plays roles that are predominantly anti-tumorigenic. The activation of PPARγ inhibits growth, and either promotes apoptosis or induces the differentiation of a number of cancer cell lines *in vitro* and *in vivo*, including liposarcoma, prostate, breast, bladder and colon cancer cells [178]. Several mechanisms are involved in these actions, including the repression of cyclin D1, the induction of cell cycle inhibitors (e.g. p21, p27 and PTEN) or the up-regulation of pro-apoptotic factors (e.g. BAX and BAD) [178]. An indirect mechanism affecting cell proliferation relies on the insulin sensitizing activity of the PPARγ agonists thiazolidinediones (TZDs), which may decrease hyperinsulinemia in patients of metabolic diseases associated to insulin resistance, and hence, prevent the mitogenic effects of insulin on malignant cells [216].

In addition to direct actions on transformed cells, inhibitory effects on the inflammatory response and on angiogenesis within the tumour microenvironment may contribute to the anti-tumorigenic activities of ligand-activated PPARγ [217]. Also, PPARγ activation counteracts the cancer-associated up-regulation of the WNT/β-catenin pathway, which is important in cancer-related chronic inflammation and oxidative stress [218].

Of note, the tumour inhibitory action of TZDs was shown to be independent or only partially dependent on PPARγ, and initial clinical trials using TZDs as monotherapy failed to show significant effectiveness against cancer [219]. However, epidemiological studies have evidenced a reduction in the development of different types of cancer in diabetic patients treated with TZDs [220]. As mentioned above, it must be taken into account that the beneficial effect of TZDs-PPARγ may not be a consequence of direct anti-tumour actions alone, but result also from indirect effects leading to the amelioration of pro-tumorigenic conditions [217]. However, relevant discordances have emerged such as the increased risk of bladder cancer, non-Hodgkin lymphoma and melanoma in type 2 diabetes patients long-time treated with pioglitazone [221,222]. Currently, numerous clinical trials are ongoing (in phase II and III) for the use of PPARγ ligands, including rosiglitazone, pioglitazone, efatutazone and troglitazone, as mono- or combined therapeutic agents for a long list of cancers with different clinical outputs [175] (Table 1).

In summary, the effects of PPARs on tumour progression are diverse and depend on the type of tissue and/or the PPAR ligand evaluated. Several variables, including the levels of expression of PPAR isotypes in the tumour microenvironment, the specific sets of genes regulated by each isotype, the relative abundance of coactivators and corepressors, and the possibility that ligands exert both PPAR-dependent and -independent activities, influence the capability of such ligands to modulate tumour growth.

9. The modulation of lipid metabolism by liver X receptors as a promising strategy in cancer therapy

Two subtypes of LXRs exist, LXRα (NR1H3) and LXRβ (NR1H2), with established roles in the control of lipid and glucose metabolism, and in the regulation of immune responses. LXRα is highly expressed in the liver, adipose tissue, intestine, kidney and lung, whereas LXRβ is present in most tissues. They can be naturally activated by specific endogenous oxysterols (e.g. 22(R)-HC, 24(S)-HC, 27-HC, and 24(S),25-epoxycholesterol) or the cholesterol precursor desmosterol. In addition, they can be activated by synthetic high-affinity agonists [223].

The effects of LXR activation as a potential therapy in cancer have been studied with raising interest in the last decade. Synthetic LXR ligands (e.g. TO901317 and GW3965) have potent anti-proliferative, cytostatic and pro-apoptotic activities in several cancer cell lines [224–227]. These effects translate into reduced tumour progression in murine models [225,226,228–232]. A number of molecular mechanisms involved in intracellular signalling and cell cycle progression have been shown to be affected by LXR activation in a cell-autonomous manner in different cancer cell types. These include the repression of positive cell cycle regulators (eg. cyclins and/or CDKs) [225,227,232–234], the up-regulation of cell cycle inhibitors (e.g. p21 or p27) [225,227,235–237], the induction of suppressor of cytokine signalling 3 [228], and the interference with the β-catenin/WNT pathway [238]. In addition, LXR agonists are able to inhibit AKT pro-survival signalling [226] and to induce caspase-dependent cell death in several cancer cell lines [225,226,229,230].

The accumulated evidences indicate that the transcriptional activation of genes involved in lipid homeostasis within cancer cells plays an essential role in the anti-tumoral actions of the LXR pathway. In this sense, the induction of the cholesterol and phospholipid transporter ATP-binding cassette (ABC)G1, and the subsequent reduction in intracellular cholesterol levels, contribute to the anti-proliferative actions of LXRs in cancer cells [239]. Indeed, alterations in the structural characteristics of lipid rafts caused by the increased expression of ABCG1 was proposed as the basis for the reduced AKT phosphorylation in pancreatic cancer cells stimulated with LXR agonists [226].

In particular, glioblastoma cells are highly dependent on the uptake of cholesterol for survival due to dysregulated endogenous cholesterol synthesis [231]. LXR-623, which is a partial agonist for LXRα and a full agonist for LXRβ, induced glioblastoma cell death through LXRβ-dependent intracellular cholesterol depletion. This effect was consistent with the capacity of LXR-623 to induce the expression of two sets of targets in parallel: the E3 ubiquitin ligase inducible degrader of the low-density lipoprotein receptor (IDOL), a mechanism that limits the uptake of cholesterol-rich low-density lipoproteins, and the cholesterol transporters ABCA1 and ABCG1 that mediate cholesterol efflux.

The discovery of a different partial agonist for LXRs, dendrogenin A, allowed the identification of an additional role for LXRβ as inducer of lethal autophagy in human melanoma and AML cells [240]. Dendrogenin A is a naturally occurring steroidal alkaloid product of the enzymatic conjugation of a cholesterol derivative (5,

6α-epoxycholesterol) and histamine [241]. Although the potential contribution of LXRα was not discarded, the partial agonism of LXRβ was proposed to mediate a complex combination of actions, including the direct transcriptional activation of other members of the NR family (nuclear receptor 77 (Nur77/NR4A1) and neuron-derived orphan receptor 1 (NOR1/NR4A3)) and of microtubule associated LC3, which is an important mediator of autophagosome formation [240]. In addition, dendrogenin A de-repressed the expression of transcription factor EB, a master transcriptional regulator of autophagy and lysosome biogenesis, by blocking the inhibitory action of LXRβ on this gene. These combinatorial effects were selective for dendrogenin A and were not observed in response to conventional full LXR agonists. Moreover, dendrogenin A induced characteristics of differentiation in human thyroid carcinoma cells in an LXRβ-dependent manner [242].

In addition, the LXR pathway is able to promote a lipogenic program involving the increased expression of fatty acid synthase (FAS), which results in triacylglyceride accumulation. Induction of this program in prostate cancer cells resulted in cell cycle arrest [234]. However, an LXR inverse agonist, SR9243, which induces LXR-corepressor interaction and reduces the expression of genes controlling glycolysis and lipogenesis, inhibited the Warburg effect and caused apoptosis in a broad range of cancer cells [243]. These contrasting observations reflect the need to further clarify the exact role of LXR-mediated lipogenesis in tumour growth control.

The importance of secreted apolipoprotein E (ApoE), another direct LXR transcriptional target, as an inhibitor of the invasive capacity of melanoma cells has been consistently demonstrated [244]. ApoE secretion resulted in inhibitory effects on melanoma progression, angiogenesis and metastasis to the brain. Of note, the selective knockdown of LXRβ, but not LXRα, in melanoma cells blocked the ability of LXR agonists to suppress invasion and endothelial cell recruitment.

Apart from cancer cell-autonomous effects, LXR activation can also impact other cells within the tumour microenvironment. The activation of the LXR pathway altered endothelial cell cholesterol homeostasis, which affected the organization of lipid rafts in the plasma membrane [245]. These effects impaired VEGF receptor signalling and correlated with reduced tumour angiogenesis. In addition, stromal and endothelial cells may also contribute to the increase in ApoE levels in response to pharmacological LXR activation [244]. ApoE produced *in vitro* by LXR-treated macrophages reduced the proliferation and increased apoptosis of a human breast cancer cell line [246]. Moreover, the increase in ApoE levels following LXR agonism with GW3965 or a novel LXR agonist, RGX-104, induced the apoptosis of myeloid-derived suppressor cells (MDSC), resulting in decreased systemic and intratumoral levels of these cells in the context of melanoma [247]. These effects were associated with an augmented infiltration of activated cytotoxic T cells and pro-inflammatory helper T cells within the tumours, thus suggesting that the LXR-ApoE axis can exert anti-metastatic actions on cancer cells [244] while enhancing immune-mediated anti-tumoral responses [247]. In fact, RGX-104 and checkpoint blockade by anti-programmed cell death protein 1 (PD-1) antibodies displayed synergistic inhibitory effects on tumour growth in mice [247].

Whereas conventional LXR agonists have failed translation to the clinic due to adverse effects, RGX-104 is currently being tested in a phase 1, first-in-human, dose escalation and expansion study in patients with lymphoma or advanced solid malignancies under standard treatments, including anti-PD-1 immunotherapy [248] (Table 1). Preliminary data indicate that RGX-104 reduce the abundance of MDSCs in cancer patients with diverse forms of metastatic cancer [247]. In addition, the importance of the LXR pathway in tumour growth control is supported by the potential prognostic value of LXRα expression in HCC patients [249].

In contrast to the generalized anti-tumoral effects of synthetic LXR agonists, the LXR pathway has been also shown to participate in mechanisms for tumour immune evasion. In this sense, factors released by the tumour, potentially endogenous oxysterols, inhibited the expression of C-C chemokine receptor type 7 (CCR7) in dendritic cells through the activation of LXRα. This mechanism impaired dendritic cell migration to lymph nodes, thus interfering with antigen presentation to T cells [250]. Other studies, however, have shown a requirement for LXR activation for efficient CCR7-dependent chemotaxis of dendritic cells and other myeloid cells [251,252], and the multifunctional enzyme CD38 emerged as an LXR transcriptional target mediating these effects in dendritic cells [251]. Therefore, these contrasting observations on the role of LXRs in dendritic cell chemotaxis raise the need to further investigate the type of molecules that are secreted in the tumour microenvironment and the way they act on the LXR pathway. On the other hand, hyaluronic acid produced within tumours has been recently shown to induce an increase in cholesterol efflux in tumour-associated macrophages through the activity of the cholesterol transporters ABCA1 and ABCG1. Increased cholesterol efflux promoted a pro-tumoral macrophage reprograming in response to IL-4 [253]. However, while these cholesterol transporters are conventional LXR targets, the involvement of LXRs in the actions of hyaluronic acid has not been established.

In summary, many of the actions of the LXR pathway translate into inhibitory effects on cancer cell proliferation or on the pro-tumoral activities of stromal cells, which directly or indirectly, derive from local changes in lipid metabolism. Therefore, the designing of improved ligands, and/or delivery strategies, that promote selective metabolic actions of LXRs within the tumour microenvironment deserve further investigation.

10. Future perspectives

Several NRs present opportunities for therapeutic intervention in cancer. As described in this review, established therapies or ongoing clinical trials exist based on the activation of several NRs (Table 1), either as monotherapy or in combination with chemotherapy or immunotherapy. In the case of PPARs, due to the fact that different isotypes exert complementary roles, a concept that has recently emerged is that dual- or pan-PPAR agonists may be more beneficial than agents targeting a single PPAR subtype. A candidate compound is bezafibrate, a pan-PPAR agonist, shown to reduce the development of colon cancer [254].

One of the major challenges in the field, however, is the development of side effects, as it often occurs after prolonged treatment with, for example, GCs [83], TZDs [174], or bexarotene [255]. This obstacle is also the reason why the progression of first-generation ligands for LXRs were stopped before reaching the clinics [256]. Novel synthetic ligands with less adverse effects and an improved therapeutic profile are currently under development for several NRs. In the case of GR, most of the side effects of GCs seem to rely on the GR transactivation function. For this reason, the designing of compounds able to selectively activate GR transrepression function, the so-called selective GR agonists and modulators (SEGRAMs), has become a major research focus and some of these molecules are currently in clinical trials [83]. In addition, a novel rexinoid capable of activating RXR with minimal toxicity offers therapeutic potential for the treatment of medulloblastoma [257]. Furthermore, the simultaneous activation of PPARα and/or PPARδ may be also a strategy to bypass the side effects of PPARγ agonists [174].

The second major obstacle in the field is the development of resistance to NR ligands. As described in this review, mutations and other mechanisms contribute to the acquired unresponsiveness of NRs to agonists or antagonists (Fig. 3). However, the molecular mechanisms underlying such resistance are far from being well understood and more detailed knowledge about the structural and functional properties of NRs in the context of cancer will facilitate the discovery and development of improved ligands. In this regard, the recently solved low-resolution of the full length AR structure may be an opportunity to develop novel anti-androgens with improved characteristics to avoid the development of resistance [258]. The use of alternative combinatorial

therapies also needs to be addressed. Tumour heterogeneity implies that within the same tumour, not all cancer cells express the receptor, as shown with breast cancer stem cells lacking ER, thus enabling their capacity to avoid the effects of endocrine therapy [55]. This illustrates the need to combine therapies in order to target such complexity. Another strategy is to reduce the content of cancer stem cells through differentiation. An example discussed here that strengthens this concept is the effectiveness of rexinoids in inducing the terminal differentiation of AML cells that are resistant to ATRA [161].

Taken together, further studies are required to identify genes targeted by each NR agonist in a cell type-specific manner, and to define potential interactions between NRs and coregulators that selectively promote the beneficial functions of each NR. In addition, molecular studies exploring the structural aspects that govern NR-coregulator interaction in response to a given ligand and the acquisition of resistance are fundamental. These aspects may not only provide avenues for the designing of compounds with improved therapeutic potential, but may also help define combinatorial strategies that address the heterogeneity of tumours.

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Declaration of Competing Interest

The authors report no declarations of interest.

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References

[1] R.M. Evans, D.J. Mangelsdorf, Nuclear receptors, RXR, and the big bang, Cell 157 (2014) 255–266, https://doi.org/10.1016/j.cell.2014.03.012.
[2] M.D. Long, M.J. Campbell, Pan-cancer analyses of the nuclear receptor superfamily, Nucl. Recept. Res. 2 (2015), https://doi.org/10.11131/2015/101182.
[3] P. Fuentes-Prior, A. Rojas, A.T. Hagler, E. Estébanez-Perpiñá, Diversity of quaternary structures regulates nuclear receptor activities, Trends Biochem. Sci. (2019), https://doi.org/10.1016/j.tibs.2018.09.005.
[4] R. Sever, C.K. Glass, Signaling by nuclear receptors, Cold Spring Harb. Perspect. Biol. 5 (2013), https://doi.org/10.1101/cshperspect.a016709.
[5] X. Yu, P. Yi, R. Hamilton, H. Shen, M. Chen, C. Foulds, M. Mancini, S. Ludtke, Z. Wang, B. O'Malley, Structural insights of transcriptionally active, full-length androgen receptor coactivator complexes, Mol. Cell (2020), https://doi.org/10.1016/j.molcel.2020.06.031.
[6] L. Zhao, S. Zhou, J.Å. Gustafsson, Nuclear receptors: recent drug discovery for cancer therapies, Endocr. Rev. 40 (2019) 1207–1249, https://doi.org/10.1210/er.2018-00222.
[7] C. Carlberg, A. Muñoz, An update on vitamin D signaling and cancer, Semin. Cancer Biol. S1044-579X (20) (2020) 30114, https://doi.org/10.1016/j.semcancer.2020.05.018.
[8] R.A. Davey, M. Grossmann, Androgen receptor structure, function and biology: from bench to bedside, Clin. Biochem. Rev. 37 (2016) 3–15.
[9] E.C. Bolton, A.Y. So, C. Chaivorapol, C.M. Haqq, H. Li, K.R. Yamamoto, Cell- and gene-specific regulation of primary target genes by the androgen receptor, Genes Dev. 21 (2007) 2005–2017, https://doi.org/10.1101/gad.1564207.
[10] C. Cai, H. Wang, Y. Xu, S. Chen, S.P. Balk, Reactivation of androgen receptor-regulated TMPRSS2:ERG gene expression in castration-resistant prostate cancer, Cancer Res. 69 (2009) 6027–6032, https://doi.org/10.1158/0008-5472.CAN-09-0395.
[11] J. Yu, J. Yu, R.S. Mani, Q. Cao, C.J. Brenner, X. Cao, X. Wang, L. Wu, J. Li, M. Hu, Y. Gong, H. Cheng, B. Laxman, A. Vellaichamy, S. Shankar, Y. Li, S. M. Dhanasekaran, R. Morey, T. Barrette, R.J. Lonigro, S.A. Tomlins, S. Varambally, Z.S. Qin, A.M. Chinnaiyan, An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression, Cancer Cell 17 (2010) 443–454, https://doi.org/10.1016/j.ccr.2010.03.018.
[12] E. Nevedomskaya, S.J. Baumgart, B. Haendler, Recent advances in prostate cancer treatment and drug discovery, Int. J. Mol. Sci. 19 (2018), https://doi.org/10.3390/ijms19051359.
[13] C.E. Bohl, W. Gao, D.D. Miller, C.E. Bell, J.T. Dalton, Structural basis for antagonism and resistance of bicalutamide in prostate cancer, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6201–6206, https://doi.org/10.1073/pnas.0500381102.
[14] W.H. Bisson, A.V. Cheltsov, N. Bruey-Sedano, B. Lin, J. Chen, N. Goldberger, L. T. May, A. Christopoulos, J.T. Dalton, P.M. Sexton, X.K. Zhang, R. Abagyan, Discovery of antiandrogen activity of nonsteroidal scaffolds of marketed drugs, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 11927–11932, https://doi.org/10.1073/pnas.0609752104.
[15] M. Crumbaker, L. Khoja, A.M. Joshua, AR signaling and the PI3K pathway in prostate cancer, Cancers (Basel) 9 (2017), https://doi.org/10.3390/cancers9040034.
[16] S.C. Chan, Y. Li, S.M. Dehm, Androgen receptor splice variants activate androgen receptor target genes and support aberrant prostate cancer cell growth independent of canonical androgen receptor nuclear localization signal, J. Biol. Chem. 287 (2012) 19736–19749, https://doi.org/10.1074/jbc.M112.352930.
[17] M. Marín-Aguilera, N. Jiménez, Ò. Reig, R. Montalbo, A.K. Verma, G. Castellano, L. Mengual, I. Victoria, M.V. Pereira, M. Milà-Guasch, S. García-Recio, D. Benítez-Ribas, R. Cabezón, A. González, M. Juan, A. Prat, B. Mellado, Androgen receptor and its splicing variant 7 expression in peripheral blood mononuclear cells and in circulating tumor cells in metastatic castration-resistant prostate cancer, Cells 9 (2020) 203, https://doi.org/10.3390/cells9010203.
[18] Y. Wang, X. Jiang, F. Feng, W. Liu, H. Sun, Degradation of proteins by PROTACs and other strategies, Acta Pharm. Sin. B 10 (2020) 207–238, https://doi.org/10.1016/j.apsb.2019.08.001.
[19] T. Neklesa, L.B. Snyder, R.R. Willard, N. Vitale, J. Pizzano, D.A. Gordon, M. Bookbinder, J. Macaluso, H. Dong, C. Ferraro, G. Wang, J. Wang, C.M. Crews, J. Houston, A.P. Crew, I. Taylor, ARV-110: an oral androgen receptor PROTAC degrader for prostate cancer, J. Clin. Oncol. 37 (2019) 259, https://doi.org/10.1200/jco.2019.37.7_suppl.259.
[20] Proof-of-concept with PROTACs in prostate cancer, Cancer Discov. 10 (2020) 1084, https://doi.org/10.1158/2159-8290.CD-NB2020-054.
[21] M. Alyamani, Z. Li, M. Berk, J. Li, J. Tang, S. Upadhyay, R.J. Auchus, N. Sharifi, Steroidogenic metabolism of galeterone reveals a diversity of biochemical activities, Cell Chem. Biol. 24 (2017) 825–832, https://doi.org/10.1016/j.chembiol.2017.05.020, e6.
[22] D.A. Bastos, E.S. Antonarakis, Galeterone for the treatment of advanced prostate cancer: the evidence to date, Drug Des. Devel. Ther. 10 (2016) 2289–2297, https://doi.org/10.2147/DDDT.S93941.
[23] R.R. McKay, L. Werner, M. Fiorillo, J. Roberts, E.I. Heath, G.J. Bubley, R. B. Montgomery, M.E. Taplin, Efficacy of therapies after galeterone in patients with castration-resistant prostate cancer, Clin. Genitourin. Cancer 15 (2017) 463–471, https://doi.org/10.1016/j.clgc.2016.10.006.
[24] Z. Yu, C. Cai, S. Gao, N.I. Simon, H.C. Shen, S.P. Balk, Galeterone prevents androgen receptor binding to chromatin and enhances degradation of mutant androgen receptor, Clin. Cancer Res. 20 (2014) 4075–4085, https://doi.org/10.1158/1078-0432.CCR-14-0292.
[25] V.K. Arora, E. Schenkein, R. Murali, S.K. Subudhi, J. Wongvipat, M.D. Balbas, N. Shah, L. Cai, E. Efstathiou, C. Logothetis, D. Zheng, C.L. Sawyers, Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade, Cell 155 (2013) 1309–1322, https://doi.org/10.1016/j.cell.2013.11.012.
[26] J. Hu, Q. Chen, The role of glucocorticoid receptor in prostate cancer progression: from bench to bedside, Int. Urol. Nephrol. 49 (2017) 369–380, https://doi.org/10.1007/s11255-016-1476-8.
[27] S.D. Kaffenberger, C.E. Barbieri, Molecular subtyping of prostate cancer, Curr. Opin. Urol. 26 (2016) 213–218, https://doi.org/10.1097/MOU.0000000000000285.

[28] S.B. Brown, S.E. Hankinson, Endogenous estrogens and the risk of breast, endometrial, and ovarian cancers, Steroids 99 (2015) 8–10, https://doi.org/10.1016/j.steroids.2014.12.013.
[29] Y. Zhou, X. Liu, The role of estrogen receptor beta in breast cancer, Biomark. Res. 8 (2020) 39, https://doi.org/10.1186/s40364-020-00223-2.
[30] S. Yan, P. Dey, Y. Ziegler, X. Jiao, S.H. Kim, J.A. Katzenellenbogen, B. S. Katzenellenbogen, Contrasting activities of estrogen receptor beta isoforms in triple negative breast cancer, Breast Cancer Res. Treat. (2020), https://doi.org/10.1007/s10549-020-05948-0.
[31] C. Fougner, H. Bergholtz, J.H. Norum, T. Sørlie, Re-definition of claudin-low as a breast cancer phenotype, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-020-15574-5.
[32] T. Sørlie, C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M. B. Eisen, M. van de Rijn, S.S. Jeffrey, T. Thorsen, H. Quist, J.C. Matese, P. O. Brown, D. Botstein, P.E. Lønning, A.L. Børresen-Dale, Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10869–10874, https://doi.org/10.1073/pnas.191367098.
[33] S.E. Hankinson, A.H. Eliassen, Circulating sex steroids and breast cancer risk in premenopausal women, Horm. Cancer 1 (2010) 2–10, https://doi.org/10.1007/s12672-009-0003-0.
[34] J. Ragaz, A. Coldman, Survival impact of adjuvant tamoxifen on competing causes of mortality in breast cancer survivors, with analysis of mortality from contralateral breast cancer, cardiovascular events, endometrial cancer, and thromboembolic episodes, J. Clin. Oncol. 16 (1998), https://doi.org/10.1200/JCO.1998.16.6.2018, 2018-24.
[35] A. Howell, J.F.R. Robertson, J.Q. Albano, A. Aschermannova, L. Mauriac, U. R. Kleeberg, I. Vergote, B. Erikstein, A. Webster, C. Morris, Fulvestrant, formerly ICI 182,780, is as effective as anastrozole in postmenopausal women with advanced breast cancer progressing after prior endocrine treatment, J. Clin. Oncol. 20 (2002), https://doi.org/10.1200/JCO.2002.10.057, 3396-403.
[36] M.R. Nathan, P. Schmid, A review of fulvestrant in breast cancer, Oncol. Ther. 5 (2017) 17–29, https://doi.org/10.1007/s40487-017-0046-2.
[37] I.E. Smith, M. Dowsett, Aromatase inhibitors in breast cancer, N. Engl. J. Med. 348 (2003) 2431–2442, https://doi.org/10.1056/NEJMra023246.
[38] M.D. Brooks, M.L. Burness, M.S. Wicha, Therapeutic implications of cellular heterogeneity and plasticity in breast cancer, Cell Stem Cell 17 (2015) 71–260, https://doi.org/10.1016/j.stem.2015.08.014.
[39] C.X. Ma, T. Reinert, I. Chmielewska, M.J. Ellis, Mechanisms of aromatase inhibitor resistance, Nat. Rev. Cancer 15 (2015) 261–275, https://doi.org/10.1038/nrc3920.
[40] H. Pan, R. Gray, J. Braybrooke, C. Davies, C. Taylor, P. McGale, R. Peto, K. I. Pritchard, J. Bergh, M. Dowsett, D.F. Hayes, 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years, N. Engl. J. Med. 377 (2017) 1836–1846, https://doi.org/10.1056/NEJMoa1701830.
[41] F. Bertucci, C.K.Y. Ng, A. Patsouris, N. Droin, S. Piscuoglio, N. Carbuccia, J. C. Soria, A.T. Dien, Y. Adnani, M. Kamal, S. Garnier, G. Meurice, M. Jimenez, S. Dogan, B. Verret, M. Chaffanet, T. Bachelot, M. Campone, C. Lefeuvre, H. Bonnefoi, F. Dalenc, A. Jacquet, M.R. De Filippo, N. Babbar, D. Birnbaum, T. Filleron, C. Le Tourneau, F. André, Genomic characterization of metastatic breast cancers, Nature 569 (2019) 560–564, https://doi.org/10.1038/s41586-019-1056-z.
[42] B. Verret, J. Cortes, T. Bachelot, F. Andre, M. Arnedos, Efficacy of PI3K inhibitors in advanced breast cancer, Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 30 (Suppl. 10) (2019) x12–x20, https://doi.org/10.1093/annonc/mdz381.
[43] D.A. Olsen, T. Bechmann, B. Østergaard, P.A. Wamberg, E.H. Jakobsen, I. Brandslund, Increased concentrations of growth factors and activation of the EGFR system in breast cancer, Clin. Chem. Lab. Med. 50 (2012) 1809–1818, https://doi.org/10.1515/cclm-2011-0823.
[44] T. Zhang, F. Feng, Y. Yao, L. Qi, J. Tian, C. Zhou, S. Dong, X. Wang, C. Sun, Efficacy and acceptability of neoadjuvant endocrine therapy in patients with hormone receptor-positive breast cancer: a network meta-analysis, J. Cell. Physiol. 234 (2019) 12393–12403, https://doi.org/10.1002/jcp.28068.
[45] K. Tryfonidis, G. Basaran, J. Bogaerts, M. Debled, L. Dirix, J.C. Thery, V.C.G. Tjan-Heijnen, D. Van Den Weyngaert, T. Cufer, M. Piccart, D. Cameron, A European Organisation for Research and Treatment of Cancer randomized, double-blind, placebo-controlled, multicentre phase II trial of anastrozole in combination with gefitinib or placebo in hormone receptor-positive advanced breast cancer (NCT00066378), Eur. J. Cancer 53 (2016) 144–154, https://doi.org/10.1016/j.ejca.2015.10.012.
[46] S. Pancholi, R. Ribas, N. Simigdala, E. Schuster, J. Nikitorowicz-Buniak, A. Ressa, Q. Gao, M.F. Leal, A. Bhamra, A. Thornhill, L. Morisset, E. Montaudon, L. Sourd, M. Fitzpatrick, M. Altelaar, S.R. Johnston, E. Marangoni, M. Dowsett, L.A. Martin, Tumour kinome re-wiring governs resistance to palbociclib in oestrogen receptor positive breast cancers, highlighting new therapeutic modalities, Oncogene 39 (2020) 4781–4797, https://doi.org/10.1038/s41388-020-1284-6.
[47] A. Basudan, N. Priedigkeit, R.J. Hartmaier, E.S. Sokol, A. Bahreini, R.J. Watters, M.M. Boisen, R. Bhargava, K.R. Weiss, M.M. Karsten, C. Denkert, J.-U. Blohmer, J. P. Leone, R.L. Hamilton, A.M. Brufsky, E. Elishaev, P.C. Lucas, A.V. Lee, S. Oesterreich, Frequent ESR1 and CDK pathway copy-number alterations in metastatic breast cancer, Mol. Cancer Res. 17 (2019) 457–468, https://doi.org/10.1158/1541-7786.MCR-18-0946.
[48] F. Holst, P.R. Stahl, C. Ruiz, O. Hellwinkel, Z. Jehan, M. Wendland, A. Lebeau, L. Terracciano, K. Al-Kuraya, F. Jänicke, G. Sauter, R. Simon, Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer, Nat. Genet. 39 (2007) 655–660, https://doi.org/10.1038/ng2006.
[49] S.W. Fanning, C.G. Mayne, V. Dharmarajan, K.E. Carlson, T.A. Martin, S. J. Novick, W. Toy, B. Green, S. Panchamukhi, B.S. Katzenellenbogen, E. Tajkhorshid, P.R. Griffin, Y. Shen, S. Chandarlapaty, J.A. Katzenellenbogen, G. L. Greene, Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation, Elife 5 (2016), https://doi.org/10.7554/eLife.12792.
[50] R. Jeselsohn, J.S. Bergholz, M. Pun, M. Cornwell, W. Liu, A. Nardone, T. Xiao, W. Li, X. Qiu, G. Buchwalter, A. Feiglin, K. Abell-Hart, T. Fei, P. Rao, H. Long, N. Kwiatkowski, T. Zhang, N. Gray, D. Melchers, R. Houtman, X.S. Liu, O. Cohen, N. Wagle, E.P. Winer, J. Zhao, M. Brown, Allele-specific chromatin recruitment and therapeutic vulnerabilities of ESR1 activating mutations, Cancer Cell 33 (2018) 173–186, https://doi.org/10.1016/j.ccell.2018.01.004, e5.
[51] J. Park, Y.J. Lee, Hypoxia induced phosphorylation of estrogen receptor at serine 118 in the absence of ligand, J. Steroid Biochem. Mol. Biol. 174 (2017) 146–152, https://doi.org/10.1016/j.jsbmb.2017.08.013.
[52] M. Anbalagan, B.G. Rowan, Estrogen receptor alpha phosphorylation and its functional impact in human breast cancer, Mol. Cell. Endocrinol. 418 Pt 3 (2015) 72–264, https://doi.org/10.1016/j.mce.2015.01.016.
[53] O. Iriondo, M. Rábano, G. Domenici, O. Carlevaris, J.A. López-Ruiz, I. Zabalza, E. Berra, M. dM Vivanco, Distinct breast cancer stem/progenitor cell populations require either HIF1α or loss of PHD3 to expand under hypoxic conditions, Oncotarget 6 (2015) 31721–31739, https://doi.org/10.18632/oncotarget.5564.
[54] A. Jögi, A. Ehinger, L. Hartman, S. Alkner, Expression of HIF-1α is related to a poor prognosis and tamoxifen resistance in contralateral breast cancer, PLoS One 14 (2019) e0226150, https://doi.org/10.1371/journal.pone.0226150.
[55] M. Piva, G. Domenici, O. Iriondo, M. Rábano, B.M. Simões, V. Comaills, I. Barredo, J.A. López-Ruiz, I. Zabalza, R. Kypta, M.D.M. Vivanco, Sox2 promotes tamoxifen resistance in breast cancer cells, EMBO Mol. Med. 6 (2014) 66–79, https://doi.org/10.1002/emmm.201303411.
[56] X. Cui, R. Schiff, G. Arpino, C.K. Osborne, A.V. Lee, Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy, J. Clin. Oncol. 23 (2005) 7721–7735, https://doi.org/10.1200/JCO.2005.09.004.
[57] D. Chi, H. Singhal, L. Li, T. Xiao, W. Liu, M. Pun, R. Jeselsohn, H. He, E. Lim, R. Vadhi, P. Rao, H. Long, J. Garber, M. Brown, Estrogen receptor signaling is reprogrammed during breast tumorigenesis, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 11437–11443, https://doi.org/10.1073/pnas.1819155116.
[58] M. Umetani, H. Domoto, A.K. Gormley, I.S. Yuhanna, C.L. Cummins, N.B. Javitt, K.S. Korach, P.W. Shaul, D.J. Mangelsdorf, 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen, Nat. Med. 13 (2007) 92–1185, https://doi.org/10.1038/nm1641.
[59] C.D. DuSell, M. Umetani, P.W. Shaul, D.J. Mangelsdorf, D.P. McDonnell, 27-Hydroxycholesterol is an endogenous selective estrogen receptor modulator, Mol. Endocrinol. 22 (2008) 65–77, https://doi.org/10.1210/me.2007-0383.
[60] L. Cedó, S.T. Reddy, E. Mato, F. Blanco-Vaca, J.C. Escolà-Gil, HDL and LDL: potential new players in breast cancer development, J. Clin. Med. 8 (2019) 853, https://doi.org/10.3390/jcm8060853.
[61] H. Clayton, I. Titley, Md.M. Vivanco, Growth and differentiation of progenitor/stem cells derived from the human mammary gland, Exp. Cell Res. 297 (2004) 444–460, https://doi.org/10.1016/j.yexcr.2004.03.029.
[62] C. Ginestier, M.H. Hur, E. Charafe-Jauffret, F. Monville, J. Dutcher, M. Brown, J. Jacquemier, P. Viens, C.G. Kleer, S. Liu, A. Schott, D. Hayes, D. Birnbaum, M. S. Wicha, G. Dontu, ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome, Cell Stem Cell 1 (2007) 555–567, https://doi.org/10.1016/j.stem.2007.08.014.
[63] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 3983–3988, https://doi.org/10.1073/pnas.0530291100.
[64] B.M. Simões, M.D. Vivanco, Cancer stem cells in the human mammary gland and regulation of their differentiation by estrogen, Future Oncol. 7 (2011) 995–1006, https://doi.org/10.2217/fon.11.80.
[65] M.G. Scioli, G. Storti, F. D'amico, P. Gentile, G. Fabbri, V. Cervelli, A. Orlandi, The role of breast cancer stem cells as a prognostic marker and a target to improve the efficacy of breast cancer therapy, Cancers (Basel) 11 (2019), https://doi.org/10.3390/cancers11071021.
[66] B.M. Simões, M. Piva, O. Iriondo, V. Comaills, J.A. López-Ruiz, I. Zabalza, J. A. Mieza, O. Acinas, M.D.M. Vivanco, Effects of estrogen on the proportion of stem cells in the breast, Breast Cancer Res. Treat. 129 (2011) 23–35, https://doi.org/10.1007/s10549-010-1169-4.
[67] Y. Yin, C.-M. Xie, H. Li, M. Tan, G. Chen, R. Schiff, X. Xiong, Y. Sun, The FBXW2-MSX2-SOX2 axis regulates stem cell property and drug resistance of cancer cells, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 20528–20538, https://doi.org/10.1073/pnas.1905973116.
[68] G. Domenici, I. Aurrekoetxea-Rodríguez, B.M. Simões, M. Rábano, S.Y. Lee, J. S. Millán, V. Comaills, E. Oliemuller, J.A. López-Ruiz, I. Zabalza, B.A. Howard, R. M. Kypta, Md.M. Vivanco, A Sox2–Sox9 signalling axis maintains human breast luminal progenitor and breast cancer stem cells, Oncogene 38 (2019) 3151–3169, https://doi.org/10.1038/s41388-018-0656-7.
[69] B. ózsvári, F. Sotgia, M.P. Lisanti, First-in-class candidate therapeutics that target mitochondria and effectively prevent cancer cell metastasis: mitoriboscins and TPP compounds, Aging (Albany. NY) 12 (2020) 10162–10179, https://doi.org/10.18632/aging.103336.
[70] S.D. Conzen, Nuclear receptors and breast cancer, Mol. Endocrinol. 22 (2008) 2215–2228, https://doi.org/10.1210/me.2007-0421.
[71] L.S. Schwartzberg, D.A. Yardley, A.D. Elias, M. Patel, P. Lorusso, H.A. Burris, A. Gucalp, A.C. Peterson, M.E. Blaney, J.L. Steinberg, J.A. Gibbons, T.A. Traina, A phase I/Ib study of enzalutamide alone and in combination with endocrine

therapies in women with advanced breast cancer, Clin. Cancer Res. 23 (2017) 4046–4054, https://doi.org/10.1158/1078-0432.CCR-16-2339.
[72] S. Timmermans, J. Souffriau, C. Libert, A general introduction to glucocorticoid biology, Front. Immunol. 10 (2019) 1545, https://doi.org/10.3389/fimmu.2019.01545.
[73] L. Escoter-Torres, G. Caratti, A. Mechtidou, J. Tuckermann, N.H. Uhlenhaut, S. Vettorazzi, Fighting the fire: mechanisms of inflammatory gene regulation by the glucocorticoid receptor, Front. Immunol. 10 (2019) 1859, https://doi.org/10.3389/fimmu.2019.01859.
[74] J. Vandewalle, A. Luypaert, K. De Bosscher, C. Libert, Therapeutic mechanisms of glucocorticoids, Trends Endocrinol. Metab. 29 (2018) 42–54, https://doi.org/10.1016/j.tem.2017.10.010.
[75] D.W. Cain, J.A. Cidlowski, Immune regulation by glucocorticoids, Nat. Rev. Immunol. 17 (2017) 233–247, https://doi.org/10.1038/nri.2017.1.
[76] M.A. Pufall, Glucocorticoids and cancer, in: J.-C. Wang, C. Harris (Eds.), Glucocorticoid Signal. From Mol. to Mice to Man, Springer Science+Business Media, New York, 2015, pp. 315–333.
[77] K.T. Lin, L.H. Wang, New dimension of glucocorticoids in cancer treatment, Steroids 111 (2016) 84–88, https://doi.org/10.1016/j.steroids.2016.02.019.
[78] T.H. Truong, C.A. Lange, Deciphering steroid receptor crosstalk in hormone-driven cancers, Endocrinology 159 (2018) 3897–3907, https://doi.org/10.1210/en.2018-00831.
[79] A. Jiménez-Panizo, P. Pérez, A.M. Rojas, P. Fuentes-Prior, E. Estébanez-Perpiñá, Non-canonical dimerization of the androgen receptor and other nuclear receptors: implications for human disease, Endocr. Relat. Cancer 26 (2019) R479–R497, https://doi.org/10.1530/ERC-19-0132.
[80] I.V. Budunova, D. Kowalczyk, P. Pérez, Y.J. Yao, J.L. Jorcano, T.J. Slaga, Glucocorticoid receptor functions as a potent suppressor of mouse skin carcinogenesis, Oncogene 22 (2003) 3279–3287, https://doi.org/10.1038/sj.onc.1206383.
[81] V. Latorre, L.M. Sevilla, A. Sanchis, P. Pérez, Selective ablation of glucocorticoid receptor in mouse keratinocytes increases susceptibility to skin tumorigenesis, J. Invest. Dermatol. 133 (2013) 2771–2779, https://doi.org/10.1038/jid.2013.255.
[82] D. Chebotaev, A. Yemelyanov, I. Budunova, The mechanisms of tumor suppressor effect of glucocorticoid receptor in skin. Mol. Carcinog., Mol Carcinog, 2007, pp. 732–740, https://doi.org/10.1002/mc.20349.
[83] N. Sundahl, J. Bridelance, C. Libert, K. De Bosscher, I.M. Beck, Selective glucocorticoid receptor modulation: new directions with non-steroidal scaffolds, Pharmacol. Ther. 152 (2015) 28–41, https://doi.org/10.1016/j.pharmthera.2015.05.001.
[84] T.R. Flint, D.T. Fearon, T. Janowitz, Connecting the metabolic and immune responses to cancer, Trends Mol. Med. 23 (2017) 451–464, https://doi.org/10.1016/j.molmed.2017.03.001.
[85] S. Sengupta, B. Wasylyk, Physiological and pathological consequences of the interactions of the p53 tumor suppressor with the glucocorticoid, androgen, and estrogen receptors. Ann. N. Y. Acad. Sci., New York Academy of Sciences, 2004, pp. 54–71, https://doi.org/10.1196/annals.1321.005.
[86] M.H. Aziz, H. Shen, C.G. Maki, Glucocorticoid receptor activation inhibits p53-induced apoptosis of MCF10Amyc cells via induction of protein kinase Cε, J. Biol. Chem. 287 (2012) 29825–29836, https://doi.org/10.1074/jbc.M112.393256.
[87] P. Fan, D.R. Siwak, B. Abderrahman, F.A. Agboke, S. Yerrum, V.C. Jordan, Suppression of nuclear factor-kB by glucocorticoid receptor blocks estrogen-induced apoptosis in estrogen-deprived breast cancer cells, Mol. Cancer Ther. 18 (2019) 1684–1695, https://doi.org/10.1158/1535-7163.MCT-18-1363.
[88] J. Ling, R. Kumar, Crosstalk between NFkB and glucocorticoid signaling: a potential target of breast cancer therapy, Cancer Lett. 322 (2012) 119–126, https://doi.org/10.1016/j.canlet.2012.02.033.
[89] W. Wu, T. Pew, M. Zou, D. Pang, S.D. Conzen, Glucocorticoid receptor-induced MAPK phosphatase-1 (MPK-1) expression inhibits paclitaxel-associated MAPK activation and contributes to breast cancer cell survival, J. Biol. Chem. 280 (2005) 4117–4124, https://doi.org/10.1074/jbc.M411200200.
[90] M. Vilasco, L. Communal, N. Mourra, A. Courtin, P. Forgez, A. Gompel, Glucocorticoid receptor and breast cancer, Breast Cancer Res. Treat. 130 (2011) 1–10, https://doi.org/10.1007/s10549-011-1689-6.
[91] E. Ayroldi, L. Cannarile, D.V. Delfino, C. Riccardi, A dual role for glucocorticoid-induced leucine zipper in glucocorticoid function: tumor growth promotion or suppression? Review-article, Cell Death Dis. 9 (2018), https://doi.org/10.1038/s41419-018-0558-1.
[92] A. Yemelyanov, J. Czwornog, D. Chebotaev, A. Karseladze, E. Kulevitch, X. Yang, I. Budunova, Tumor suppressor activity of glucocorticoid receptor in the prostate, Oncogene 26 (2007) 1885–1896, https://doi.org/10.1038/sj.onc.1209991.
[93] L.C. Matthews, A.A. Berry, D.J. Morgan, T.M. Poolman, K. Bauer, F. Kramer, D. G. Spiller, R.V. Richardson, K.E. Chapman, S.N. Farrow, M.R. Norman, A.J. K. Williamson, A.D. Whetton, S.S. Taylor, J.P. Tuckermann, M.R.H. White, D. W. Ray, Glucocorticoid receptor regulates accurate chromosome segregation and is associated with malignancy, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 5479–5484, https://doi.org/10.1073/pnas.1411356112.
[94] M. Voisin, P. De Medina, A. Mallinger, F. Dalenc, E. Huc-Claustre, J. Leignadier, N. Serhan, R. Soules, G. Ségala, A. Mougel, E. Noguer, L. Mhamdi, E. Bacquié, L. Iuliano, C. Zerbinati, M. Lacroix-Triki, L. Chaltiel, T. Filleron, V. Cavaillès, T. Al Saati, P. Rochaix, R. Duprez-Paumier, C. Franchet, L. Ligat, F. Lopez, M. Record, M. Poirot, S. Silvente-Poirot, Identification of a tumor-promoter cholesterol metabolite in human breast cancers acting through the glucocorticoid receptor, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E9346–E9355, https://doi.org/10.1073/pnas.1707965114.
[95] S. Silvente-Poirot, F. Dalenc, M. Poirot, The effects of cholesterol-derived oncometabolites on nuclear receptor function in cancer, Cancer Res. 78 (2018) 4803–4808, https://doi.org/10.1158/0008-5472.CAN-18-1487.
[96] P.J. Davis, H.-Y. Lin, A.A. Hercbergs, K.A. Keating, S.A. Mousa, How thyroid hormone works depends on cell type, receptor type, and hormone analogue: implications in cancer growth, Discov. Med. 27 (2019) 111–117.
[97] A. Pascual, A. Aranda, Thyroid hormone receptors, cell growth and differentiation, Biochim. Biophys. Acta - Gen. Subj. 1830 (2013) 3908–3916, https://doi.org/10.1016/j.bbagen.2012.03.012.
[98] A. Aranda, O. Martínez-Iglesias, L. Ruiz-Llorente, V. García-Carpizo, A. Zambrano, Thyroid receptor: roles in cancer, Trends Endocrinol. Metab. 20 (2009) 318–324, https://doi.org/10.1016/j.tem.2009.03.011.
[99] S.Y. Cheng, J.L. Leonard, P.J. Davis, Molecular aspects of thyroid hormone actions, Endocr. Rev. 31 (2010) 139–170, https://doi.org/10.1210/er.2009-0007.
[100] C. Barlow, B. Meister, M. Lardelli, U. Lendahl, B. Vennström, Thyroid abnormalities and hepatocellular carcinoma in mice transgenic for v-erbA, EMBO J. 13 (1994) 4241–4250, https://doi.org/10.1002/j.1460-2075.1994.tb06744.x.
[101] X.G. Zhu, L. Zhao, M.C. Willingham, S.Y. Cheng, Thyroid hormone receptors are tumor suppressors in a mouse model of metastatic follicular thyroid carcinoma, Oncogene 29 (2010) 1909–1919, https://doi.org/10.1038/onc.2009.476.
[102] C.J. Guigon, D.W. Kim, M.C. Willingham, S.Y. Cheng, Mutation of thyroid hormone receptor-B in mice predisposes to the development of mammary tumors, Oncogene 30 (2011) 3384–3390, https://doi.org/10.1038/onc.2011.50.
[103] O. Martínez-Iglesias, S. Garcia-Silva, S.P. Tenbaum, J. Regadera, F. Larcher, J. M. Paramio, B. Vennström, A. Aranda, Thyroid hormone receptor β1 acts as a potent suppressor of tumor invasiveness and metastasis, Cancer Res. 69 (2009) 501–509, https://doi.org/10.1158/0008-5472.CAN-08-2198.
[104] A. Francavilla, B.I. Carr, A. Azzarone, L. Polimeno, Z. Wang, D.H. Van Thiel, V. Subbotin, J.G. Prelich, T.E. Starzl, Hepatocyte proliferation and gene expression induced by triiodothyronine in vivo and in vitro, Hepatology 20 (1994) 1237–1241, https://doi.org/10.1002/hep.1840200521.
[105] R. López-Fontal, M. Zeini, P.G. Través, M. Gómez-Ferrería, A. Aranda, G.T. Sáez, C. Cerdá, P. Martín-Sanz, S. Hortelano, L. Boscá, Mice lacking thyroid hormone receptor β show enhanced apoptosis and delayed liver commitment for proliferation after partial hepatectomy, PLoS One 5 (2010), https://doi.org/10.1371/journal.pone.0008710.
[106] G.M. Ledda-Columbano, A. Perra, R. Loi, H. Shinozuka, A. Columbano, Cell proliferation induced by triiodothyronine in rat liver is associated with nodule regression and reduction of hepatocellular carcinomas, Cancer Res. 60 (2000).
[107] A. Reddy, C. Dash, A. Leerapun, T.A. Mettler, L.M. Stadheim, K.N. Lazaridis, R. O. Roberts, L.R. Roberts, Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease, Clin. Gastroenterol. Hepatol. 5 (2007) 118–123, https://doi.org/10.1016/j.cgh.2006.07.011.
[108] C. Frau, R. Loi, A. Petrelli, A. Perra, S. Menegon, M.A. Kowalik, S. Pinna, V. P. Leoni, F. Fornari, L. Gramantieri, G.M. Ledda-Columbano, S. Giordano, A. Columbano, Local hypothyroidism favors the progression of preneoplastic lesions to hepatocellular carcinoma in rats, Hepatology 61 (2015) 249–259, https://doi.org/10.1002/hep.27399.
[109] E. Kress, S. Skah, M. Sirakov, J. Nadjar, N. Gadot, J.Y. Scoazec, J. Samarut, M. Plateroti, Cooperation between the thyroid hormone receptor TRα1 and the WNT pathway in the induction of intestinal tumorigenesis, Gastroenterology 138 (2010) 74–1863, https://doi.org/10.1053/j.gastro.2010.01.041.
[110] S. Markowitz, M. Haut, T. Stellato, C. Gerbic, K. Molkentin, Expression of the ErbA-β class of thyroid hormone receptors is selectively lost in human colon carcinoma, J. Clin. Invest. 84 (1989) 1683–1687, https://doi.org/10.1172/JCI114349.
[111] T.T. Hörkkö, K. Tuppurainen, S.M. George, P. Jernvall, T.J. Karttunen, M. J. Mäkinen, Thyroid hormone receptor β1 in normal colon and colorectal cancer-association with differentiation, polypoid growth type and K-ras mutations, Int. J. Cancer 118 (2006) 1653–1659, https://doi.org/10.1002/ijc.21556.
[112] K.J. Jerzak, J.G. Cockburn, S.K. Dhesy-Thind, G.R. Pond, K.I. Pritchard, S. Nofech-Mozes, P. Sun, S.A. Narod, A. Bane, Thyroid hormone receptor beta-1 expression in early breast cancer: a validation study, Breast Cancer Res. Treat. 171 (2018) 709–717, https://doi.org/10.1007/s10549-018-4844-5.
[113] K.J. Jerzak, J. Cockburn, G.R. Pond, K.I. Pritchard, S.A. Narod, S.K. Dhesy-Thind, A. Bane, Thyroid hormone receptor α in breast cancer: prognostic and therapeutic implications, Breast Cancer Res. Treat. 149 (2015) 293–301, https://doi.org/10.1007/s10549-014-3235-9.
[114] M. Cristofanilli, Y. Yamamura, S.W. Kau, T. Bevers, S. Strom, M. Patangan, L. Hsu, S. Krishnamurthy, R.L. Theriault, G.N. Hortobagyi, Thyroid hormone and breast carcinoma: primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma, Cancer 103 (2005) 1122–1128, https://doi.org/10.1002/cncr.20881.
[115] O. Martínez-Iglesias, S. García-Silva, J. Regadera, A. Aranda, Hypothyroidism enhances tumor invasiveness and metastasis development, PLoS One 4 (2009), https://doi.org/10.1371/journal.pone.0006428.
[116] M. Collado, M. Serrano, Senescence in tumours: evidence from mice and humans, Nat. Rev. Cancer 10 (2010) 51–57, https://doi.org/10.1038/nrc2772.
[117] A. Zambrano, V. García-Carpizo, M.E. Gallardo, R. Villamuera, M.A. Gómez-Ferrería, A. Pascual, N. Buisine, L.M. Sachs, R. Garesse, A. Aranda, The thyroid hormone receptor β induces DNA damage and premature senescence, J. Cell Biol. 204 (2014) 129–146, https://doi.org/10.1083/jcb.201305084.
[118] L. Ruiz-Llorente, S. Ardila-González, L.F. Fanjul, O. Martínez-Iglesias, A. Aranda, microRNAs 424 and 503 are mediators of the anti-proliferative and anti-invasive

action of the thyroid hormone receptor beta, Oncotarget 5 (2014) 2918–2933, https://doi.org/10.18632/oncotarget.1577.
[119] E. Alonso-Merino, R.M. Orozco, L. Ruíz-Llorente, O.A. Martínez-Iglesias, J. P. Velasco-Martín, A. Montero-Pedrazuela, L. Fanjul-Rodríguez, C. Contreras-Jurado, J. Regadera, A. Aranda, Thyroid hormones inhibit TGF-β signaling and attenuate fibrotic responses, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E3451–E3460, https://doi.org/10.1073/pnas.1506113113.
[120] O.A. Martínez-Iglesias, E. Alonso-Merino, S. Gómez-Rey, J.P. Velasco-Martín, R. M. Orozco, E. Luengo, R.G. Martín, I. Ibáñez De Cáceres, A.F. Fernández, M. F. Fraga, P. González-Peramato, C. Varona, J. Palacios, J. Regadera, A. Aranda, Autoregulatory loop of nuclear corepressor 1 expression controls invasion, tumor growth, and metastasis, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E328–E337, https://doi.org/10.1073/pnas.1520469113.
[121] O. Martínez-Iglesias, D. Olmeda, E. Alonso-Merino, S. Gómez-Rey, A.M. González-López, E. Luengo, M.S. Soengas, J. Palacios, J. Regadera, A. Aranda, The nuclear corepressor 1 and the thyroid hormone receptor β suppress breast tumor lymphangiogenesis, Oncotarget 7 (2016) 78971–78984, https://doi.org/10.18632/oncotarget.12978.
[122] A.J. Minn, G.P. Gupta, P.M. Siegel, P.D. Bos, W. Shu, D.D. Giri, A. Viale, A. B. Olshen, W.L. Gerald, J. Massagué, Genes that mediate breast cancer metastasis to lung, Nature 436 (2005) 518–524, https://doi.org/10.1038/nature03799.
[123] P.J. Stephens, P.S. Tarpey, H. Davies, P. Van Loo, C. Greenman, D.C. Wedge, S. Nik-Zainal, S. Martin, I. Varela, G.R. Bignell, L.R. Yates, E. Papaemmanuil, D. Beare, A. Butler, A. Cheverton, J. Gamble, J. Hinton, M. Jia, A. Jayakumar, D. Jones, C. Latimer, K.W. Lau, S. McLaren, D.J. McBride, A. Menzies, L. Mudie, K. Raine, R. Rad, M.S. Chapman, J. Teague, D. Easton, A. Langerød, R. Karesen, E. Schlichting, B. Naume, T. Sauer, L. Ottestad, M.T.M. Lee, C.Y. Shen, B.T.K. Tee, B.W. Huimin, A. Broeks, A.C. Vargas, G. Turashvili, J. Martens, A. Fatima, P. Miron, S.F. Chin, G. Thomas, S. Boyault, O. Mariani, S.R. Lakhani, M. Van De Vijver, L. Van't Veer, J. Foekens, C. Desmedt, C. Sotiriou, A. Tutt, C. Caldas, J. S. Reis-Filho, S.A.J.R. Aparicio, A.V. Salomon, A.L. Børresen-Dale, A. L. Richardson, P.J. Campbell, P.A. Futreal, M.R. Stratton, The landscape of cancer genes and mutational processes in breast cancer, Nature 486 (2012) 400–404, https://doi.org/10.1038/nature11017.
[124] J.E. Visvader, G.J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions, Nat. Rev. Cancer 8 (2008) 755–768, https://doi.org/10.1038/nrc2499.
[125] I. López-Mateo, E. Alonso-Merino, C. Suarez-Cabrera, J.W. Park, S.Y. Cheng, S. Alemany, J.M. Paramio, A. Aranda, Thyroid hormone receptor β inhibits self-renewal capacity of breast cancer stem cells, Thyroid 30 (2020) 116–132, https://doi.org/10.1089/thy.2019.0175.
[126] V. Duong, C. Rochette-Egly, The molecular physiology of nuclear retinoic acid receptors. From health to disease, Biochim. Biophys. Acta - Mol. Basis Dis. 1812 (2011) 1023–1031, https://doi.org/10.1016/j.bbadis.2010.10.007.
[127] L. Altucci, M.D. Leibowitz, K.M. Ogilvie, A.R. de Lera, H. Gronemeyer, RAR and RXR modulation in cancer and metabolic disease, Nat. Rev. Drug Discov. 6 (2007) 793–810, https://doi.org/10.1038/nrd2397.
[128] M. Liu, A. Iavarone, L.P. Freedman, Transcriptional activation of the human p21 (WAF1/CIP1) gene by retinoic acid receptor. Correlation with retinoid induction of U937 cell differentiation, J. Biol. Chem. 271 (1996) 31723–31728, https://doi.org/10.1074/jbc.271.49.31723.
[129] A.M. Jiménez-Lara, N. Clarke, L. Altucci, H. Gronemeyer, Retinoic-acid-induced apoptosis in leukemia cells, Trends Mol. Med. 10 (2004) 15–508, https://doi.org/10.1016/j.molmed.2004.08.006.
[130] O. di Martino, J.S. Welch, Retinoic acid receptors in acute myeloid leukemia therapy, Cancers (Basel) 11 (2019), https://doi.org/10.3390/cancers11121915.
[131] F.F. Ferrara, F. Fazi, A. Bianchini, F. Padula, V. Gelmetti, S. Minucci, M. Mancini, P.G. Pelicci, F. Lo Coco, C. Nervi, Histone deacetylase-targeted treatment restores retinoic acid signaling and differentiation in acute myeloid leukemia, Cancer Res. 61 (2001) 2–7.
[132] N. Bushue, Y.J.Y. Wan, Retinoid pathway and cancer therapeutics, Adv. Drug Deliv. Rev. 62 (2010) 98–1285, https://doi.org/10.1016/j.addr.2010.07.003.
[133] X.C. Xu, Tumor-suppressive activity of retinoic acid receptor-β in cancer, Cancer Lett. 253 (2007) 14–24, https://doi.org/10.1016/j.canlet.2006.11.019.
[134] A. Leder, A. Kuo, R.D. Cardiff, E. Sinn, P. Leder, v-Ha-ras transgene abrogates the initiation step in mouse skin tumorigenesis: effects of phorbol esters and retinoic acid, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 82–9178, https://doi.org/10.1073/pnas.87.23.9178.
[135] L. Costantini, R. Molinari, B. Farinon, N. Merendino, Retinoic acids in the treatment of most lethal solid cancers, J. Clin. Med. 9 (2020) 360, https://doi.org/10.3390/jcm9020360.
[136] I.P. Uray, E. Dmitrovsky, P.H. Brown, Retinoids and rexinoids in cancer prevention: from laboratory to clinic, Semin. Oncol. 43 (2016) 49–64, https://doi.org/10.1053/j.seminoncol.2015.09.002.
[137] A. Papi, S. De Carolis, S. Bertoni, G. Storci, V. Sceberras, D. Santini, C. Ceccarelli, M. Taffurelli, M. Orlandi, M. Bonafé, PPARγ and RXR ligands disrupt the inflammatory cross-talk in the hypoxic breast cancer stem cells niche, J. Cell. Physiol. 229 (2014) 1595–1606, https://doi.org/10.1002/jcp.24601.
[138] M. Cao, D.B. Royce, R. Risingsong, C.R. Williams, M.B. Sporn, K.T. Liby, The rexinoids LG100268 and LG101506 inhibit inflammation and suppress lung carcinogenesis in A/J mice, Cancer Prev. Res. Phila. (Phila) 9 (2016) 105–114, https://doi.org/10.1158/1940-6207.CAPR-15-0325.
[139] R. Ocadiz-Delgado, E. Castaneda-Saucedo, A.K. Indra, R. Hernandez-Pando, P. Gariglio, Impaired cervical homeostasis upon selective ablation of RXRα in epithelial cells, Genesis 46 (2008) 19–28, https://doi.org/10.1002/dvg.20357.
[140] J. Huang, W.C. Powell, A.C. Khodavirdi, J. Wu, T. Makita, R.D. Cardiff, M. B. Cohen, H.M. Sucov, P. Roy-Burman, Prostatic intraepithelial neoplasia in mice with conditional disruption of the retinoid X receptor α allele in the prostate epithelium, Cancer Res. 62 (2002).
[141] C. Zhong, S. Yang, J. Huang, M.B. Cohen, P. Roy-Burman, Aberration in the expression of the retinoid receptor, RXRα, in prostate cancer, Cancer Biol. Ther. 2 (2003) 179–184, https://doi.org/10.4161/cbt.2.2.281.
[142] N. Ando, M. Shimizu, M. Okuno, R. Matsushima-Nishiwaki, H. Tsurumi, T. Tanaka, H. Moriwaki, Expression of retinoid X receptor α is decreased in 3′-methyl-4-dimethylaminoazobenzene-induced hepatocellular carcinoma in rats, Oncol. Rep. 18 (2007) 879–884, https://doi.org/10.3892/or.18.4.879.
[143] Y. Takiyama, N. Miyokawa, A. Sugawara, S. Kato, K. Ito, K. Sato, K. Oikawa, H. Kobayashi, S. Kimura, M. Tateno, Decreased expression of retinoid X receptor isoforms in human thyroid carcinomas, J. Clin. Endocrinol. Metab. 89 (2004) 5851–5861, https://doi.org/10.1210/jc.2003-032036.
[144] K. Yamazaki, M. Shimizu, M. Okuno, R. Matsushima-Nishiwaki, N. Kanemura, H. Araki, H. Tsurumi, S. Kojima, I.B. Weinstein, H. Moriwaki, Synergistic effects of RXRα and PPARγ ligands to inhibit growth in human colon cancer cells - phosphorylated RXRα is a critical target for colon cancer management, Gut 56 (2007) 1557–1563, https://doi.org/10.1136/gut.2007.129858.
[145] R. Matsushima-Nishiwaki, M. Okuno, S. Adachi, T. Sano, K. Akita, H. Moriwaki, S. L. Friedman, S. Kojima, Phosphorylation of retinoid X receptor α at serine 260 impairs its metabolism and function in human hepatocellular carcinoma, Cancer Res. 61 (2001).
[146] G. Guo, X. Sun, C. Chen, S. Wu, P. Huang, Z. Li, M. Dean, Y. Huang, W. Jia, Q. Zhou, A. Tang, Z. Yang, X. Li, P. Song, X. Zhao, R. Ye, S. Zhang, Z. Lin, M. Qi, S. Wan, L. Xie, F. Fan, M.L. Nickerson, X. Zou, X. Hu, L. Xing, Z. Lv, H. Mei, S. Gao, C. Liang, Z. Gao, J. Lu, Y. Yu, C. Liu, L. Li, X. Fang, Z. Jiang, J. Yang, C. Li, X. Zhao, J. Chen, F. Zhang, Y. Lai, Z. Lin, F. Zhou, H. Chen, H.C. Chan, S. Tsang, D. Theodorescu, Y. Li, X. Zhang, J. Wang, H. Yang, Y. Gui, J. Wang, Z. Cai, Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation, Nat. Genet. 45 (2013) 63–1459, https://doi.org/10.1038/ng.2798.
[147] J.N. Weinstein, R. Akbani, B.M. Broom, W. Wang, R.G.W. Verhaak, D. McConkey, et al., Comprehensive molecular characterization of urothelial bladder carcinoma, Nature 507 (2014) 22–315, https://doi.org/10.1038/nature12965.
[148] J.T. Goldstein, A.C. Berger, J. Shih, F.F. Duke, L. Furst, D.J. Kwiatkowski, A. D. Cherniack, M. Meyerson, C.A. Strathdee, Genomic activation of PPARG reveals a candidate therapeutic axis in bladder cancer, Cancer Res. 77 (2017) 6987–6998, https://doi.org/10.1158/0008-5472.CAN-17-1701.
[149] R. Matsushima-Nishiwaki, Y. Shidoji, S. Nishiwaki, T. Yamada, H. Moriwaki, Y. Muto, Aberrant metabolism of retinoid X receptor proteins in human hepatocellular carcinoma, Mol. Cell. Endocrinol. 121 (1996) 179–190, https://doi.org/10.1016/0303-7207(96)03863-4.
[150] K. Prüfer, C. Schröder, K. Hegyi, J. Barsony, Degradation of RXRs influences sensitivity of rat osteosarcoma cells to the antiproliferative effects of calcitriol, Mol. Endocrinol. 16 (2002) 961–976, https://doi.org/10.1210/mend.16.5.0821.
[151] H. Zhou, W. Liu, Y. Su, Z. Wei, J. Liu, S.K. Kolluri, H. Wu, Y. Cao, J. Chen, Y. Wu, T. Yan, X. Cao, W. Gao, A. Molotkov, F. Jiang, W.G. Li, B. Lin, H.P. Zhang, J. Yu, S.P. Luo, J.Z. Zeng, G. Duester, P.Q. Huang, X.K. Zhang, NSAID Sulindac and Its Analog Bind RXRα and Inhibit RXRα-Dependent AKT Signaling, Cancer Cell 17 (2010) 560–573, https://doi.org/10.1016/j.ccr.2010.04.023.
[152] X. Ye, H. Wu, L. Sheng, Y. xin Liu, F. Ye, M. Wang, H. Zhou, Y. Su, X. kun Zhang, Oncogenic potential of truncated RXRα during colitis-associated colorectal tumorigenesis by promoting IL-6-STAT3 signaling, Nat. Commun. 10 (2019), https://doi.org/10.1038/s41467-019-09375-8.
[153] N.J. Bodsworth, M. Block, M. Bower, D. Donnell, R. Yocum, Phase III vehicle-controlled, multi-centered study of topical alitretinoin gel 0.1% in cutaneous AIDS-related Kaposi's sarcoma, Am. J. Clin. Dermatol. 2 (2001) 77–87, https://doi.org/10.2165/00128071-200102020-00004.
[154] F. Recchia, A. Lalli, M. Lombardo, S. De Filippis, G. Saggio, F. Fabbri, M. Rosselli, E. Capomolla, S. Rea, Ifosfamide, cisplatin, and 13-Cis retinoic acid for patients with advanced or recurrent squamous cell carcinoma of the head and neck: a phase I-II study, Cancer 92 (2001) 814–821, https://doi.org/10.1002/1097-0142(20010815)92:4<814::AID-CNCR1387>3.0.CO;2-8.
[155] L. Amoroso, R. Haupt, A. Garaventa, M. Ponzoni, Investigational drugs in phase II clinical trials for the treatment of neuroblastoma, Expert Opin. Investig. Drugs 26 (2017) 1281–1293, https://doi.org/10.1080/13543784.2017.1380625.
[156] M. Duvic, K. Hymes, P. Heald, D. Breneman, A.G. Martin, P. Myskowski, C. Crowley, R.C. Yocum, Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous t-cell lymphoma: multinational phase II-III trial results, J. Clin. Oncol. 19 (2001) 2456–2471, https://doi.org/10.1200/JCO.2001.19.9.2456.
[157] T.W. Hermann, W.C. Yen, P. Tooker, B. Fan, K. Roegner, A. Negro-Vilar, W. W. Lamph, R.P. Bissonnette, The retinoid X receptor agonist bexarotene (Targretin) synergistically enhances the growth inhibitory activity of cytotoxic drugs in non-small cell lung cancer cells, Lung Cancer 50 (2005) 9–18, https://doi.org/10.1016/j.lungcan.2005.05.008.
[158] W.C. Yen, M.R. Corpuz, R.Y. Prudente, T.A. Cooke, R.P. Bissonnette, A. Negro-Vilar, W.W. Lamph, A selective retinoid X receptor agonist bexarotene (Targretin) prevents and overcomes acquired paclitaxel (Taxol) resistance in human non-small cell lung cancer, Clin. Cancer Res. 10 (2004) 8656–8664, https://doi.org/10.1158/1078-0432.CCR-04-0979.
[159] G.R. Blumenschein, F.R. Khuri, J. Von Pawel, U. Gatzemeier, W.H. Miller, R. M. Jotte, J. Le Treut, S.L. Sun, J.K. Zhang, Z.E. Dziewanowska, A. Negro-Vilar, Phase III trial comparing carboplatin, paclitaxel, and bexarotene with carboplatin

and paclitaxel in chemotherapy-naïve patients with advanced or metastatic non-small-cell lung cancer: SPIRIT II, J. Clin. Oncol. 26 (2008) 1879–1885, https://doi.org/10.1200/JCO.2007.12.2689.
[160] R. Ramlau, P. Zatloukal, J. Jassem, P. Schwarzenberger, S.V. Orlov, M. Gottfried, J.R. Pereira, G. Temperley, R. Negro-Vilar, S. Rahal, J.K. Zhang, A. Negro-Vilar, Z. E. Dziewanowska, Randomized phase III trial comparing bexarotene (L1069-49)/ cisplatin/ vinorelbine with cisplatin/vinorelbine in chemotherapy-naïve patients with advanced or metastatic non-small-cell lung cancer: SPIRIT I, J. Clin. Oncol. 26 (2008) 1886–1892, https://doi.org/10.1200/JCO.2007.12.2614.
[161] L. Altucci, A. Rossin, O. Hirsch, A. Nebbioso, D. Vitoux, E. Wilhelm, F. Guidez, M. De Simone, E.M. Schiavone, D. Grimwade, A. Zelent, H. De Thé, H. Gronemeyer, Rexinoid-triggered differentiation and tumor-selective apoptosis of acute myeloid leukemia by protein kinase A-mediated desubordination of retinoid X receptor, Cancer Res. 65 (2005) 8754–8765, https://doi.org/10.1158/0008-5472.CAN-04-3569.
[162] J.S. Welch, H. Niu, G.L. Uy, P. Westervelt, C.N. Abboud, R. Vij, K.E. Stockerl-Goldstein, M. Jacoby, I. Pusic, M.A. Schroeder, J.F. Dipersio, A.F. Cashen, A phase I dose escalation study of oral bexarotene in combination with intravenous decitabine in patients with AML, Am. J. Hematol. 89 (2014), https://doi.org/10.1002/ajh.23735.
[163] P.V. Sanchez, S.T. Glantz, S. Scotland, M.T. Kasner, M. Carroll, Induced differentiation of acute myeloid leukemia cells by activation of retinoid X and liver X receptors, Leukemia 28 (2014) 749–760, https://doi.org/10.1038/leu.2013.202.
[164] M. Shimizu, Y. Shirakami, K. Imai, K. Takai, H. Moriwaki, Acyclic retinoid in chemoprevention of hepatocellular carcinoma: targeting phosphorylated retinoid X receptor for prevention of liver carcinogenesis, J. Carcinog. 11 (2012), https://doi.org/10.4103/1477-3163.100398.
[165] M. Honda, T. Yamashita, T. Yamashita, K. Arai, Y. Sakai, A. Sakai, M. Nakamura, E. Mizukoshi, S. Kaneko, Peretinoin, an acyclic retinoid, improves the hepatic gene signature of chronic hepatitis C following curative therapy of hepatocellular carcinoma, BMC Cancer 13 (2013), https://doi.org/10.1186/1471-2407-13-191.
[166] G.H. Wang, F.Q. Jiang, Y.H. Duan, Z.P. Zeng, F. Chen, Y. Dai, J.B. Chen, J.X. Liu, J. Liu, H. Zhou, H.F. Chen, J.Z. Zeng, Y. Su, X.S. Yao, X.K. Zhang, Targeting truncated retinoid X receptor-α by CF31 induces TNF-α-dependent apoptosis, Cancer Res. 73 (2013) 307–318, https://doi.org/10.1158/0008-5472.CAN-12-2038.
[167] J. Zhu, R. Nasr, L. Pérès, F. Riaucoux-Lormière, N. Honoré, C. Berthier, D. Kamashev, J. Zhou, D. Vitoux, C. Lavau, H. de Thé, RXR is an essential component of the oncogenic PML/RARA complex in vivo, Cancer Cell 12 (2007) 23–35, https://doi.org/10.1016/j.ccr.2007.06.004.
[168] B.B. Zeisig, C. Kwok, A. Zelent, P. Shankaranarayanan, H. Gronemeyer, S. Dong, C.W.E. So, Recruitment of RXR by homotetrameric RARα fusion proteins is essential for transformation, Cancer Cell 12 (2007) 36–51, https://doi.org/10.1016/j.ccr.2007.06.006.
[169] J.H.A. Martens, A.B. Brinkman, F. Simmer, K.J. Francoijs, A. Nebbioso, F. Ferrara, L. Altucci, H.G. Stunnenberg, PML-RARα/RXR alters the epigenetic landscape in acute promyelocytic leukemia, Cancer Cell 17 (2010) 173–185, https://doi.org/10.1016/j.ccr.2009.12.042.
[170] M. Kiss, Z. Czimmerer, G. Nagy, P. Bieniasz-Krzywiec, M. Ehling, A. Pap, S. Poliska, P. Boto, P. Tzerpos, A. Horvath, Z. Kolostyak, B. Daniel, I. Szatmari, M. Mazzone, L. Nagy, Retinoid X receptor suppresses a metastasis-promoting transcriptional program in myeloid cells via a ligand-insensitive mechanism, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 10725–10730, https://doi.org/10.1073/pnas.1700785114.
[171] M. Casanova-Acebes, M.P. Menéndez-Gutiérrez, J. Porcuna, D. Álvarez-Errico, Y. Lavin, A. García, S. Kobayashi, J. Le Berichel, V. Núñez, F. Were, D. Jiménez-Carretero, F. Sánchez-Cabo, M. Merad, M. Ricote, RXRs control serous macrophage neonatal expansion and identity and contribute to ovarian cancer progression, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-020-15371-0.
[172] M.P. Menendez-Gutierrez, T. Roszer, M. Ricote, Biology and therapeutic applications of peroxisome proliferator- activated receptors, Curr. Top. Med. Chem. 12 (2012) 548–584, https://doi.org/10.2174/156802612799436669.
[173] J.N. Feige, L. Gelman, L. Michalik, B. Desvergne, W. Wahli, From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions, Prog. Lipid Res. 45 (2006) 120–159, https://doi.org/10.1016/j.plipres.2005.12.002.
[174] I. Takada, M. Makishima, Peroxisome proliferator-activated receptor agonists and antagonists: a patent review (2014-present), Expert Opin. Ther. Pat. 30 (2020) 1–13, https://doi.org/10.1080/13543776.2020.1703952.
[175] H.S. Cheng, W.R. Tan, Z.S. Low, C. Marvalim, J.Y.H. Lee, N.S. Tan, Exploration and development of PPAR modulators in health and disease: an update of clinical evidence, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20205055.
[176] S.R. Pyper, N. Viswakarma, S. Yu, J.K. Reddy, PPARalpha: energy combustion, hypolipidemia, inflammation and cancer, Nucl. Recept. Signal. 8 (2010), https://doi.org/10.1621/nrs.08002.
[177] L. Michalik, B. Desvergne, W. Wahli, Peroxisome-proliferator-activated receptors and cancers: complex stories, Nat. Rev. Cancer 4 (2004) 61–70, https://doi.org/10.1038/nrc1254.
[178] J.M. Peters, Y.M. Shah, F.J. Gonzalez, The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention, Nat. Rev. Cancer 12 (2012) 181–195, https://doi.org/10.1038/nrc3214.
[179] S.S. Lee, T. Pineau, J. Drago, E.J. Lee, J.W. Owens, D.L. Kroetz, P.M. Fernandez-Salguero, H. Westphal, F.J. Gonzalez, Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators, Mol. Cell. Biol. 15 (1995) 3012–3022, https://doi.org/10.1128/mcb.15.6.3012.
[180] J. Ashby, A. Brady, C.R. Elcombe, B.M. Elliott, J. Ishmael, J. Odum, J. D. Tugwood, S. Kettle, I.F.H. Purchase, Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis, Hum. Exp. Toxicol. 13 (1994), https://doi.org/10.1177/096032719401300201.
[181] S.A. Saha, L.G. Kizhakepunnur, A. Bahekar, R.R. Arora, The role of fibrates in the prevention of cardiovascular disease-a pooled meta-analysis of long-term randomized placebo-controlled clinical trials, Am. Heart J. 154 (2007) 943–953, https://doi.org/10.1016/j.ahj.2007.07.011.
[182] J.C. Corton, J.M. Peters, J.E. Klaunig, The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions, Arch. Toxicol. 92 (2018) 83–119, https://doi.org/10.1007/s00204-017-2094-7.
[183] Y. Xi, Y. Zhang, S. Zhu, Y. Luo, P. Xu, Z. Huang, PPAR-mediated toxicology and applied pharmacology, Cells 9 (2020) 352, https://doi.org/10.3390/cells9020352.
[184] K. Tachibana, D. Yamasaki, K. Ishimoto, T. Doi, The role of PPARs in cancer, PPAR Res. 2008 (2008), https://doi.org/10.1155/2008/102737.
[185] K. Morimura, C. Cheung, J.M. Ward, J.K. Reddy, F.J. Gonzalez, Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor a to Wy-14,643-induced liver tumorigenesis, (n.d.). https://doi.org/10.1093/carcin/bgi329.
[186] Q. Yang, T. Nagano, Y. Shah, C. Cheung, S. Ito, F.J. Gonzalez, The PPARa-Humanized mouse: a model to investigate species differences in liver toxicity mediated by PPARa, Toxicol. Sci. 101 (2008) 132–139, https://doi.org/10.1093/toxsci/kfm206.
[187] Y.M. Shah, K. Morimura, Q. Yang, T. Tanabe, M. Takagi, F.J. Gonzalez, Peroxisome proliferator-activated receptor α regulates a MicroRNA-mediated signaling cascade responsible for hepatocellular proliferation, Mol. Cell. Biol. 27 (2007) 4238–4247, https://doi.org/10.1128/mcb.00317-07.
[188] S.P. Lakshmi, A.T. Reddy, A. Banno, R.C. Reddy, PPAR Agonists for the Prevention and Treatment of Lung Cancer, 2017, https://doi.org/10.1155/2017/8252796.
[189] L. Wu, W. Wang, M. Dai, H. Li, C. Chen, D. Wang, PPARα ligand, AVE8134, and cyclooxygenase inhibitor therapy synergistically suppress lung cancer growth and metastasis, BMC Cancer 19 (2019), https://doi.org/10.1186/s12885-019-6379-5.
[190] A. Fidoamore, L. Cristiano, C. Laezza, R. Galzio, E. Benedetti, B. Cinque, A. Antonosante, M. d'Angelo, V. Castelli, M.G. Cifone, R. Ippoliti, A. Giordano, A. Cimini, Energy metabolism in glioblastoma stem cells: PPARα a metabolic adaptor to intratumoral microenvironment, Oncotarget 8 (2017) 108430–108450, https://doi.org/10.18632/oncotarget.19086.
[191] A. Pozzi, M.R. Ibanez, A.E. Gatica, S. Yang, S. Wei, S. Mei, J.R. Falck, J. H. Capdevila, Peroxisomal proliferator-activated receptor-α-dependent inhibition of endothelial cell proliferation and tumorigenesis, J. Biol. Chem. 282 (2007) 17685–17695, https://doi.org/10.1074/jbc.M701429200.
[192] D. Panigrahy, A. Kaipainen, S. Huang, C.E. Butterfield, C.M. Barnés, M. Fannon, A.M. Laforme, D.M. Chaponis, J. Folkman, M.W. Kieran, PPARα agonist fenofibrate suppresses tumor growth through direct and indirect angiogenesis inhibition, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 985–990, https://doi.org/10.1073/pnas.0711281105.
[193] M. Ricote, C.K. Glass, PPARs and molecular mechanisms of transrepression, Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1771 (2007) 926–935, https://doi.org/10.1016/j.bbalip.2007.02.013.
[194] T. Aoyama, J.M. Peters, N. Iritani, T. Nakajima, K. Furihata, T. Hashimoto, F. J. Gonzalez, Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPARα), J. Biol. Chem. 273 (1998) 5678–5684, https://doi.org/10.1074/jbc.273.10.5678.
[195] S. Kersten, S. Mandard, P. Escher, F.J. Gonzalez, S. Tafuri, B. Desvergne, W. Wahli, The peroxisome proliferator-activated receptor α regulates amino acid metabolism, FASEB J. 15 (2001) 1971–1978, https://doi.org/10.1096/fj.01-0147com.
[196] Y. Luo, C. Xie, C.N. Brocker, J. Fan, X. Wu, L. Feng, Q. Wang, J. Zhao, D. Lu, M. Tandon, M. Cam, K.W. Krausz, W. Liu, F.J. Gonzalez, Intestinal PPARα protects against colon carcinogenesis via regulation of methyltransferases DNMT1 and PRMT6, Gastroenterology 157 (2019) 744–759, https://doi.org/10.1053/j.gastro.2019.05.057, e4.
[197] X. Zuo, W. Xu, M. Xu, R. Tian, M.J. Moussalli, F. Mao, X. Zheng, J. Wang, J. S. Morris, M. Gagea, C. Eng, S. Kopetz, D.M. Maru, A. Rashid, R. Broaddus, D. Wei, M.-C. Hung, A.K. Sood, I. Shureiqi, Metastasis regulation by PPARD expression in cancer cells, JCI Insight 2 (2017), https://doi.org/10.1172/jci.insight.91419.
[198] M. Yoshinaga, K. Taki, S. Somada, Y. Sakiyama, N. Kubo, T. Kaku, S. Tsuruta, T. Kusumoto, H. Sakai, K. Nakamura, R. Takayanagi, Y. Muto, The expression of both peroxisome proliferator-activated receptor delta and cyclooxygenase-2 in tissues is associated with poor prognosis in colorectal cancer patients, Dig. Dis. Sci. 56 (2011) 1194–1200, https://doi.org/10.1007/s10620-010-1389-9.
[199] O. Takayama, H. Yamamoto, B. Damdinsuren, Y. Sugita, C.Y. Ngan, X. Xu, T. Tsujino, I. Takemasa, M. Ikeda, M. Sekimoto, N. Matsuura, M. Monden, Expression of PPARδ in multistage carcinogenesis of the colorectum: implications of malignant cancer morphology, Br. J. Cancer 95 (2006) 889–895, https://doi.org/10.1038/sj.bjc.6603343.
[200] A. Abdollahi, C. Schwager, J. Kleeff, I. Esposito, S. Domhan, P. Peschke, K. Hauser, P. Hahnfeldt, L. Hlatky, J. Debus, J.M. Peters, H. Friess, J. Folkman, P. E. Huber, Transcriptional network governing the angiogenic switch in human pancreatic cancer, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12890–12895, https://doi.org/10.1073/pnas.0705505104.

[201] T.V. Pedchenko, A.L. Gonzalez, D.Z. Wang, R.N. DuBois, P.P. Massion, Peroxisome proliferator-activated receptor β/δ expression and activation in lung cancer, Am. J. Respir. Cell Mol. Biol. 39 (2008) 689–696, https://doi.org/10.1165/rcmb.2007-0426OC.
[202] R. Müller, PPARβ/δ in human cancer, Biochimie 136 (2017) 90–99, https://doi.org/10.1016/j.biochi.2016.10.019.
[203] X. Zuo, M. Xu, J. Yu, Y. Wu, M.J. Moussalli, G.C. Manyam, S. Il Lee, S. Liang, M. Gagea, J.S. Morris, R.R. Broaddus, I. Shureiqi, Potentiation of Colon Cancer Susceptibility in Mice by Colonic Epithelial PPAr-δ/β Overexpression, 2014, p. 106, https://doi.org/10.1093/jnci/dju052.
[204] D. Wang, L. Fu, J. Wei, Y. Xiong, R.N. DuBois, PPARD mediates the effect of dietary fat in promoting colorectal cancer metastasis, Cancer Res. 79 (2019) 4480–4490, https://doi.org/10.1158/0008-5472.CAN-19-0384.
[205] S. Beyaz, M.D. Mana, J. Roper, D. Kedrin, A. Saadatpour, S.J. Hong, K.E. Bauer-Rowe, M.E. Xifaras, A. Akkad, E. Arias, L. Pinello, Y. Katz, S. Shinagare, M. Abu-Remaileh, M.M. Mihaylova, D.W. Lamming, R. Dogum, G. Guo, G.W. Bell, M. Selig, G.P. Nielsen, N. Gupta, C.R. Ferrone, V. Deshpande, G.C. Yuan, S. H. Orkin, D.M. Sabatini, Ö.H. Yilmaz, High-fat diet enhances stemness and tumorigenicity of intestinal progenitors, Nature 531 (2016) 53–58, https://doi.org/10.1038/nature17173.
[206] L. Yang, H. Zhang, Z.G. Zhou, H. Yan, G. Adell, X.F. Sun, Biological function and prognostic significance of peroxisome proliferator-activated receptor δ in rectal cancer, Clin. Cancer Res. 17 (2011) 3760–3770, https://doi.org/10.1158/1078-0432.CCR-10-2779.
[207] N. Martín-Martín, A. Zabala-Letona, S. Fernandez-Ruiz, L. Arreal, L. Camacho, M. Castillo-Martin, A.R. Cortazar, V. Torrano, I. Astobiza, P. Zuñiga-García, A. Ugalde-Olano, A. Loizaga-Iriarte, M. Unda, L. Valcarcel-Jim enez, A. Arruabarrena-Aristorena, M. Piva, P. Sanchez-Mosquera, A.M. Aransay, A. Gomez-Muñoz, R. Barrio, J.D. Sutherland, A. Carracedo, PPARd elicits ligand-independent repression of trefoil factor family to limit prostate cancer growth, Cancer Res. 78 (2018) 399–409, https://doi.org/10.1158/0008-5472.CAN-17-0908.
[208] B. Zhu, R. Bai, M.J. Kennett, B.H. Kang, F.J. Gonzalez, J.M. Peters, Chemoprevention of chemically induced skin tumorigenesis by ligand activation of peroxisome proliferator-activated receptor-β/δ and inhibition of cyclooxygenase 2, Mol. Cancer Ther. 9 (2010) 3267–3277, https://doi.org/10.1158/1535-7163.MCT-10-0820.
[209] M.T. Bility, M.K. Devlin-Durante, N. Blazanin, A.B. Glick, J.M. Ward, H. Kang, M. J. Kennett, F.J. Gonzalez, J.M. Peters, Ligand activation of peroxisome proliferator-activated receptor b/d (PPARb/d) inhibits chemically induced skin tumorigenesis, Carcinogenesis 29 (2008) 2406–2414, https://doi.org/10.1093/carcin/bgn219.
[210] F.S. Harman, C.J. Nicol, H.E. Marin, J.M. Ward, F.J. Gonzalez, J.M. Peters, Peroxisome proliferator-activated receptor-δ attenuates colon carcinogenesis, Nat. Med. 10 (2004) 481–483, https://doi.org/10.1038/nm1026.
[211] X. Zuo, Z. Peng, M.J. Moussalli, J.S. Morris, R.R. Broaddus, S.M. Fischer, I. Shureiqi, Targeted genetic disruption of peroxisome proliferator-activated receptor-δ and colonic tumorigenesis, J. Natl. Cancer Inst. 101 (2009) 7–762, https://doi.org/10.1093/jnci/djp078.
[212] D. Wang, H. Wang, Y. Guo, W. Ning, S. Katkuri, W. Wahli, B. Desvergne, S.K. Dey, R.N. DuBois, Crosstalk between peroxisome proliferator-activated receptor δ and VEGF stimulates cancer progression, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 19069–19074, https://doi.org/10.1073/pnas.0607948103.
[213] S. Müller-Brüsselbach, M. Kömhoff, M. Rieck, W. Meissner, K. Kaddatz, J. Adamkiewicz, B. Keil, K.J. Klose, R. Moll, A.D. Burdick, J.M. Peters, R. Müller, Deregulation of tumor angiogenesis and blockade of tumor growth in PPARβ-deficient mice, EMBO J. 26 (2007) 98–3686, https://doi.org/10.1038/sj.emboj.7601803.
[214] M. Ghosh, Y. Ai, K. Narko, Z. Wang, J.M. Peters, T. Hla, PPARδ is pro-tumorigenic in a mouse model of COX-2-induced mammary cancer, Prostaglandins Other Lipid Mediat. 88 (2009) 97–100, https://doi.org/10.1016/j.prostaglandins.2008.11.004.
[215] S.A. Ham, T. Yoo, J.S. Hwang, E.S. Kang, W.J. Lee, K.S. Paek, C. Park, J.-H. Kim, J.T. Do, D.-S. Lim, H.G. Seo, Ligand-activated PPARδ modulates the migration and invasion of melanoma cells by regulating Snail expression, Am. J. Cancer Res. 4 (2014) 674.
[216] R. Vigneri, L. Sciacca, P. Vigneri, Rethinking the relationship between insulin and cancer, Trends Endocrinol. Metab. 31 (2020), https://doi.org/10.1016/j.tem.2020.05.004.
[217] A. Bundscherer, A. Reichle, C. Hafner, S. Meyer, T. Vogt, Targeting the tumor stroma with peroxisome proliferator activated receptor (PPAR) agonists, Anticancer Agents Med. Chem. 9 (2012) 21–816, https://doi.org/10.2174/187152009789056912.
[218] A. Vallée, Y. Lecarpentier, J.-N. Vallée, Targeting the canonical WNT/β-catenin pathway in cancer treatment using non-steroidal anti-inflammatory drugs, Cells 8 (2019) 726, https://doi.org/10.3390/cells8070726.
[219] E. Fröhlich, R. Wahl, Chemotherapy and chemoprevention by thiazolidinediones, Biomed Res. Int. 2015 (2015) 845340, https://doi.org/10.1155/2015/845340.
[220] G.T. Robbins, D. Nie, PPAR gamma, bioactive lipids, and cancer progression, Front. Biosci. 17 (2012) 1816–1834, https://doi.org/10.2741/4021.
[221] R.E. Soccio, E.R. Chen, M.A. Lazar, Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes, Cell Metab. 20 (2014) 91–573, https://doi.org/10.1016/j.cmet.2014.08.005.
[222] A. Ferrara, J.D. Lewis, C.P. Quesenberry, T. Peng, B.L. Strom, S.K. Van Den Eeden, S.F. Ehrlich, L.A. Habel, Cohort study of pioglitazone and cancer incidence in patients with diabetes, Diabetes Care 34 (2011) 923–929, https://doi.org/10.2337/dc10-1067.
[223] B. Wang, P. Tontonoz, Liver X receptors in lipid signalling and membrane homeostasis, Nat. Rev. Endocrinol. 14 (2018) 452–463, https://doi.org/10.1038/s41574-018-0037-x.
[224] C.P. Chuu, H.P. Lin, Antiproliferative effect of LXR agonists T0901317 and 22(R)-hydroxycholesterol on multiple human cancer cell lines, Anticancer Res. 30 (2010) 3643–3648.
[225] G. Lo Sasso, F. Bovenga, S. Murzilli, L. Salvatore, G. Di Tullio, N. Martelli, A. D'Orazio, S. Rainaldi, M. Vacca, A. Mangia, G. Palasciano, A. Moschetta, Liver X receptors inhibit proliferation of human colorectal cancer cells and growth of intestinal tumors in mice, Gastroenterology 144 (2013) 1497–1507, https://doi.org/10.1053/j.gastro.2013.02.005, e13.
[226] A.J.C. Pommier, G. Alves, E. Viennois, S. Bernard, Y. Communal, B. Sion, G. Marceau, C. Damon, K. Mouzat, F. Caira, S. Baron, J.M.A. Lobaccaro, Liver X Receptor activation downregulates AKT survival signaling in lipid rafts and induces apoptosis of prostate cancer cells, Oncogene 29 (2010) 2712–2723, https://doi.org/10.1038/onc.2010.30.
[227] L.L. Vedin, J.A. Gustafsson, K.R. Steffensen, The oxysterol receptors lxrα and lxrβ suppress proliferation in the colon, Mol. Carcinog. 52 (2013) 835–844, https://doi.org/10.1002/mc.21924.
[228] W. Fu, J. Yao, Y. Huang, Q. Li, W. Li, Z. Chen, F. He, Z. Zhou, J. Yan, LXR agonist regulates the carcinogenesis of PCa via the SOCS3 pathway, Cell. Physiol. Biochem. 33 (2014) 195–204, https://doi.org/10.1159/000356662.
[229] V. Derangère, A. Chevriaux, F. Courtaut, M. Bruchard, H. Berger, F. Chalmin, S. Z. Causse, E. Limagne, F. Végran, S. Ladoire, B. Simon, W. Boireau, A. Hichami, L. Apetoh, G. Mignot, F. Ghiringhelli, C. Rébé, Liver X receptor β activation induces pyroptosis of human and murine colon cancer cells, Cell Death Differ. 21 (2014) 1914–1924, https://doi.org/10.1038/cdd.2014.117.
[230] W. Zhang, H. Jiang, J. Zhang, Y. Zhang, A. Liu, Y. Zhao, X. Zhu, Z. Lin, X. Yuan, Liver X receptor activation induces apoptosis of melanoma cell through caspase pathway, Cancer Cell Int. 14 (2014), https://doi.org/10.1186/1475-2867-14-16.
[231] G.R. Villa, J.J. Hulce, C. Zanca, J. Bi, S. Ikegami, G.L. Cahill, Y. Gu, K.M. Lum, K. Masui, H. Yang, X. Rong, C. Hong, K.M. Turner, F. Liu, G.C. Hon, D. Jenkins, M. Martini, A.M. Armando, O. Quehenberger, T.F. Cloughesy, F.B. Furnari, W. K. Cavenee, P. Tontonoz, T.C. Gahman, A.K. Shiau, B.F. Cravatt, P.S. Mischel, An LXR-cholesterol axis creates a metabolic co-dependency for brain cancers, Cancer Cell 30 (2016) 683–693, https://doi.org/10.1016/j.ccell.2016.09.008.
[232] C. Hu, D. Liu, Y. Zhang, G. Lou, G. Huang, B. Chen, X. Shen, M. Gao, W. Gong, P. Zhou, S. Dai, Y. Zeng, F. He, LXRα-mediated downregulation of FOXM1 suppresses the proliferation of hepatocellular carcinoma cells, Oncogene 33 (2014) 2888–2897, https://doi.org/10.1038/onc.2013.250.
[233] L.-L. Vedin, S.A. Lewandowski, P. Parini, J.-A. Gustafsson, K.R. Steffensen, The oxysterol receptor LXR inhibits proliferation of human breast cancer cells, Carcinogenesis 30 (2009) 575–579, https://doi.org/10.1093/carcin/bgp029.
[234] K.H. Kim, G.Y. Lee, J.I. Kim, M. Ham, J. Won Lee, J.B. Kim, Inhibitory effect of LXR activation on cell proliferation and cell cycle progression through lipogenic activity, J. Lipid Res. 51 (2010) 3425–3433, https://doi.org/10.1194/jlr.M007989.
[235] H. Xiong, Y. Zhang, S. Chen, Z. Ni, J. He, X. Li, B. Li, K. Zhao, F. Yang, Y. Zeng, B. Chen, F. He, Induction of SOCS3 by liver X receptor suppresses the proliferation of hepatocellular carcinoma cells, Oncotarget 8 (2017) 64083–64094, https://doi.org/10.18632/oncotarget.19321.
[236] J. Fukuchi, J.M. Kokontis, R.A. Hiipakka, C.-P. Chuu, S. Liao, Antiproliferative effect of liver X receptor agonists on LNCaP human prostate cancer cells, Cancer Res. 64 (2004) 7686–7689, https://doi.org/10.1158/0008-5472.CAN-04-2332.
[237] N.R. Candelaria, S. Addanki, J. Zheng, T. Nguyen-Vu, H. Karaboga, P. Dey, C. Gabbi, L.L. Vedin, K. Liu, W. Wu, P.K. Jonsson, J.Z. Lin, F. Su, L.R. Bollu, S. E. Hodges, A.L. McElhany, M.A. Issazadeh, W.E. Fisher, M.M. Ittmann, K. R. Steffensen, J.Å. Gustafsson, C.Y. Lin, Antiproliferative effects and mechanisms of liver X receptor ligands in pancreatic ductal adenocarcinoma cells, PLoS One 9 (2014), https://doi.org/10.1371/journal.pone.0106289.
[238] K. Youlin, Z. Li, H. Weiyang, K. Jian, L. Siming, G. Xin, Liver X receptor activation inhibits PC-3 prostate cancer cells via the beta-catenin pathway, Pathol. Res. Pract. 213 (2017) 267–270, https://doi.org/10.1016/j.prp.2016.04.013.
[239] A. El Roz, J.M. Bard, J.M. Huvelin, H. Nazih, LXR agonists and ABCG1-dependent cholesterol efflux in MCF-7 breast cancer cells: relation to proliferation and apoptosis, Anticancer Res. 32 (2012) 3007–3013.
[240] G. Segala, M. David, P. De Medina, M.C. Poirot, N. Serhan, F. Vergez, A. Mougel, E. Saland, K. Carayon, J. Leignadier, N. Caron, M. Voisin, J. Cherier, L. Ligat, F. Lopez, E. Noguer, A. Rives, B. Payré, T. Al Saati, A. Lamaziere, G. Despres, J. M. Lobaccaro, S. Baron, C. Demur, F. De Toni, C. Larrue, H. Boutzen, F. Thomas, J.E. Sarry, M. Tosolini, D. Picard, M. Record, C. Récher, M. Poirot, S. Silvente-Poirot, Dendrogenin A drives LXR to trigger lethal autophagy in cancers, Nat. Commun. 8 (2017) 1–17, https://doi.org/10.1038/s41467-017-01948-9.
[241] P. De Medina, M.R. Paillasse, G. Segala, M. Voisin, L. Mhamdi, F. Dalenc, M. Lacroix-Triki, T. Filleron, F. Pont, T. Al Saati, C. Morisseau, B.D. Hammock, S. Silvente-Poirot, M. Poirot, Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties, Nat. Commun. 4 (2013), https://doi.org/10.1038/ncomms2835.
[242] M. Bauriaud-Mallet, L. Vija-Racaru, S. Brillouet, A. Mallinger, P. de Medina, A. Rives, B. Payre, M. Poirot, F. Courbon, S. Silvente-Poirot, The cholesterol-derived metabolite dendrogenin A functionally reprograms breast adenocarcinoma and undifferentiated thyroid cancer cells, J. Steroid Biochem. Mol. Biol. 192 (2019) 105390, https://doi.org/10.1016/j.jsbmb.2019.105390.

[243] C.A. Flaveny, K. Griffett, B.E.D.M. El-Gendy, M. Kazantzis, M. Sengupta, A. L. Amelio, A. Chatterjee, J. Walker, L.A. Solt, T.M. Kamenecka, T.P. Burris, Broad anti-tumor activity of a small molecule that selectively targets the warburg effect and lipogenesis, Cancer Cell 28 (2015) 42–56, https://doi.org/10.1016/j.ccell.2015.05.007.

[244] N. Pencheva, C.G. Buss, J. Posada, T. Merghoub, S.F. Tavazoie, Broad-spectrum therapeutic suppression of metastatic melanoma through nuclear hormone receptor activation, Cell 156 (2014) 986–1001, https://doi.org/10.1016/j.cell.2014.01.038.

[245] A. Noghero, A. Perino, G. Seano, E. Saglio, G. Lo Sasso, F. Veglio, L. Primo, E. Hirsch, F. Bussolino, F. Morello, Liver X receptor activation reduces angiogenesis by impairing lipid raft localization and signaling of vascular endothelial growth factor receptor-2, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 2280–2288, https://doi.org/10.1161/ATVBAHA.112.250621.

[246] A. El Roz, J.M. Bard, S. Valin, J.M. Huvelin, H. Nazih, Macrophage apolipoprotein e and proliferation of MCF-7 breast cancer cells: role of LXR, Anticancer Res. 33 (2013) 3783–3790.

[247] M.F. Tavazoie, I. Pollack, R. Tanqueco, B.N. Ostendorf, B.S. Reis, F.C. Gonsalves, I. Kurth, C. Andreu-Agullo, M.L. Derbyshire, J. Posada, S. Takeda, K. N. Tafreshian, E. Rowinsky, M. Szarek, R.J. Waltzman, E.A. Mcmillan, C. Zhao, M. Mita, A. Mita, B. Chmielowski, M.A. Postow, A. Ribas, D. Mucida, S. F. Tavazoie, LXR/ApoE activation restricts innate immune suppression in cancer, Cell 172 (2018) 825–840, https://doi.org/10.1016/j.cell.2017.12.026, e18.

[248] A Study of RGX-104 in Patients With Advanced Solid Malignancies and Lymphoma - Full Text View - ClinicalTrials.gov, (n.d.). https://clinicaltrials.gov/ct2/show/NCT02922764. (Accessed 18 June 2020).

[249] H. Long, X. Guo, S. Qiao, Q. Huang, Tumor LXR expression is a prognostic marker for patients with hepatocellular carcinoma, Pathol. Oncol. Res. 24 (2018) 339–344, https://doi.org/10.1007/s12253-017-0249-8.

[250] E.J. Villablanca, L. Raccosta, D. Zhou, R. Fontana, D. Maggioni, A. Negro, F. Sanvito, M. Ponzoni, B. Valentinis, M. Bregni, A. Prinetti, K.R. Steffensen, S. Sonnino, J. Gustafsson, C. Doglioni, C. Bordignon, C. Traversari, V. Russo, Tumor - mediated liver X receptor - α activation inhibits CC chemokine receptor - 7 expression on dendritic cells and dampens antitumor responses, Nat. Med. 16 (2009) 98–105, https://doi.org/10.1038/nm.2074.

[251] S. Beceiro, A. Pap, Z. Czimmerer, T. Sallam, J.A. Guillén, G. Gallardo, C. Hong, N. A-Gonzalez, C. Tabraue, M. Diaz, F. Lopez, J. Matalonga, A.F. Valledor, P. Dominguez, C. Ardavin, C. Delgado-Martin, S. Partida-Sanchez, J.L. Rodriguez-Fernandez, L. Nagy, P. Tontonoz, A. Castrillo, Liver X receptor nuclear receptors are transcriptional regulators of dendritic cell chemotaxis, Mol. Cell. Biol. 38 (2018), https://doi.org/10.1128/mcb.00534-17 e00534-17.

[252] J.E. Feig, I. Pineda-Torra, M. Sanson, M.N. Bradley, Y. Vengrenyuk, D. Bogunovic, E.L. Gautier, D. Rubinstein, C. Hong, J. Liu, C. Wu, N. van Rooijen, N. Bhardwaj, M. Garabedian, P. Tontonoz, E.A. Fisher, LXR promotes the maximal egress of monocyte-derived cells from mouse aortic plaques during atherosclerosis regression, J. Clin. Invest. 120 (2010) 4415–4424, https://doi.org/10.1172/JCI38911.

[253] P. Goossens, J. Rodriguez-Vita, A. Etzerodt, M. Masse, O. Rastoin, V. Gouirand, T. Ulas, O. Papantonopoulou, M. Van Eck, N. Auphan-Anezin, M. Bebien, C. Verthuy, T.P. Vu Manh, M. Turner, M. Dalod, J.L. Schultze, T. Lawrence, Membrane cholesterol efflux drives tumor-associated macrophage reprogramming and tumor progression, Cell Metab. 29 (2019) 1376–1389, https://doi.org/10.1016/j.cmet.2019.02.016, e4.

[254] T. Tanaka, Preclinical cancer chemoprevention studies using animal model of inflammation-associated colorectal carcinogenesis, Cancers (Basel) 4 (2012) 673–700, https://doi.org/10.3390/cancers4030673.

[255] F. Lalloyer, T.Å. Pedersen, B. Gross, S. Lestavel, S. Yous, E. Vallez, J.Å. Gustafsson, S. Mandrup, C. Fiévet, B. Staels, A. Tailleux, Rexinoid bexarotene modulates triglyceride but not cholesterol metabolism via gene-specific permissivity of the RXR/LXR heterodimer in the liver, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1488–1495, https://doi.org/10.1161/ATVBAHA.109.189506.

[256] J.R. Schultz, H. Tu, A. Luk, J.J. Repa, J.C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D.J. Mangelsdorf, K.D. Lustig, B. Shan, Role of LXRs in control of lipogenesis, Genes Dev. 14 (2000) 2831–2838.

[257] E.F. Garner, L.L. Stafman, A.P. Williams, J.M. Aye, C. Goolsby, V.R. Atigadda, B. P. Moore, L. Nan, J.E. Stewart, A.B. Hjelmeland, G.K. Friedman, E.A. Beierle, UAB30, a novel RXR agonist, decreases tumorigenesis and leptomeningeal disease in group 3 medulloblastoma patient-derived xenografts, J. Neurooncol. 140 (2018) 209–224, https://doi.org/10.1007/s11060-018-2950-1.

[258] X. Yu, P. Yi, R.A. Hamilton, H. Shen, M. Chen, C. Foulds, M. Mancini, S. Ludtke, Z. Wang, B. O'Malley, Structural insights of transcriptionally active, full-length androgen receptor coactivator complexes, SSRN Electron. J. (2020), https://doi.org/10.2139/ssrn.3578148.

[259] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674, https://doi.org/10.1016/j.cell.2011.02.013.