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Combining precision oncology and immunotherapy by targeting the MALT1 protease

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ABSTRACT

An innovative strategy for cancer therapy is to combine the inhibition of cancer cell-intrinsic oncogenic signaling with cancer cell-extrinsic immunological activation of the tumor microenvironment (TME). In general, such approaches will focus on two or more distinct molecular targets in the malignant cells and in cells of the surrounding TME. In contrast, the protease Mucosa-associated lymphoid tissue protein 1 (MALT1) represents a candidate to enable such a dual approach by engaging only a single target. Originally identified and now in clinical trials as a lymphoma drug target based on its role in the survival and proliferation of malignant lymphomas addicted to chronic B cell receptor signaling, MALT1 proteolytic activity has recently gained additional attention through reports describing its tumor-promoting roles in several types of non-hematological solid cancer, such as breast cancer and glioblastoma. Besides cancer cells, regulatory T (Treg) cells in the TME are particularly dependent on MALT1 to sustain their immune-suppressive functions, and MALT1 inhibition can selectively reprogram tumor-infiltrating Treg cells into Foxp3-expressing proinflammatory antitumor effector cells. Thereby, MALT1 inhibition induces local inflammation in the TME and synergizes with anti-PD-1 checkpoint blockade to induce antitumor immunity and facilitate tumor control or rejection. This new concept of boosting tumor immunotherapy in solid cancer by MALT1 precision targeting in the TME has now entered clinical evaluation. The dual effects of MALT1 inhibitors on cancer cells and immune cells therefore offer a unique opportunity for combining precision oncology and immunotherapy to simultaneously impair cancer cell growth and neutralize immunosuppression in the TME. Further, MALT1 targeting may provide a proof of concept that modulation of Treg cell function in the TME represents a feasible strategy to augment the efficacy of cancer immunotherapy. Here, we review the role of MALT1 protease in physiological and oncogenic signaling, summarize the landscape of tumor indications for which MALT1 is emerging as a therapeutic target, and consider strategies to increase the chances for safe and successful use of MALT1 inhibitors in cancer therapy.

INTRODUCTION

Mucosa-associated lymphoid tissue protein 1 (MALT1), also known as paracaspase 1 (PCASP1), is a human immune protease that is attracting attention as an emerging drug target for cancer therapy. Interest in targeting MALT1 originated from its cell-intrinsic role as a driver of cancer cell survival and proliferation especially in hematological malignancies, such as diffuse large B cell lymphoma (DLBCL).[1–4] Beyond being an oncogenic driver in cancer cells, MALT1 executes key functions in the immune system and recent research uncovered that its protease function in Treg cells is critical for maintaining an immunosuppressive tumor microenvironment (TME) in solid cancer.[5 6] Based on these cancer cell-intrinsic and cell-extrinsic functions, clinical trials are now testing the safety and efficacy of MALT1 inhibitors for cancer therapy. While several trials explore the direct targeting of MALT1 in B cell receptor (BCR)-addicted non-Hodgkin's lymphoma (NHL) (NCT03900598, NCT04876092 and NCT04657224), another trial investigates the use of MALT1 inhibitors to reprogram tumor-infiltrating Treg cells into proinflammatory effector cells to boost antitumor immune responses in non-hematological cancers (NCT04859777).

MALT1 PARACASPASE: A UNIQUE ROLE IN BOTH IMMUNE ACTIVATION AND TOLERANCE

MALT1 is ubiquitously expressed in most human tissues and cells, but genetic deficiency or loss-of-function mutations in mice and humans revealed its primary role in controlling the activity of lymphocytes and thus adaptive immunity.[7 8] In T and B cells, assembly of the higher-order CBM (CARD11-BCL10-MALT1) signalosome, consisting of the core subunits CARD11 (caspase recruitment domain 11, also termed CARMA1), BCL10 (B cell lymphoma/leukemia protein 10) and MALT1, bridges T and BCR (TCR/BCR) signaling to the nuclear factor-kappaB (NF-κB) and Jun N-terminal kinase (JNK) pathways, which trigger lymphocyte activation, differentiation and effector functions.[9] MALT1 localizes to the outer surface of the

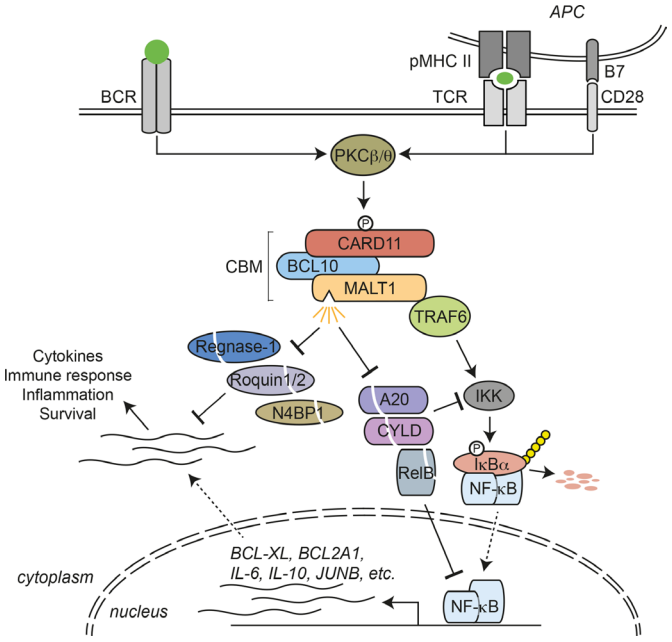

Figure 1 CBM complex signaling to NF-κB and the role of MALT1 protease function following TCR or BCR stimulation in T or B cells, respectively. APC, antigen-presenting cell; BCR, B cell receptor; MALT1, Mucosa-associated lymphoid tissue protein 1; NF-κB, nuclear factor-kappaB; TCR, T cell receptor; CBM, CARD11-BCL10-MALT1 complex.

CBM signalosome and thereby provides an accessible platform that serves a dual function[10] (figure 1): First, by recruiting TNF receptor associated factor 6 (TRAF6), MALT1 exerts non-catalytic molecular scaffolding function to drive activation of downstream signaling pathways, including NF-κB and JNK.[11 12] Second, recruitment to the CBM complex activates the MALT1 protease so that its paracaspase domain catalyzes the cleavage of a range of substrate proteins.[13] The latter is not critical for initial NF-κB or JNK signaling, but modulates NK-κB activity and other immune cell functions by cleaving regulators involved in cell signaling (eg, BCL10, A20, CYLD, HOIL-1), transcriptional activation (RelB), and RNA stability/metabolism (Regnase-1, Roquin-1/2 and N4BP1).[9 13 14]

Analyses of MALT1-deficient mice revealed critical functions in both immune activation and immune tolerance, which has been attributed to its roles in conventional effector as well as regulatory T cells, respectively.[15–17] Absence of MALT1 does not cause major disruptions of early lymphocyte development, but a complete block in thymic Treg (tTreg), yet only a partial block in peripheral Treg (pTreg) cell development, in addition to a severe reduction in innate B cells. Antigen-induced NF-κB signaling is nearly abolished in T cells and impaired in B cells, which explains the severe defect in mounting an appropriate response to T cell-dependent or T cell-independent antigens.[16–18] Accordingly, human germline mutations associated with defective MALT1 lead to combined immunodeficiencies that predispose patients to bacterial, viral and fungal infections.[7] The impairment of conventional lymphocyte effector function likely explains the absence of the early-onset inflammatory syndromes that would otherwise be expected with absent tTreg cells and reduced pTreg cell numbers.[19]

Mice expressing a catalytically inactive mutant MALT1 protease also have a block in tTreg cell and innate B cell development. In contrast to MALT1-deficient mice, however, they develop a lymphoproliferative, IFNγ-driven autoimmune inflammatory syndrome, although more variably and with delayed onset compared with mice devoid of Treg cells due to a lack of the Foxp3 transcription factor.[20–24] This indicates that in contrast to thymic Treg cell development, effector lymphocyte function only partially depends on MALT1 protease activity.

Conditional deletion of MALT1, CARD11, or BCL10 in mature Treg cells following completion of their development does not cause a significant decline in their overall frequency in blood and peripheral lymphoid tissues, indicating that these proteins do not control Treg cell survival.[5 6 25 26] However, deletion of each CBM component or selective inactivation of MALT1 paracaspase in mature Treg cells revealed the cell-intrinsic role of the CBM complex in maintaining peripheral immune tolerance by enabling the full differentiation and maintenance of activated effector Treg (eTreg) cells. Thereby, MALT1, CARD11, and BCL10 are required for the suppressive activity of Treg cells, explaining why their Treg cell-specific deletion leads to a Scurfy-like, fatal autoimmune inflammatory syndrome.[5 6] Importantly, selective genetic inactivation of either MALT1 protease or scaffolding function demonstrated that protease activity, but not TRAF6 recruitment, is required for Treg cell maturation and their sustained suppressive functions in vivo.[5 11 20 22–24] Thus, the catalytic and non-catalytic functions of MALT1 balance the activation of conventional and regulatory effector T cells, which is critical for maintaining peripheral tolerance and allows productive immune activation upon challenge. While the necessity of the MALT1 protease for maintaining the suppressive function of Treg cells represents an opportunity to enhance anti-tumor immunity by pharmacological MALT1 targeting, autoimmune-related side effects need to be considered in the clinical use of highly effective, long-term MALT1 inhibition, as will be discussed below.

THERAPEUTIC TARGETING OF CANCER CELL-INTRINSIC MALT1

MALT1 function and targeting in hematological malignancies

NHL represents a heterogeneous group of lymphoid neoplasms originating from mature or precursor B or T cells. B cell lymphomas are often characterized by oncogenic mutations affecting key components of BCR and downstream NF-κB signaling pathways.[27] Even before it was demonstrated that MALT1 is a functional protease,[28 29] the oncogenic API2-MALT1 fusion protein in MALT lymphoma and the critical prosurvival function of MALT1 in DLBCL raised strong interest in MALT1 as a precision target for the treatment of aggressive lymphomas.[30 31] Importantly, MALT1 protease is

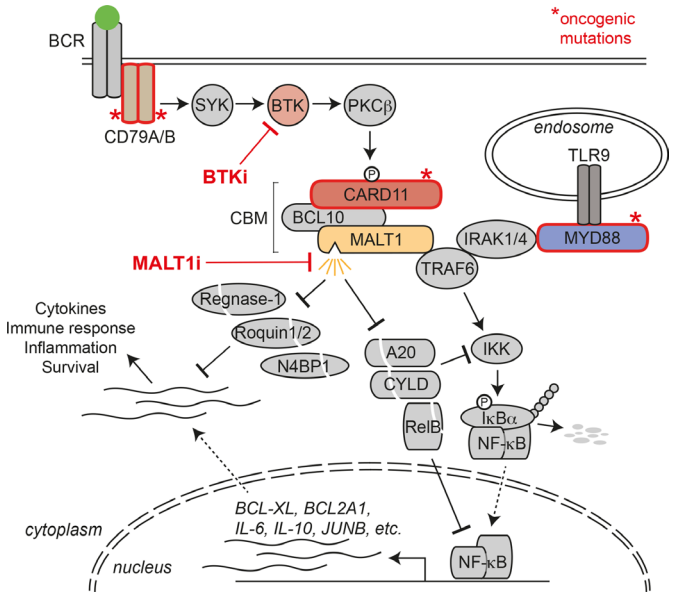

Figure 2 Chronic BCR and TLR signaling and oncogenic driver mutations impacting MALT1 protease activation in ABC DLBCL. ABC, activated B cell; BCR, B cell receptor; DLBCL, diffuse large B cell lymphoma; TLR, Toll-like receptor.

not active in all lymphoma subtypes, because it relies on chronic BCR signaling or oncogenic activation of BCR signaling mediators. Here, we will summarize lymphoma entities for which genetic and pharmacological evidence point to a functional role of MALT1 and thus a potential benefit of therapeutic targeting.

MALT lymphoma is a usually slow growing, extranodal marginal zone B cell lymphoma that manifests most frequently in the mucosa of the stomach. While the early stages of MALT lymphoma are in general sensitive to eradication of *Helicobacter pylori*, aggressive late stage MALT lymphomas, in which the translocation breakpoint t(11;18)(q21;q21) results in the generation of the oncogenic fusion protein API2-MALT1,[31 32] grow independently of bacterial infection and are resistant to antimicrobial treatments.[33] The API2-MALT1 fusion activates NF-κB signaling and especially the non-canonical NF-κB pathway relies on MALT1 catalytic activity.[34] Importantly, in the context of the API2-MALT1 fusion, substrate specificity of MALT1 is expanded to include the non-canonical NF-κB regulator NIK (NF-κB inducing kinase) and the tumor suppressor LIMA1 (LIM domain and actin-binding protein 1), demonstrating that oncogenic mutations can influence substrate selection.[34 35] Cleavage of NIK enhances non-canonical NF-κB signaling and LIMA1 inactivation promotes NF-κB-independent tumor growth, suggesting that aberrant MALT1 protease activity may be a target in the relatively small subset of MALT lymphoma patients (<10%) with late stage, antibiotic-resistant MALT lymphomas expressing by the API2-MALT1 fusion oncogene.

The concept of cancer cell-intrinsic therapeutic MALT1 targeting originated from research on the role of chronic BCR signaling for survival and growth of DLBCL, the most prevalent subtype of NHL.[36] Molecularly, DLBCL represents a heterogeneous disease, and cell-of-origin analyses based on gene expression profiling defined the activated B cell (ABC)-type and the germinal center B cell (GCB)-type as the two main entities that account for approximately 85% of all DLBCL cases.[37 38] Overall patient survival significantly improved with the introduction of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) immune-chemotherapy, but response rates are significantly worse for the ABC- compared with the GCB-type.[39] Loss-of-function RNAi screens demonstrated the critical prosurvival functions of BCR-proximal protein kinases spleen tyrosine kinase, BTK (Bruton's tyrosine kinase), and PKCβ as well as the entire CBM complex including MALT1 for ABC DLBCL tumor growth[30] (figure 2). Oncogenic driver mutations in the BCR adaptors CD79A and B are frequently found in ABC DLBCL and often occur in association with the MYD88 L265P variant, which provokes chronic Toll-like receptor 9 (TLR9) signaling.[40–43] In these cases, BCR and TLR signals converge in the formation of a CBM-MYD88-containing super-activation cluster, which fosters oncogenic signaling.[44] The predominant clinical relevance of chronic BCR signaling was emphasized in a phase III trial that demonstrated vastly superior outcomes by combining R-CHOP treatment with the BTK inhibitor ibrutinib in younger DLBCL patients (≤60 years) carrying CD79B and/or MYD88 mutations.[45] However, patients with oncogenic lesions downstream of BTK, as frequently found in CARD11, do not benefit from BTK targeting.[43 46 47] Further, multiple genetic and non-genetic adaptations can lead to secondary ibrutinib resistances and lymphoma relapse, underscoring that targeting BTK alone may often not be sufficient to achieve long-term responses.[48]

Since MALT1 acts downstream of BTK, MALT1 protease inhibition represents an alternative strategy to overcome ibrutinib resistance or to increase clinical efficacy by combinatorial treatment. Initial in vitro studies with the irreversible peptide inhibitor VRPR-FMK demonstrated that MALT1 inhibition is toxic to human ABC DLBCL-derived, but not MALT1-independent GCB DLBCL-derived cancer cell lines.[3 4] First-in-class non-competitive (eg, phenothiazines mepazine and thioridazine) or irreversible (MI-2 and compound 3) small molecule MALT1 inhibitors validated these in vitro findings and effectively and selectively killed xenotransplanted ABC DLBCL tumors in vivo.[1 2 49] Expression of the allosteric inhibitor-resistant MALT1 E397A variant renders ABC DLBCL cell lines resistant to mepazine or thioridazine induced cytotoxicity, proving that cancer cell-intrinsic targeting of MALT1 is necessary for killing the lymphoma cells.[50] Indeed, the optimized MALT1 inhibitor S-mepazine is toxic to CARD11 mutant and ibrutinib-resistant ABC DLBCL and combinatorial BTK and MALT1 inhibition augments killing of CD79 mutant DLBCL.[51] Killing of BTK inhibitor resistant ABC DLBCL cells was also confirmed with the more potent and selective allosteric

MALT1 inhibitors MLT-985 and JNJ-67856633 in vitro and after xenotransplantation.[52 53]

The mechanistic function of MALT1 protease activity in driving aberrant survival and proliferation of ABC DLBCL are not yet fully understood. As mentioned above, the non-catalytic MALT1 scaffolding directly triggers activation of the canonical IKK/NF-κB pathway, whereas the MALT1 protease is thought to enhance NF-κB signaling by cleaving and inactivating inhibitory factors such as the tumor suppressors A20 or CYLD.[2 4] Further, MALT1-catalyzed cleavage and destabilization of the non-canonical NF-κB family member RelB augments DNA binding of the canonical NF-κB proteins RelA and c-Rel, which in turn induces antiapoptotic gene expression in ABC DLBCL.[54] In line with the concept that the MALT1 protease removes negative regulators to release brakes on NF-κB activation, MALT1 inhibition suppressed global NF-κB-dependent gene expression in ABC DLBCL cells.[1 3] However, MALT1 also constitutively cleaves positive NF-κB regulators such as HOIL-1, MALT1 (autocleavage) and BCL10 in ABC DLBCL cells, with unknown consequences for lymphomagenesis.[3 4 55 56] Moreover, MALT1 protease activity may not only modulate transcriptional induction in DLBCL, but also post-transcriptional gene regulation through the cleavage of Roquin-1/2.[49] How strong this contributes to aberrant activation in B cell lymphomas needs to be addressed. Collectively, these data provided strong conceptual support for the clinical application of allosteric MALT1 inhibitors to treat highly malignant ABC DLBCL.

Mantle cell lymphoma (MCL) is a relatively rare and highly aggressive subtype of NHL. Despite high initial response rates with frontline R-CHOP immunochemotherapy, nearly all patients develop a relapsing-remitting disease course and only few patients can be cured.[57] Similar to ABC DLBCL, MCL are characterized by an NF-κB gene signature that is highly sensitive to upstream BCR pathway inhibitors.[58 59] Small molecule BTK inhibitors have been approved for relapsed MCL, but primary or acquired resistances are nearly universal.[60 61] At least a subset of MCLs is addicted to MALT1 protease activity, which stabilizes MYC protein by a yet undefined cellular mechanism and thereby induces MYC-induced target gene expression.[62] MCLs also display recurrent translocations of the CCND1, 2 and 3 genes resulting in overexpression of the cell cycle promoting factors cyclin D1, D2 and D3.[63] Interestingly, expression of cyclin D2 during early hematopoiesis in mice drives an MCL-like lymphoma with chronic BCR/NF-κB pathway activation and increased MALT1 protease activity.[64] Inhibition of MALT1 protease activity is highly toxic to these cyclin D2-driven MCL-like tumors in vitro and in vivo, providing an additional rationale for targeting of MALT1 in MCL patients.

Chronic lymphocytic leukemias (CLL) is the most prevalent neoplasia in Western countries. Its growth and survival relies on antigen-independent 'tonic' BCR signaling[65] and interference with BCR signaling through BTK inhibitors induces death of CLL cells and strong therapeutic responses. Targeting BTK provides in many cases long-term control of the disease, but in-class toxicities and acquired resistances remain common complications,[66] bringing attention to MALT1 inhibitors as promising candidates to treat BTK-resistant CLL. However, evidence that MALT1 is required for survival of CLL cells remains scarce and mainly relies on the use of the covalent MALT1 inhibitor MI-2.[67 68] While MI-2 inhibits MALT1 protease function and NF-κB signaling and reduces cell viability in CLL cells, it also disrupts several other biological networks to a similar degree and decreases MALT1-independent PI3K/AKT and MAPK/ERK signaling in CLL. Given the non-selectivity of MI-2,[69 70] it remains to be seen if these findings can be reproduced with more potent and specific MALT1 inhibitors.

Besides the well-studied role of MALT1 in B cell lymphomas, aberrant MALT1 protease activation has also been implicated in T cell malignancies. CARD11 activating mutations have been identified in some T cell-derived lymphomas, indicative of CBM-triggered MALT1 protease activation.[71–73] While in most cases the therapeutic potential of MALT1 protease inhibition has not yet been explored, MALT1 protease inhibition was shown to impair growth and survival of aggressive adult T cell leukemia and T cell acute lymphoblastic leukemia.[74 75] As for CLL, however, these studies relied on the poorly selective MALT1 inhibitor MI-2 and further analyses using more potent and selective inhibitors are warranted to firmly establish the utility of MALT1 inhibitors for the treatment of T cell lymphomas.

Taken together, strong biological evidence links MALT1 protease activity to aberrant antigen receptor signaling in various hematological malignancies. Since MALT1 protease acts downstream of BTK, which is a target of several approved drugs for the treatment of BCR-addicted NHL, direct targeting of MALT1 is a promising strategy to overcome BTK resistances or to enhance efficacy in combination treatment of lymphomas. Accordingly, clinical trials have been initiated to evaluate the safety and efficacy of either single arm treatment of NHL with MALT1 inhibitor JNJ-67856633 (NCT03900598) or the combination of JNJ-67856633 with first-generation and second-generation BTK inhibitors ibrutinib and JNJ-64264681, respectively (NCT04876092 and NCT04657224).

MALT1 function and targeting in non-hematological solid cancer

Besides the well-established prosurvival function of MALT1 and the CBM complex downstream of the BCR in hematological malignancies, a number of recent studies also demonstrated oncogenic roles of MALT1 in several non-hematological cancers. While CARD11 expression is confined to lymphoid cells, alternative CBM complexes comprizing either CARD9, CARD10 (CARMA3) or CARD14 (CARMA2) subunits mediate NF-κB signaling on ligand binding to innate immune receptors, G-protein-coupled receptors (GPCRs), or receptor tyrosine kinases

(RTKs) in various other cell types.[76 77] Especially CARD10 is broadly expressed, and can assemble an alternative CARD10-BCL10-MALT1 (C10BM) complex after ligand engagement by GPCRs and RTKs.[78] The GPCRs lysophosphatidic acid receptors, type 1 angiotensin II receptor (AT1R), and thrombin-induced protease-activated receptor 1 (PAR1) trigger NF-κB-dependent inflammatory responses via these C10BM complexes.[79–83] In addition, CARD10 also channels RTK signaling downstream of EGFR and HER2 to the canonical NF-κB pathway.[84 85] Since expression and activation of these GPCRs or RTKs is linked to cancer cell transformation, survival, and metastasis, a tumor-promoting function of MALT1 protease has been proposed for various solid cancers, but it is best characterized in the case of breast cancer.

High expression of the GPCRs AT1R and PAR1 correlates with increased metastasis and poor clinical outcome in breast cancer.[86 87] Both, AT1R-induced and PAR1-induced NF-κB activation and target gene expression in breast cancer cells strictly rely on CARD10, BCL10, and MALT1. In addition, angiotensin II and thrombin induce MALT1 protease activation and substrate cleavage.[86 88] Similar to the known function of NF-κB in BCR-addicted lymphomas, C10BM-mediated NF-κB activation via GPCRs promotes proliferation and survival of breast cancer cells.[86 87] Furthermore, the C10BM complex controls cell migration and invasion, hallmarks of tumor metastasis. Mechanistically, expression of AT1R and PAR1 drives epithelial-to-mesenchymal transition (EMT), which relies on MALT1 protease activity.[88] Especially in triple-negative breast cancer (TNBC), characterized by the absence of estrogen and progesterone receptors and low HER2 expression, MALT1 protease and NF-κB are required for the transcriptional EMT program, suggesting that MALT1 targeting may affect not only cancer cell survival but also metastasis. Accordingly, while MALT1 inhibition by mepazine reduced growth of orthotopic PAR1-positive breast cancer xenografts, it even more strongly impaired metastatic dissemination of TNBC cells.[88] Of note, AT1R and HER2 overexpression are mutually exclusive in patients with invasive breast cancer, suggesting that the receptors may serve redundant functions in tumorigenesis.[87] Indeed, heregulin (HRG) stimulation of HER2-positive breast cancer cells induces NF-κB activation via the C10BM signalosome, contributing to proliferation, anchorage-independent growth and cell migration, and tumor invasiveness.[85] Collectively, these data suggest that MALT1 inhibition counteracts the tumor-promoting and prometastatic functions GPCRs and RTKs, providing a rationale for tumor-cell intrinsic targeting of MALT1 in breast cancer. Of note, CARD10-dependent MALT1 activation in cancer cells acts not only in a cell-intrinsic, but also cell-extrinsic manner by inducing their expression and secretion of proinflammatory cytokines, chemokines, and growth factors that have paracrine, tumor growth-promoting effects on the TME, for example, through endothelial cell chemotaxis.[87] Besides breast cancer, CARD10, BCL10, and MALT1-dependent processes have been suggested to operate downstream of GPCRs or RTKs in glioblastoma, lung cancer, osteosarcoma, melanoma, pancreatic cancer, oral cancer, ovarian cancer, and prostate cancer.[85 86 89–95] In general, however, the role of MALT1 protease activation in these cancers has not been explored in detail.

A recent study suggested a novel mechanism through which MALT1 promotes glioma cell survival, implicating MALT1 protease as a potential target for the treatment of glioblastoma.[96] MALT1 is highly expressed in glioblastoma multiforme and, especially in patient-derived glioblastoma stem cells (GSCs), displays basal protease activity. Either knock-down or pharmacological inhibition of MALT1 by phenothiazine-derivatives attenuates growth and reduces viability of GSCs, which again relies on the CARD10-containing CBM complex present in non-immune cells.[96] While the upstream mechanisms responsible for MALT1 activity remained unclear, its inhibition impairs autophagic flux leading to lysosomal-mediated cell death, which is linked to a displacement of mTOR from lysosomes. Thus, disruption of endo-lysosomal homeostasis appears to be the main cause of cell death on MALT1 inhibition in GSCs and a new mechanism by which MALT1 inhibition exerts its therapeutic effects. The findings are of particular interest, because phenothiazine-derived MALT1 inhibitors like mepazine are able to cross the blood–brain barrier.[96 97]

Taken together, MALT1 protease activity has been shown to control proliferation, survival, migration, and/or invasiveness in many non-hematological solid cancers, even though the underlying mechanisms and therapeutic relevance in most cases still require closer inspection. However, in combination with augmented antitumor immunity on MALT1 inhibition in the TME, direct inhibition of MALT1 in cancer cells may further enhance treatment efficacy (see next chapter).

MODULATING THE TME BY MALT1 PROTEASE INHIBITORS

Involvement of tumor infiltrating Treg cells in cancer immunotherapy resistance

Therapeutic antibodies that block so-called immune checkpoints, such as CTLA-4 or the PD-1/PD-L1 pathway, produce long-term disease-free survival and cures in some patients with previously hard to treat or untreatable forms of cancer. Consequently, immune checkpoint therapies (ICTs) are now approved as front- or second-line treatment for a wide range of human cancer types.[98] However, the majority of patients still do not respond to these treatments (‘primary resistance’) or relapse after exhibiting an initial response (‘acquired resistance’).[99] Therapy resistance is often correlated with an a priori insufficient immune and inflammatory reaction to the cancerous growth. Defects in tumor-reactive effector T cell priming, their infiltration, or their survival in the TME can lead to such ‘cold’ tumors, but in addition, the local immunosuppressive activity of tumor-infiltrating Treg cells is thought to be a major cause for the lack of an

effective either spontaneous or ICT-induced antitumor immune response.[99 100] Even in inflamed, so-called 'hot' tumors, which are characterized by abundant infiltration of cytotoxic CD8$^+$ T cells and natural killer (NK) cells, high densities of Treg cells in the TME are associated with weaker responses to ICT and poorer prognosis.[100] Given the critical role of the CBM complex and MALT1 protease activity for the immunosuppressive function of Treg cells,[5 6 25 26] pharmacological MALT1 inhibitors present an intriguing opportunity to overcome ICT resistance by breaking Treg cell-mediated tolerance in both immunologically 'cold' or 'hot' tumors.

Treg cells physiologically serve to maintain immune homeostasis. Whereas tTreg cells recognize self antigens, pTreg cells respond to (eg, commensal- and food-derived) non-self-antigens. Both subsets functionally complement each other, but are transcriptionally similar and capable of deploying the same mechanisms of suppression, including secretion of immune-regulatory cytokines such as TGF-β, IL-10, and IL-35, metabolization of extracellular immune-stimulatory ATP into immune-suppressive adenosine, and CTLA-4-mediated downregulation of costimulatory molecules on antigen-presenting cells.[101 102] Tumor-infiltrating Treg cells contribute to both primary and acquired immunotherapy resistance through the same mechanisms of suppression, preventing elimination of the cancer cells. Both mouse and human studies suggest, based on largely non-overlapping TCR usage by tumor-infiltrating Treg and conventional effector T cells, a predominant role for self-antigen-specific tTreg in immunological tumor tolerance,[103–106] even though evidence for intratumoral pTreg generation is also found in some human tumors.[107 108]

Treg cell reprogramming by MALT1 inhibition in the TME

The above-mentioned block in tTreg cell development in the constitutive, global absence of CBM proteins or MALT1 protease activity initially precluded an examination of their role in tumor infiltrating Treg cells. However, selective CBM complex disruption in mature Treg in more recent studies yielded some unexpected results.[5 6 25 26] Whereas the impaired generation and maintenance of eTreg cells upon conditional deletion of CARD11 in Foxp3$^+$ Treg cells causes early-onset, fatal immune pathology, partial reduction of CARD11 protein in Treg cells through heterozygous deletion of only one *Card11* allele does not cause detectable immune pathology. Mice with heterozygous *Card11* deletion are healthy and show normal life expectancy, but implanted solid cancers grow more slowly in these animals.[6] Importantly, partial CARD11 reduction in Treg cells provokes their production of the proinflammatory cytokines IFNγ and TNF in the TME. These proinflammatory Treg cells maintain Foxp3 expression, suggesting a state of Treg cell 'fragility' rather than the loss of lineage identity referred to as Treg cell 'instability'.[109] Proinflammatory Treg cell conversion is confined to the tumor tissue and not observed in lymphoid or in healthy non-lymphoid tissues, suggesting a therapeutic window for the modulation of CBM complex activity that avoids systemic autoimmune toxicity. The basis for this selectivity remains unclear, but eTreg may have an elevated need for CBM signaling to withstand the exposure to strong TCR and costimulatory signals, cytokines, and the metabolic conditions of the TME, rendering them more sensitive to partial CBM complex disruption than their counterparts that maintain immune homeostasis in uninflamed tissues.[6] Importantly, deletion of one allele of CARD11 in only 50% of Treg cells is sufficient to reduce tumor growth.[6] Since a mere loss of immune-suppressive function by a reduction in CARD11 in only half of Treg cells would be compensated for by the remaining CARD11-sufficient cells, these data indicate an active antitumor function of the CARD11-deficient Treg cells. In fact, antitumor activity relies on IFNγ production of Treg cells with reduced CARD11 expression, which causes classical activation of tumor-associated macrophages and elevated expression of antigen-presenting MHC class I proteins on cancer cells. These preclinical observations suggest a dominant mechanism, in which the proinflammatory reprogramming of a fraction of Treg cells by partial inactivation of the CBM complex is sufficient to obtain an antitumor effect, which will be also of clinical relevance for enhancement of ICT through MALT1 inhibition, as discussed below.

Similar antitumor activity is also observed following deletion of BCL10 in Treg cells[5] and in mice that lack MALT1 protease activity either globally or specifically in Treg cells.[26] Since MALT1 protease activity, but not its recruitment of TRAF6 to induce TCR-driven NF-κB signaling is required for maintaining the suppressive function of mature Treg cells,[5 11] MALT1 protease inhibitors are promising candidates to reduce the function or even to reprogram tumor infiltrating Treg cells into antitumor effectors. Accordingly, treatment with the MALT1 inhibitor mepazine slows mouse melanoma growth in immune-competent hosts.[5 6] However, it is ineffective in lymphocyte-deficient animals, indicating an immune-mediated mechanism. It is also ineffective in MALT1-deficient hosts, ruling out a requirement for direct activity on MALT1-sufficient cancer cells in this setting.[5 6] MALT1 inhibition acts on multiple layers to enhance antitumor immunity. By reducing Treg cell suppressive activity, it increases the number of IFNγ-producing conventional CD4$^+$ and CD8$^+$ effector T cells.[5] However, antitumor effects are also observed upon depletion of CD8$^+$ T cells.[6] Since MALT1 inhibition, similar to partial CARD11 deletion, converts tumor-infiltrating Treg cells into IFNγ-producing cells, this suggests a critical role of proinflammatory Treg cell reprogramming[6] (figure 3). Not unexpectedly, the Th1 inflammatory response resulting from MALT1 inhibition also induces PD-L1 expression on cancer cells, which likely limits the treatment-induced antitumor effect by engaging PD-1 on activated effector lymphocytes. Consequently, PD-1 pathway-targeted ICT synergizes with MALT1 inhibition to reduce the growth of poorly immunogenic ('cold') and to prevent relapse

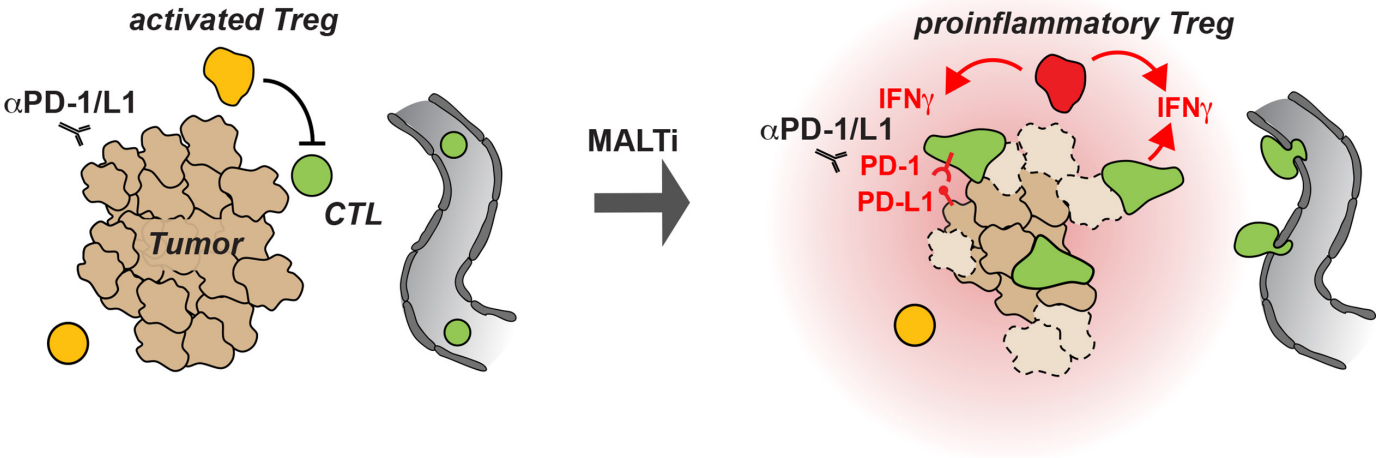

Figure 3 Concept of MALT1 inhibitor induced Treg cell reprogramming in the TME. Activated Treg cells (yellow) that suppress CTL (green) in immune-cold tumors are reprogrammed by MALT1 inhibitors (MALTi) into IFNγ-producing Treg cells (red), which enhance local inflammation in the TME. This and loss of Treg cell suppressive function both promote the recruitment and function of CTL, but also the upregulation of PD-L1 on cancer cells, causing acquired resistance that is overcome by synergistic anti-PD1 ICT to boost antitumor immunity. CTL, cytotoxic T cells; ICT, immune checkpoint therapy; MALT1, Mucosa-associated lymphoid tissue protein 1; TME, tumor microenvironment.

following rejection of immunogenic ('hot') tumors in mice.[6]

Importantly, spontaneous conversion of tumor-infiltrating Treg cells into IFNγ-secreting cells has been observed in human patients with cancer, for example, in patients with colorectal cancer, where it is correlated with favorable disease outcomes,[110] and in glioblastoma patients.[111] When Treg cells are rendered resistant to proinflammatory conversion in mouse models of cancer, for example, by deleting their receptors for either IL-6[112] or IFN-γ,[109] otherwise potent immunotherapy regimen, including PD-1 targeted ICT, become ineffective. This suggests that spontaneous Treg cell conversion is not only a by-product, but in fact critical for therapeutically induced antitumor immunity. It needs further investigations to understand how the inhibition of MALT1 protease or other experimental perturbations of Treg cells that have similar effects[6 109 113] amplify basal proinflammatory Treg conversion on a molecular level.

In summary, MALT1 inhibitors may have clinical antitumor activity by reprogramming preferentially self-reactive, immunosuppressive Treg cells into IFNγ-secreting antitumor effector cells selectively in the TME to produce a local Th1-type autoimmune response and render patients responsive to PD-1/PD-L1-targeteted ICT. This may increase the range of patients and solid cancer types that respond to established forms of immunotherapy, a concept that is currently being evaluated using the MALT1 inhibitor MPT-0118 either as a single agent or in combination with the anti-PD1 antibody pembrolizumab (NCT04859777).

OPPORTUNITIES FOR SIMULTANEOUS CANCER CELL-INTRINSIC AND CELL-EXTRINSIC TARGETING OF MALT1

Ideally, MALT1 targeting will take advantage of both cancer cell-intrinsic and cell-extrinsic therapeutic effects. While the ongoing NHL trial primarily aims to evaluate the lymphoma cell-intrinsic vulnerability of MALT1 protease activity, MALT1 inhibition may also have beneficial effects on the TME of these hematological tumors. The majority of DLBCL are infiltrated by Treg cells, but contrary to most non-hematological cancers, high Treg cell density is generally associated with a favorable prognosis.[114] However, the presence specifically of strongly activated eTreg cells characterized by high CTLA-4 expression in the TME correlates with poor prognosis, highlighting the importance of the functional state of DLBCL-infiltrating Treg cells.[114] Since MALT1 inhibition preferentially affects activated, suppressive eTreg cells, patients with the latter group of DLBCL may selectively benefit from Treg cell reprogramming that could synergize with ICT, which has otherwise so far yielded disappointing results in DLBCL.[115] Finally, increased Treg cell infiltration also correlated with an inferior clinical outcome specifically in non-GCB type DLBCL,[116] which primarily represent ABC-type patients with elevated MALT1 protease activation. Thus, particularly in the subset of patients with MALT1-dependent ABC DLBCL, the simultaneous targeting of MALT1 in the tumor as well as in tumor-infiltrating Treg cells may together yield a beneficial clinical response. Unfortunately, the scarcity of syngeneic MALT1-dependent lymphoma models has made it difficult to assess the effects of MALT1 inhibition on the TME in preclinical settings. Fully immune-competent mouse models of MALT1-dependent lymphoma, such as the cyclin D2-driven MCL-like tumor model,[64] will be essential to assess the concurrent impact of MALT1 inhibition on the TME.

Combined cancer cell-intrinsic and cell-extrinsic MALT1 targeting may also amplify therapeutic effects in non-hematological solid cancer, as best exemplified for breast cancer. Proliferation, survival and invasiveness of breast cancer relies on MALT1 proteolytic activity.[86–88] Especially in xenograft models of TNBC, MALT1 inhibition reduces tumor growth and metastasis in a cell-intrinsic manner.[88] Traditionally, breast cancers have been considered to be poorly immunogenic, but it has become clear that, like many other cancers, they are embedded in a complex TME with a network of immunosuppressive cells, including Treg cells, that cause immune escape and tumor progression.[117–119] Treg cell infiltration is associated with more aggressive behavior and unfavorable prognosis in advanced breast cancer[117] and particularly TNBC harbor numerous activated Treg cells with potent suppressor function.[120] While early clinical trials evaluating anti-PD1 (pembrolizumab) monotherapy showed promising responses in patients with advanced metastatic and PD-L1-positive TNBC, randomized controlled trials failed to demonstrate a significant improvement in overall survival of pre-treated metastatic TNBC patients compared with chemotherapy.[121–123] Of note, there was a trend for improved pembrolizumab responses in TNBC patients to correlate with higher PD-L1 expression.[124] Since MALT1 inhibition reprograms activated eTreg cells to secrete IFNγ and induce expression PD-L1 on cancer cells,[6] and given the density of highly suppressive intratumoral eTreg especially in TNBC,[120] proinflammatory Treg cell reprogramming by MALT1 inhibition may improve ICT responses in TNBC concurrent with cancer cell-intrinsic effects on progression and metastasis.[88] This provides a rationale for the combination of MALT1 inhibitors and ICT especially in TNBC, but also many other non-hematological solid cancer types, including glioblastoma, melanoma, lung cancer, and ovarian cancer, with suggested cell-intrinsic dependence on MALT protease activity.

MALT1 INHIBITOR PROFILES AND CONSIDERATIONS FOR CANCER CELL-INTRINSIC VERSUS CELL-EXTRINSIC TARGETING

The demonstration that MALT1 confers a unique enzymatic activity to the CBM complex in activated lymphocytes and lymphoma cells has initiated an intensive quest for MALT1 inhibitory compounds not only for the treatment of BCR-addicted lymphomas, but also for mitigation of antigen receptor-triggered immune responses in autoimmune and inflammatory diseases.[3 4 28 29] Drug discovery programs in academia and industry led to the development of candidate drugs whose chemical classes and structures have recently been summarized.[125] Structure-guided drug research revealed a favorable allosteric mechanism of MALT1 inhibition leading to the development of potent, selective, and reversible MALT1 inhibitors,[50 52 126] which has yielded currently two clinical candidates, JNJ-67856633[53 127] and MPT-0118.[128] Clinical programs using JNJ-67856633 aim at cancer cell-intrinsic targeting of MALT1 in NHL and CLL as a single agent or in combination with the BTK inhibitor ibrutinib (NCT03900598 and NCT04876092). The MPT-0118 trial evaluates MALT1 inhibition for tumor-cell extrinsic proinflammatory reprogramming of Treg cells in the TME to boost antitumor immunity in solid cancer as a single agent or in combination with the anti-PD1 checkpoint blocker pembrolizumab (NCT04859777). Given the dual role of MALT1 in the cancer cells and the immune TME, as well as potential adverse effects on long-term MALT1 inhibition, several considerations apply in choosing MALT1 inhibitors with optimal profiles for different clinical settings.

For precision oncology, it is in general desirable to engage the target with drugs that show high in vivo potency and selectivity. MALT1 inhibitors such as MLT-985 or JNJ-67856633 meet these criteria.[52 53] However, even in the targeted therapy of lymphomas, on-target effects on the immune system must be considered. Although effector T cells maintain residual function in the absence of MALT1 protease activity, highly potent MALT1 inhibition impairs T cell activation[69 129] and may thus also reduce beneficial antitumor immunity in hematological cancers, an effect that would not be captured in immunodeficient xenograft models commonly used for drug testing in this setting. Second, sustained potent MALT1 inhibition may not only reprogram lymphoma-infiltrating Treg cells to aid the therapy of solid cancers, but is also expected to affect the systemic Treg cell pool that maintains immune homeostasis. This has indeed been observed in animal studies, where circulating Treg cell numbers declined within a week and autoimmune toxicity developed soon after.[130] Thus, adverse on-target effects such as Treg cell depletion need to be monitored and autoimmune toxicity may limit the maximal dose levels and/or duration of continuous MALT1 inhibitor treatment in patients. Importantly, peripheral Treg cell depletion and immune pathologies depend on the strength of MALT1 inhibition and are fully reversible,[129–132] suggesting that inhibitors with moderate potency, lower dosing, or treatment pausing between cycles may open a therapeutic window that avoids adverse effects (figure 4).

The risk of systemic autoimmune toxicity through systemic Treg cell depletion is also of concern when MALT1 inhibition is intended to reprogram tumor-infiltrating Treg cells in solid cancers to amplify PD-1-targeted ICT efficacy. In this context, immune suppression through MALT1 inhibition in antitumor effector lymphocytes, whose recruitment to the TME is an intended consequence of proinflammatory Treg cell reprogramming, would predictably also off-set treatment efficacy. However, since partial inactivation of the CBM complex through heterozygous CARD11 deficiency is well tolerated and suffices to reprogram tumor infiltrating Treg cells,[6] full MALT1 protease inhibition may not be required to achieve the desired treatment effect. Intermittent dosing or dose reduction of highly potent MALT1 inhibitors,[129] or the use of inhibitors with intermediate potency may

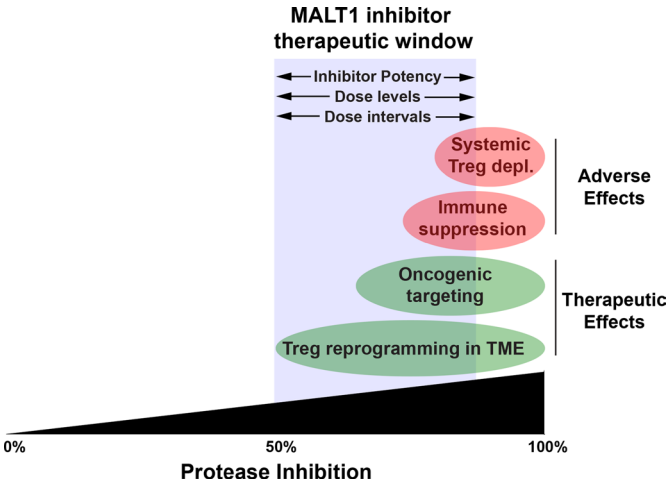

Figure 4 For cancer therapy, judicious selection of MALT1 inhibitor in vivo potency, dose, and dosing schedule can optimally balance treatment efficacy with undesirable T cell-dependent immune-suppression and the risk of autoimmune toxicity through systemic Treg cell depletion. MALT1, mucosa-associated lymphoid tissue protein 1.

therefore mitigate the risk of both autoimmune toxicity and immune suppression without compromising on their antitumor activity through proinflammatory Treg cell reprogramming in the TME (figure 4). Thus, in contrast to cancer cell-intrinsic targeting, high in vivo potency of MALT1 inhibitors may not be the key factor, which is in line with the ability of moderately potent MALT1 inhibitors, such as the phenothiazines mepazine or MPT-0118 to reprogram tumor-infiltrating Treg cells and induce effective antitumor immunity in preclinical models despite any potential immune-suppressive effects, and without detectable impact on Treg cells in blood and healthy tissues.[6 128 130 132] The efficacy of these phenothiazines may in part also be owed to their pharmacokinetic properties that are reflected in an asymmetric distribution between tumor tissue and plasma and their tumor tissue retention, producing effective doses in the TME while likely not achieving the strong and persistent systemic exposures required to deplete circulating Treg cells.[128 130] This favorable profile may also explain their robust activity against ABC-DLBCL xenografts, despite their only intermediate potency.[1] However, since higher dosing may be required for MALT1 inhibitors with moderate potencies, adverse effects through off-target activity may be more prevalent and this needs to be closely monitored. On the other hand, it will need to be determined if dosing of highly potent inhibitors can be titrated to reduce systemic Treg depletion[129] while still effectively reprogramming tumor-infiltrating Treg cells.

Taken together, preclinical data clearly point to a therapeutic window for the use of cancer cell-intrinsic or cell-extrinsic targeting of MALT1. However, differential requirements and potential adverse events emphasize that distinct MALT1 inhibitor profiles may be suitable depending on the clinical setting.

CONCLUSIONS AND OUTLOOK

The critical role of MALT1 protease activity in modulating immune responses and driving progression of lymphomas and non-hematological solid cancer has inspired intensive drug research, resulting in the discovery of different classes of small molecule MALT1 inhibitors with distinct pharmacological properties. Tremendous progress has been made in elucidating cancer cell-intrinsic MALT1 protease functions, but also how MALT1 activity in Treg cells maintains an immune-suppressive TME. Preclinical evidence strongly advocates that MALT1 targeting in cancer cells as well as in Treg cells in the TME may yield beneficial antitumor responses. Especially the ability of MALT1 inhibitors to reprogram tumor-infiltrating suppressive into proinflammatory Treg cells opens a new avenue for converting immunologically 'cold' into 'hot' tumors, which thereby are sensitized for ICTs. It will be interesting to explore synergies with immunotherapies other than PD-1 blockade, including those that act primarily in the TME, but also those that are thought to primarily amplify the induction of antitumor immunity in tumor-draining lymph nodes, such as anti-CTLA4 antibodies. The latter could potentially even antagonize the effectiveness of proinflammatory Treg cell reprogramming through their Treg cell-depleting activity reported in some settings.[133]

First-in-class MALT1 inhibitors have entered clinical evaluation for cancer cell-intrinsic MALT1 targeting in malignant lymphomas and cancer cell-extrinsic MALT1 inhibition in solid tumors. Precise biomarkers will be essential to obtain clinical proof of mechanisms, to enable patient stratification, and to facilitate the design of combinatorial treatment protocols. While oncogenic lesions in lymphomas can provide a rationale for combining BTK and MALT1 inhibitors, infiltration of Treg cells in solid cancers implies local immune tolerance and constitutes the basis for combining MALT1 and immune checkpoint inhibitors. In all cases, appropriate biomarkers will allow for accurate monitoring of systemic immune alterations, which can serve as early signs of reversible adverse events that may result from efficient and long-term MALT1 protease inhibition. Taken together, MALT1 inhibition can combine direct targeting of cancer cells with augmentation of antitumor immunity and trials have been started to obtain clinical proof of concept. Beyond the utility specifically of MALT1 inhibitors for cancer therapy, these studies may validate the general concept of therapeutic reprogramming of suppressive into proinflammatory Treg cells and, more broadly, of drug-induced modulation of distinct immune cell subsets based on their distinct metabolic and signaling dependencies in the TME, for enhancing antitumor immunity to improve the treatment of patients with cancer.

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