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REVIEW – CLINICAL ONCOLOGY

Blocking antibody-mediated phosphatidylserine enhances cancer immunotherapy

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Abstract

Cancer immunotherapy is a major breakthrough in tumor therapy and has been used in monotherapy or combination therapy. However, it has been associated with poor immune tolerance in some patients or immune-related adverse events. Therefore, ideal and reliable tumor elimination strategies are urgently needed to overcome these shortcomings. Phosphatidylserine (PS) is a negatively charged phospholipid, usually present in the inner lobules of eukaryotic cell membranes. Under certain physiological or pathological conditions, PS may be exposed on the outer leaflets of apoptotic cells serving as recognition signals by phagocytes and modulating the immune response. On the contrary, increased exposure of PS in the tumor microenvironment can significantly antagonize the body's anti-tumor immunity, thereby promoting tumor growth and metastasis. During radiotherapy and chemotherapy, PS-mediated immunosuppression increases the PS levels in necrotic tissue in the tumor microenvironment, further suppressing tumor immunity. PS-targeted therapy is a promising strategy in cancer immunotherapy. It inhibits tumor growth and improves the anti-tumor activity of immune checkpoint inhibitors. A comprehensive understanding of the mechanism of PS-targeted therapy opens up a new perspective for future cancer immunotherapies.

Keywords Phosphatidylserine · Cancer · Immunotherapy · Tumor microenvironment

Abbreviations

ADCC	Antibody-dependent cytotoxicity
Ceacam-1	Carcinoembryonic Antigen Cell Adhesion Molecule-1
DC	Dendritic cells
FAS1	Fascicle protein I
FcRs	Fc receptors
HMGB1	High Mobility Group Histone B1
ICIs	Immune checkpoint inhibitors
IDO	Indoleamine 2,3-dioxygenase 1
IFN-γ	Interferon γ
IL-2	Interleukin 2
1N11	Monoclonal antibodies
mAbs	Monoclonal antibodies
MDSCs	Myeloid-derived suppressor cells
NSCLC	Non-small cell lung cancer
PS	Phosphatidylserine
Rac1	Rac family small GTPase 1
RTKs	Receptor tyrosine kinases
TAM	Tyro, Axl, and Mertk
TIM	T cells, immunoglobulin, and mucin
TME	Tumor microenvironment
TNF	Tumor necrosis factor
TIL	Tumor-infiltrating lymphocytes
VCAM-1	Vascular cell adhesion molecule 1
β2GP1	β2 Glycoprotein 1

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Background

Cancer is a serious public health challenge associated with high morbidity and mortality. Researchers have continuously explored new tumor targets in the past few decades. In recent

years, immunotherapy has become the focus of new treatment approaches and has been shown to achieve remarkable results. However, the benefits achieved with monotherapy using immune checkpoint inhibitors (ICIs) were not ideal (Barrueto et al. 2020). Therefore, a clinical decision was made to use simultaneous blockade of CTLA-4 and PD-1. The possibility of this therapy inducing high-grade immune-related adverse events should thus be considered (Tang et al. 2020; Zhou et al. 2020). Further, immunotherapy may affect the tumor microenvironment (TME) to a large extent (Lei et al. 2020). Accumulating evidence reveals that TME plays a more prominent role in tumor immunity than ICIs (Kamal et al. 2020). Other tumor-related immunomodulatory therapies targeting TME or new treatment strategies that combine existing therapies have also been explored. Researchers have also gained interest in phosphatidylserine (PS)-targeted therapy. Phospholipids in eukaryotic cells have an asymmetric distribution. The negatively charged phosphatidylserine is mainly located in the inner membrane leaflet. Researchers have shown that the exposed PS is recognized during cell apoptosis, triggering phagocytosis. Activation of the PS receptor may have immunosuppressive effects by attenuation of the immune response. PS exposure in the tumor microenvironment may lead to immunosuppression and facilitate tumor growth (Shurin et al. 2009; Zohar and Shoenfeld 2018). Therefore, the location of PS on the cell membrane affects cell viability, tumor invasion, and metastasis (Iida et al. 2015). Therefore, targeting exposed PS in the TME with ICIs may offer a new strategy for improving tumor treatment success.

The basic mechanism of targeting PS

PS is generally located on the inner leaflet of the plasma membrane. Under normal physiological conditions, PS exposure on the outer membrane releases an "eat me" signal, inducing macrophages to engulf the cell (Calianese and Birge 2020). Although PS can also be transiently exposed on the surface of some immune cells including B cells, T cells and dendritic cells (DC), they may have a higher PS exposure threshold to circumvent autoimmunity (Fischer et al. 2006). Externalized PS is a cell marker, mainly responsible for the recognition and uptake of apoptotic cells by phagocytes, and inhibits the potential autoimmune response (Ishii et al. 2005). However, in TME, apoptosis-related key factors, such as hypoxia, adenosine, lactate, vascular endothelial growth factor, activated caspase, Ca^{2+} influx, among others, promote the externalization of PS to the outer leaflet of the plasma membrane (Francis et al. 2013; Vaupel and Multhoff 1887). The exposed PS binds to PS receptors on immune cells and weakens the innate and adaptive immune response by activating the immunosuppressive pathway, conducive to tumor immune escape (Freeman et al. 2010). Furthermore, PS exposure often occurs on the premise of cell necrosis or apoptosis in TME (Li et al. 2019). However, this phenomenon has also been observed in endothelial cells or extracellular vesicles of tumor or mesenchymal origin (Fendl et al. 2018). What is more, PS is also exposed in a variety of infectious pathogens and on the surface of infected cells, producing a weaker non-inflammatory immune response, called "apoptosis mimicry" (Hosseini et al. 2015). Presently, a variety of PS receptors have been discovered and are involved in different signal transduction pathways. Further, most PS receptors are related to the anti-inflammatory response, with some contributing to the pro-inflammatory response.

Induction and immunosuppressive effects of PS exposure on tumor cells

PS is absent on the external surface of vascular endothelial cells in normal cells. However, in the tumor microenvironment, oxidative stress can expose PS to the surface of the vascular endothelium of cancer cells (Sharma and Kanwar 2018). Generally, PS recognizes receptors on DC, macrophages, and T cells to suppress immunity (Chang et al. 2020). PS-binding to the receptors promotes the polarization of macrophages from a pro-inflammatory M1-like phenotype to a pro-tumor M2-like phenotype and leads to the secretion of immunosuppressive factors, such as IL-10 and TGF-β (Serinkan et al. 2005; Quan et al. 2018). IL-10 and TGF-β inhibit T cell activation by suppressing tumor antigen presentation by DC and induction of regulatory Tregs (Stewart et al. 2013). Meanwhile, PS on tumor-derived micro-vesicles has also been shown to inhibit T cell activation (Park and Kang 2019). PS has also been shown as an essential in mediating the combination of IFN-γ and IL-12 and prolonged inflammation (Frey and Gaipl 2011). Therefore, exposure of PS in tumor cells also has anti-tumor effects by mediating chronic inflammation.

Targeting PS receptors and cancer therapy

Several receptors recognize PS, including TIM (T cells, immunoglobulin, and mucin) gene family, stabilin-1/2, and TAM (Tyro, Axl, and Mertk) gene family members in TEM to cause immunosuppression (Dayoub and Brekken 2020). The TIM gene family, BAI1, and stabilin-1/2, directly bind to PS. However, the TAM gene family binds to PS indirectly via binding to protein ligands (Hochreiter-Hufford et al. 2013; David et al. 2012; Wu et al. 2018). We summarize the main receptors, their related characteristics and presenting cells. (Table 1).

Table 1 major PS receptors and their characteristics

PS receptors		Characteristics	Presenting cells/tissues
TIM family	TIM-1	Recognize PS through an interaction with the conserved n-terminal immunoglobulin-like extracellular domain	$CD4^+$ T cells, Th2 cells regulatory B cells
	TIM-3		Th1,Tc1, $CD8^+$ T cells, Th2 cells, Th17 cells, and regulatory T cells
		Binds to PS directly	Sarcoma, cervical, gastric cancer, myeloma, melanoma, and lung cancer cells
	TIM-4		Dendritic cells
			Macrophages
Stabilin-1		Binds to PS on the surface of apoptotic cells	Liver, spleen, lymph nodes
		Activate Rac1	Bone marrow (stabilin-2)
		Reorganize the actin cytoskeleton through Gulp1 to phagocytose apoptotic cells	Adrenal cortex (stabilin-1)
Stabilin-2		Binds to PS directly	
TAM family	Tyro	Binds to PS via γ-carboxylated bridging proteins, Gas6 or protein S, thus activating the TAM receptor	Macrophages
			Dendritic cells
	Axl	Indirectly binds to PS	NK cells, T cells
	Mertk		

TIM

The human TIM gene family mainly encodes three receptors, TIM-1, TIM-3, and TIM-4, which regulate various immune responses, such as asthma and infection (Burstyn-Cohen and Maimon 2019). Studies have shown that the TIM receptor recognizes PS by interacting with the conserved n-terminal immunoglobulin-like extracellular domain (Burstyn-Cohen and Maimon 2019). IM-1 is mainly expressed on CD4 + T cells and regulatory B cells. TIM-1 on Th2 cells is an effective costimulatory molecule for T cell activation. DC and macrophages highly express TIM-4 and participate in the inflammatory response (Kong et al. 2020).

TIM-3 is a type-I transmembrane protein, which is a specific marker for Th1 and Tc1 cells. TIM-3 is also expressed on cytotoxic CD8 + T cells, Th2 cells, Th17 cells, and regulatory T cells. It forms an interaction with PS through the extracellular IgV domain, which is a mucin stalk containing N- and o-chain glycosylation sites and an intracellular tail with a conserved tyrosine residue (Anderson 2014). In addition, TIM-3 is associated with poor prognosis in various cancers, including cervical cancer, gastric cancer, melanoma, and lung cancer. So far, Galectin-9, Carcinoembryonic Antigen Cell Adhesion Molecule-1 (Ceacam-1), High Mobility Group Histone B1 (HMGB1), and PS are the four ligands related to TIM-3 (Romero 2016; Du et al. 2017) (Fig. 1). The TIM-3/Galectin-9 pathway also promotes the production of myeloid-derived suppressor cells (MDSCs), which suppresses adaptive immunity, thus accelerating tumor growth and decrease autoimmunity (Zhou et al. 2019; Wang et al. 2014). As a molecule exposed on the surface of apoptotic cells, PS binds to TIM-3, promoting the clearance of apoptotic bodies (He et al. 2018). The number of apoptotic cells in the spleen of mice treated with TIM-3 mAb was shown to be increased, while the serum anti-dsdna antibodies were shown to be increased (Nakayama et al. 2009). The study also found that CD8 + DC expressing TIM-3 mediated phagocytosis of apoptotic cells and cross-presentation of related antigens to CD8 + T cells (Nakayama et al. 2009). In addition, allelic variants of TIM-3 have different binding affinities and phagocytic abilities to PS. The BALB/c alleles of TIM-3 have a higher binding to PS than HBA. DC and macrophages constitutively express TIM-3 and act as a negative immune regulator (Ocana-Guzman et al. 2016). An increase of the anti-inflammatory cytokines, such as IL-4 and TGF-β, polarizes the M1-like phenotype of macrophages to the M2-like phenotype, and increases the expression of TIM-3 (Ocana-Guzman et al. 2016). TIM-3^+ phagocytes and PS expression mediate the uptake of apoptotic cells, thus initiating TIM-3-mediated inhibition (Sabatos-Peyton et al. 2018). A recent research revealed that TIM-3 promotes tumor growth by weakening immune activity. According to another study, TIM-3 was synergistically expressed with PD-1 in tumor-infiltrating lymphocytes (TIL) (Jie et al. 2017; Li et al. 2016). TIM-3^+PD-1^+ TILs showed a clear T cell failure phenotype, that was, unable to secrete interleukin 2 (IL-2), tumor necrosis factor (TNF), and interferon γ (IFN-γ). Another study revealed that PD-1 + CD8 + T cells in patients with melanoma also increased the expression of TIM-3 (Lu et al. 2017). The above results suggest that blocking TIM-3 and PD-1 may be beneficial in the recovery of T cell failure.

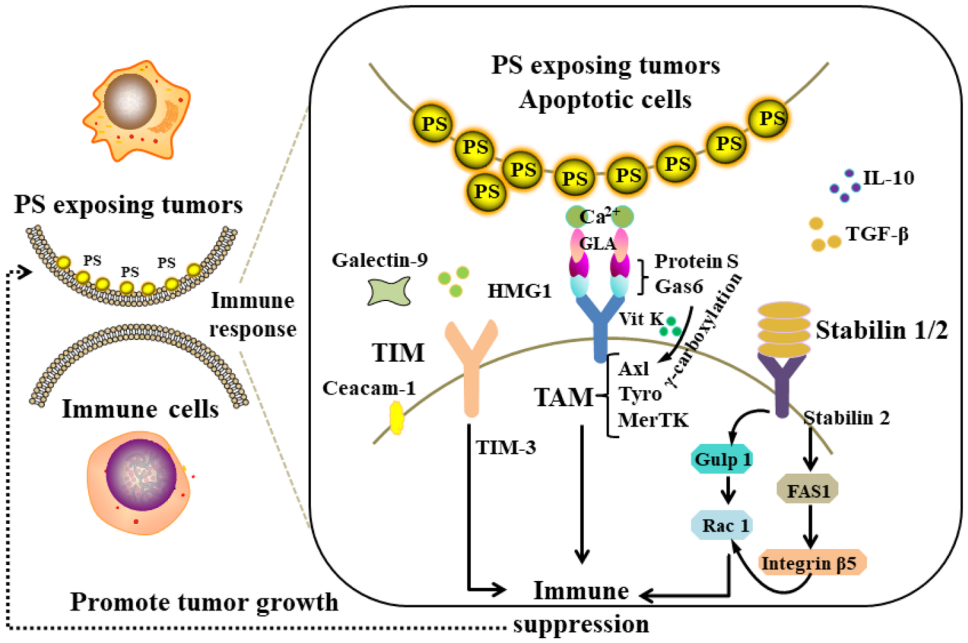

Fig. 1 The PS receptor in immunosuppression. The four ligands related to TIM-3 are Galectin-9, Ceacam-1, HMGB1, and PS. Stabilin-1 and stabilin-2 directly bind to PS on the surface of apoptotic cells to activate Rac1. In the Gulp1-independent pathway, stabilin-2 acts on integrin β5 through FAS1, to activate Rac1 and induces cytoskeletal rearrangement. The TAM gene family (Tyro, Axl, and Mertk) binds to PS via Gas6 or protein S. The γ-carboxylated GLA domain of Gas6 and protein S directly bind to PS. Blocking vitamin K-dependent Gas6 γ-carboxylation can inhibit the activation of Axl on tumor cells and reduce tumor progression and metastasis

Stabilin-1 and stabilin-2

Stabilin-1 and stabilin-2 are mostly expressed in the liver, spleen, lymph nodes, bone marrow (stabilin-2), and adrenal cortex (stabilin-1) (Park and Kim 2019). Stabilin-1 and stabilin-2 directly bind to PS on the surface of apoptotic cells, activating Rac1 (Rac family small GTPase 1) (Park and Kim 2019). They reorganize the actin cytoskeleton through Gulp1 to phagocytose apoptotic cells. In the Gulp1-independent pathway, stabilin-2 acts on integrin β5 through its fascicle protein I (FAS1), activates Rac1 and induces cytoskeletal rearrangement (Park et al. 2016; Twarda-Clapa et al. 2018). Down-regulation of endogenous Gulp1 can attenuate phagocytosis of PS-exposed erythrocytes mediated by stabilin-1 and stabilin-2 (Park et al. 2010). On the contrary, overexpression of Gulp1 can enhance phagocytosis of PS-exposed red blood cells by stabilin-2. Stabilin-1 is also expressed in selectively activated macrophages (M2-like macrophages) (David et al. 2012). M2-like macrophages activate the body's repair response and promote tumor growth. Stabilin-1 was shown to be recruited in macrophages co-cultured with apoptotic cells and eliminated the dead cells via mediation with PS (Park et al. 2012). Immunosuppressive leukopenia in TME was observed after treatment with anti-Stabilin-1 showing that stabilin-1 in macrophages was involved in immune response against tumor cells. In addition, the growth of primary tumors in TME with stabilin-1-silenced macrophages was also reduced compared with the control group. TGF-β is an immunoregulatory factor related to the differentiation of immunosuppressive regulatory T cells. Park et al. showed that intervention with PS-exposed red blood cells or anti-stabilin-2 antibodies induced the secretion of TGF-β, indicating that stabilin-2 could regulate the inflammatory response after apoptosis (Park et al. 2008).

TAM

TAM genes are receptor tyrosine kinases (RTKs) expressed on various tumors. RTKs bind to PS via the γ-carboxylated bridging proteins Gas6 or protein S (Graham et al. 2014). The γ-carboxylated GLA domain of Gas6 and protein S directly binds to PS, and the receptor-binding domain is responsible for recognizing the TAM receptor (Wu et al. 2018; Meer and Poll 2014). The activation of RTKs on tumor cells is related to chemotherapy resistance. Blocking the vitamin K-dependent γ-carboxylation of Gas6 was shown to inhibit Axl activation on tumor cells and reduce tumor progression and metastasis in preclinical tumor models (Wu et al. 2018; Stasi et al. 2020). The combination of PS and TAM RTKs on tumor cells can also enhance the expression of PD-L1 on tumor cells (Kasikara et al. 2017). Therefore, anti-tumor immunity can be enhanced by blocking PS-mediated activation of TIM and TAM RTK pathways.

Targeting PS

Ran et al. covalently bound the monoclonal antibody of vascular cell adhesion molecule 1 (VCAM-1) to the extracellular domain of coagulation-related human tissue factor forming a 'coagulant' (Ran et al. 1998). The coagulant was selectively localized to tumor blood vessels expressing VCAM-1, thus promoting thrombus formation and delaying

tumor growth. Interestingly, the coagulant was also located in the blood vessels expressing VCAM-1 in the heart and lungs of mice. However, these blood vessels did not show thrombosis (Lino et al. 2019). Further, they used immunohistochemistry to evaluate the distribution of monoclonal anti-phosphatidylserine (PS) antibodies. The results showed that VCAM-1 and PS were co-expressed in tumor blood vessels, but PS was not found on the external cell membrane of the heart and lungs (Gosk et al. 2008). They hypothesized that the difference in selectivity was due to the exposure of PS on the surface of blood vessel endothelial cells within TME, which was necessary for inducing blood coagulation (Ran et al. 1998). Ran et al. then developed a monoclonal antibody 9D2 against anionic phospholipids based on the exposure of PS on the outer leaflet of the cell membrane in tumor vasculature (Ran et al. 2005). 9D2 and Annexin V specifically localize to different epitopes in tumor blood vessels. The interaction of 9D2 and PS did not require $Ca2^{+}$, unlike with Annexin V (Ran et al. 2002). Reactive oxygen species, hypoxia, and some inflammatory mediators, such as IL-1a and IFN-γ, in TME were shown to induce a moderate increase in PS exposure (Klein et al. 2021). Therefore, targeting exposed PS in tumor blood vessels may be an effective anti-cancer strategy.

The monoclonal antibody IgG (2aG4, 3G4, bavituximab, 1N11, and mch1N11) launched by Thorpe Laboratories against PS was shown to exert anti-tumor activity by destroying tumor blood vessels (Ran et al. 2005). The PS-targeting monoclonal antibody was shown to bind to PS via the serum cofactor β2 glycoprotein 1 (β2GP1). β2GP1 is an anionic phospholipid-binding protein in serum, which acts as a bridge between PS and monoclonal antibodies (Luster et al. 2006). PS-targeting monoclonal antibody binds to dimeric β2GP1 and PS on the plasma membrane with high affinity. However, β2GP1 could trigger the production of anti-phospholipid antibodies, causing autoimmune diseases such as systemic lupus erythematosus (Martinez-Flores et al. 2015). According to Mineo et al., monoclonal antibodies (1N11) could prevent the adverse events associated with anti-phospholipid antibodies.

Macrophages mediated the inhibition of tumor angiogenesis by 3G4, which was seen as reduced blood vessel density and plasma volume. This reveals that targeting PS can change the tumor microenvironment (Chang et al. 2020). In addition, targeting PS on tumor cells can prevent the conversion of the M1-like pro-inflammatory phenotype of macrophages to the M2-like pro-tumor phenotype (Dayoub and Brekken 2020). In orthotopic human breast xenotransplantation models, 40% of tumor blood vessels were combined with monoclonal antibodies. Preclinical studies showed that infected cells increased PS exposure when treated with mouse chimeric antibody 1N11 (mch1N11), thus increasing anti-tumor activity (Budhu et al. 2021). Human chimeric monoclonal antibodies may have anti-cancer effects mediated by macrophages through antibody-dependent cytotoxicity (ADCC) directed against PS^{+} tumor endothelial cells (Belzile et al. 2018). Meanwhile, the anti-cancer effect of monoclonal antibodies is amplified by chemotherapy. In short, increased PS exposure combined with PS-targeting antibody in tumor cell damage is beneficial to enhance anti-tumor immunity. The PS-targeting antibody enhances the killing effect through ADCC and reprogramming of immunosuppressive cells (Birge et al. 2016). At the same time, this process is accompanied by the maturation of DCs and the proliferation of effector T cells. Reprogramming is considered to be mediated by the blockade of the PS receptors of immunosuppressive cells and the interaction of antibodies with activated Fc receptors (FcRs) on DCs (Masuda et al. 2009; Naqvi et al. 2016). Reprogramming can be achieved by blocking the PS receptors of immunosuppressive cells or by binding antibodies to activated FcRs on DCs (Stavenhagen et al. 2007). The binding of antibodies to activated FcRs not only promotes DC maturation and antigen presentation ability, but also promotes the progress of adaptive immunity (Bajtay et al. 2006). Further studies have found that targeting apoptotic tumor cells to FcγR could provide effective DC vaccination against tumors (Murphy et al. 2014).

Targeting PS receptors

There has been an increased interest in PS-targeted therapy and the development of antibodies against PS receptors. TIM-3 antibody combined with anti-CTLA-4 and anti-PD-1 drugs was highly sensitive and well tolerated in patients with carcinogenic sarcoma (Ngiow et al. 2011). In addition, blockade of TIM-3 relieved immune suppression by reducing regulatory T cells in head and neck tumors (Liu et al. 2018). Several clinical trials targeting TIM-3 antibodies are summarized in Table 2. For example, NCT0368050 evaluated the effect of combined anti-TIM-3 antibody (TSR-022) and anti-PD-1 antibody in treating locally advanced or metastatic liver cancer. NCT03489343 was the first study to test the safety of Sym023 (Anti-TIM-3) in patients with metastatic solid malignancies or lymphomas without standard therapies. Unfortunately, most of these studies have not reported their findings. Harding et al. evaluated the effect of a new TIM-3 monoclonal antibody (LY3321367) alone or in combination with anti-PD-L1 antibodies in patients with advanced solid tumors (Harding et al. 2021). LY3321367 showed acceptable safety with good pharmacokinetics. However, it had moderate anti-tumor activity (Harding et al. 2021).

Antibodies targeting TAM have also attracted widespread attention. Tyro3 is considered an oncogene in various cancers, and it is specifically overexpressed in melanoma and

Table 2 Clinical trials targeting TIM-3

NCT number	Title	Status	Conditions	Interventions	Phases
NCT04641871	Sym021 in combination with Sym022 or Sym023 in patients with advanced solid tumors	Active, not recruiting	Metastatic cancer Solid tumor	Drug: Sym021 Drug: Sym022 Drug: Sym023	Phase 1
NCT04370704	Study of combination therapy with INCMGA00012 (anti-PD-1), INCAGN02385 (anti-LAG-3), and INCAGN02390 (anti-TIM-3) in participants with select advanced malignancies	Recruiting	Melanoma	Drug: INCAGN02385 Drug: INCAGN02390 Drug: INCMGA00012	Phase 1/2
NCT04139902	Neoadjuvant PD-1 inhibitor dostarlimab (TSR-042) vs. combination of Tim-3 inhibitor cobolimab (TSR-022) and PD-1 inhibitor dostarlimab (TSR-042) in melanoma	Recruiting	Melanoma stage III Melanoma Stage IV	Drug: dostarlimab (TSR-042) Drug: dostarlimab (TSR-042) and TSR-022 (combination)	Phase 2
NCT03961971	Trial of anti-Tim-3 in combination with anti-PD-1 and SRS in recurrent GBM	Recruiting	Glioblastoma multiforme	Drug: MBG453	Phase 1
NCT03752177	A study of LY3415244 in participants with advanced solid tumors	Terminated	Solid tumor	Drug: LY3415244	Phase 1
NCT03708328	A dose escalation and expansion study of RO7121661, a PD-1/TIM-3 bispecific antibody, in participants with advanced and/or metastatic solid tumors	Recruiting	Solid tumors Metastatic melanoma NSCLC/SCLC ESCC	Drug: RO7121661	Phase 1
NCT03680508	TSR-022 (anti-TIM-3 antibody) and TSR-042 (anti-PD-1 antibody) in patients with liver cancer	Recruiting	Adult primary liver cancer	Drug: TSR-022 and TSR-042	Phase 2
NCT03489343	Sym023 (anti-TIM-3) in Patients With Advanced Solid Tumor Malignancies or Lymphomas	Completed	Metastatic cancer Solid tumor Lymphoma	Drug: Sym023	Phase 1
NCT03311412	Sym021 monotherapy, in combination with Sym022 or Sym023, and in combination with Both Sym022 and Sym023 in patients with advanced solid tumor malignancies or lymphomas	Recruiting	Metastatic cancer Solid tumor Lymphoma	Drug: Sym021 Drug: Sym022 Drug: Sym023	Phase 1
NCT03307785	Study of niraparib, TSR-022, bevacizumab, and platinum-based doublet chemotherapy in combination with TSR-042	Active, not recruiting	Neoplasms Metastatic cancer Advanced cancer Solid tumor NSCLC	Drug: niraparib|Drug: TSR-042 Drug: carboplatin-paclitaxel Drug: bevacizumab|Drug: TSR-022 Drug: carboplatin-pemetrexed Drug: carboplatin-nab-paclitaxel	Phase 1
NCT03099109	A study of LY3321367 alone or with LY3300054 in participants with advanced relapsed/refractory Solid tumors pancreatic cancer	Active, not recruiting	Solid tumor	Drug: LY3321367 Drug: LY3300054	Phase 1

Table 2 (continued)

NCT number	Title	Status	Conditions	Interventions	Phases
NCT02817633	A Study of TSR-022 in participants with advanced solid tumors (AMBER)	Recruiting	Neoplasms	Drug: TSR-022|Drug: nivolumab Drug: TSR-042|Drug: TSR-033 Drug: docetaxel|Drug: pemetrexed Drug: cisplatin|Drug: carboplatin	Phase 1

colorectal cancer. Demarest et al. used monoclonal antibodies (mAbs) against Tyro3 to test their effect on the survival of melanoma cell lines. The results indicated that Tyro3 could increase the survival of melanoma cells and could be blocked by monoclonal antibodies (Demarest et al. 2013). Another study by Chien et al. found that targeting Tyro3 inhibited colon cancer epithelial–mesenchymal transition and increased drug sensitivity (Chien et al. 2016). Other studies developed monoclonal antibodies directed against Axl. AXL can activate oncogenic signaling pathways, leading to the migration of leukemic cancer cells. According to Duan et al., anti-AXL high-affinity antibody DAXL-88 could block the binding of AXL to its ligand GAS6 and inhibit GAS6-induced tumor cell invasion (Duan et al. 2019). In addition, tyrosine kinase of the AXL receptor is also considered a potential therapeutic target for NSCLC (Okimoto and Bivona 2015). BA3011 is also a monoclonal antibody that selectively binds to AXL in NSCLC and shows good efficacy (Samuel et al. 2007). At present, researchers have also developed a variety of drugs, such as ADC and CAB-AXL-ADC. Moreover, mouse models with MERTK-deficient tumors showed increased infiltration of leukocytes and CD8 + T lymphocytes (Cook et al. 2013). Targeting MerTK could enhance adaptive immunity following radiotherapy (Tormoen et al. 2020). Therefore, studies targeting PS receptors may provide a new perspective in tumor immunotherapy.

Combination of PS-targeted therapy and immune checkpoint inhibitors

In recent years, the clinical success of immunotherapy has drawn new attention to immuno-biology. Although immune checkpoint blockers have improved the anti-tumor response and survival rate, only a few patients benefit from them (Zhang et al. 2018). Freimark et al. revealed that PS-targeted treatment of melanoma in mice enhanced the anti-tumor activity of ICIs (Freimark et al. 2016). PS-targeting antibody combined with ICIs significantly inhibited tumor growth compared with a single agent. Combined therapy also increased the ratio of $CD4^+/CD8^+$ tumor-infiltrating lymphocytes (TILs) and induced the expression of pro-inflammatory mediators including IL-2, IFN-γ, and TNF-α (Freimark et al. 2016). IFN-γ can lead to chronic inhibition of T cell signaling pathway and the production of indoleamine 2,3-dioxygenase 1 (IDO), resulting in signal attenuation (Chinnadurai et al. 2014). However, another study revealed that IFN-γ-permitted MDSCs inhibited T cell effects through PD-1 ligands without IDO (Pistillo et al. 2020). Multiple immune checkpoints can be co-expressed on activated TILs. Several studies have shown that PD-L1 combined with TILs may be more accurate than a single-agent therapy with PD-L1 in predicting the survival of colorectal cancer (Wang et al. 2020). Moreover, the expression of PD-1 and PD-L1 on TILs effectively predicts the prognosis in small cell lung cancer (Sun et al. 2020). In addition, the ratio of $CD8^+$ T cells to MDSCs and Tregs in the tumor microenvironment was also increased.

Gray et al. used single or combined therapy composed of PS-targeting and anti-PD-1 antibodies in mice carrying syngeneic EMT-6 or E0771 tumors (Gray et al. 2016). They found that treatment with PS-targeting antibodies could inhibit tumor growth and significantly enhance the anti-tumor activity of ICIs, with a statistically significant survival advantage in single-agent therapy (Gray et al. 2016). Secondary tumors in mice showed relative resistance to combination therapy. Further, the spleen cells were induced to produce large amounts of IFN-γ. An immune profile analysis showed enhanced expression of TIL and immune monitoring-related cytokines with PS-targeting and anti-PD-1 combined therapy, which was consistent with a previous study by Freimark et al. (2016). A recent study revealed that the combined therapy of PS-targeting mch1N11 and ICIs had a better anti-tumor effect than ICIs monotherapy (Freimark et al. 2016; Gray et al. 2016). The combination therapy based on mch1N11 effectively promoted the proliferation of $CD4^+/CD8^+$ TILs and induced the expression of pro-inflammatory cytokines, including IL-2, IFN-γ, and TNF-α. The ratio of MDSCs

and Treg cells in tumor and spleen tissues was also up-regulated. The above results were comparable to studies showing that ICIs' combined blockade was more effective than monotherapy (Colli et al. 2017). Lag3 is a negative regulatory target expressed on T cells (Long et al. 2018). The addition of anti-Lag-3 to mch1N11 and anti-PD-1 targeted therapy increased the tumor suppression effect to 99% and the tumor regression rate to 80% (Budhu et al. 2021; Belzile et al. 2018). Triple therapy in the animal model significantly enhanced antigen presentation and reduced tumor-promoting genes. What is more, radiotherapy and chemotherapy are inducers of PS externalization that also up-regulate the expression of PD-L1 in tumor cells.

So far, PS-targeted drugs have been launched, some of them undergoing clinical trials as combination therapy in multiple cancers. Bavituximab is an immunomodulatory chimeric monoclonal antibody that promotes immune response by inhibiting phosphatidylserine signaling. Table 3 summarizes relevant clinical trials of PS antibody (bavituximab) targeting in tumors. A single-arm phase II clinical trial evaluating combination therapy with bavituximab and sorafenib in the treatment of advanced hepatocellular carcinoma showed that the combined regimen did not aggravate the toxicity related to sorafenib (Mokdad et al. 2019). Another study evaluated the efficacy of the combined regimen in a mouse xenograft model of liver cancer (Stasi and Cappuzzo 2014). The results showed that the combined regimen was better than single-agent therapy, with sorafenib significantly increasing PS exposure in tumor blood vessels (Stasi and Cappuzzo 2014). The host immune response, such as antibody-dependent cellular cytotoxicity, is activated with the binding of bavituximab to PS. This leads to blood vessel destruction and enhancement of anti-tumor immunity.

Studies have also pointed out that combined therapy can significantly reduce the tumor micro-vessel density and levels of M2 macrophage and increase tumor endothelial cell apoptosis and the number of M1 macrophages (Gerber et al. 2015). Several preclinical studies have shown that the combination of bavituximab and chemotherapy can combat various solid tumors. Digumarti et al. evaluated the efficacy of bavituximab combined with paclitaxel and carboplatin in treating metastatic non-small cell lung cancer (NSCLC) (Digumarti et al. 2014). This combination showed tolerable safety and potential efficacy as a first-line treatment for advanced metastatic NSCLC (Digumarti et al. 2014). However, the potential risks of this combination need further evaluation. For example, blocking of MerTk (PS receptor) on T cells reduces the tumor-killing effect. In addition, the safety of combined anti-PS therapy and other anti-cancer drugs remains largely unknown.

The main limitations or risks of PS-targeted therapy

Although immunotherapy has achieved significant clinical results, only a few patients have benefited from ICIs. Moreover, immunotherapy is also associated with immune-related adverse events (Fan et al. 2021). Therefore, it is paramount to improve cancer immunotherapies. Drugs targeting PS have good tolerability and anti-tumor activity. However, there is a gap in their clinical applicability. First, the inhibition of PS receptors may reduce tumor-killing effects. Second, the safety of anti-PS therapy in combination with other ICIs is largely unknown. Third, the role of PS as a global checkpoint inhibitor in cancer needs to be further explored. Fourth, it is not known whether all PS receptors have immunosuppressive effects. Therefore, multiple studies are required to evaluate the different PS receptors in different tumors to develop new agents targeting the pathways.

Perspective and conclusion

Current cancer immunotherapy research aims to eliminate immunotherapy-related adverse events. To achieve this, research has employed a combination of PS-targeting antibodies with emerging therapies, such as radiotherapy and chemotherapy, immunotherapy, and oncolytic viruses, in various tumors. Thus, this review provides innovative and cross-cutting ideas and directions for developing PS-targeted drugs.

Table 3 Published trials of targeting PS antibody (bavituximab) in tumors

Title	Phase	Results	Experimental regimen	References
Phase Ib study of bavituximab with carboplatin and pemetrexed in chemotherapy-naive advanced non-squamous non-small-cell lung cancer	Phase Ib	Overall response rate: 35% Median progression-free survival: 4.8 months Median overall survival: 12.2 months	Bavituximab (3 mg/kg) + carboplatin/pemetrexed	Grilley-Olson et al. (2018)
A phase I clinical trial of bavituximab and paclitaxel in patients with HER2 negative metastatic breast cancer	Phase I	Overall response rate: 85% Complete response: 15%	Bavituximab (3 mg/kg) + paclitaxel	Chalasani et al. (2015)
Bavituximab plus paclitaxel and carboplatin for the treatment of advanced non-small-cell lung cancer	Phase II	Overall response rate: 41% Median progression-free survival: 6.0 months Median overall survival: 12.4 months	Bavituximab (3 mg/kg) + carboplatin–paclitaxel	Digumarti et al. (2014)
Phase I safety and pharmacokinetic study of bavituximab, a chimeric phosphatidylserine-targeting monoclonal antibody, in patients with advanced solid tumors	Phase I	Well-tolerated Pharmacokinetics support weekly dosing	Bavituximab monotherapy (0.1, 0.3, 1.0, 3.0 mg/kg)	Gerber et al. (2011)
Randomized phase III study of docetaxel plus bavituximab in previously treated advanced non-squamous non-small-cell lung cancer	Phase III	Median overall survival: 10.5 months There was no difference in progression-free survival	Docetaxel + weekly bavituximab (3 mg/kg) or placebo	Gerber et al. (2018)
Efficacy and safety of bavituximab in combination with sorafenib in advanced hepatocellular carcinoma: a single-arm, open-label, phase II clinical trial	Phase II	Median overall survival: 6.1 months Median disease-specific survival was 8.6 months	Bavituximab (3 mg/kg) weekly + sorafenib (400 mg) twice daily	Mokdad et al. (2019)

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