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# 1 Phosphatidylserine: Paving the Way for a New Era in Cancer Therapies

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21 **Abstract:** The lipid Phosphatidylserine (PS) plays a vital role in the growth and proliferation  
22 of cancer cells and has been identified as a potential target for cancer treatment. Recent research  
23 has focused on using phosphatidylserine-targeting agents in the treatment of several classes of  
24 cancer, like breast, lung, and prostate. Using PS-targeting antibodies to target cancer cells while  
25 leaving healthy cells unharmed will be selective. These antibodies are specifically targeted to  
26 phosphatidylserine molecules located on the exterior membrane of cancer cells, triggering a  
27 series of events that ultimately destroy the cancer cells. In addition to that incorporating  
28 phosphatidylserine into the liposome membrane, to specifically target cancer cells, thereby  
29 enabling more efficient drug delivery and improved cancer treatment outcomes. Mostly PS has  
30 active ingredients of currently undergoing clinical trials for potential use in treating various  
31 types of cancer. On the role of phosphatidylserine in biophysical and cancer biology, this  
32 review summarizes the latest research, as well as related prospective clinical and preclinical



33 trials such as immunotherapy and biomarkers. A new indication of future PS implementations  
34 in cancer therapy appears to be a new era.

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35 **Keywords:** Phosphatidylserine. Cancer, Biomarkers, Immune-suppression, Drug Delivery.

36

## 37 1. Introduction

38 Cancer is a multifaceted and complex disease which impacts a vast number of  
39 individuals globally. This chronic condition is marked by the uncontrolled proliferation of cells  
40 and the infiltration and dissemination of cells from their original site to other locations within  
41 the body [1-3]. According to Cancer Research UK, there exists a multitude of over 200 diverse  
42 forms of cancer that have the potential to occur in any anatomical region and can exert influence  
43 on individuals across various age groups, genders, and ethnic backgrounds [4]. This  
44 phenomenon has instigated a significant shift in the conventional methodology of medical  
45 treatment, transitioning away from a generalized approach and towards a more personalized or  
46 "customized therapy" wherein the determination of treatment options is predicated upon the  
47 specific mutational configuration of a patient's tumor [5]. Tumors can be classified as benign  
48 or malignant [6]. Benign neoplasms are non-malignant tumors that cannot spread throughout  
49 the body. However, some neoplasms can still be dangerous based on their location, such as an  
50 Intracranial neoplasm that may present challenges in surgical removal [7]. Malignant tumors,  
51 on the other hand, do not remain encapsulated, show features of invasion, and metastasize [5,  
52 8] are often more challenging to treat and can be life-threatening [9].

53 Evidence suggests that cancer is a disease of the genome at the cellular level [1-3].  
54 Interestingly, most agents accountable for the onset of cancer acknowledged as carcinogens,  
55 are agents that induce modifications in the sequence of DNA or mutations, commonly referred  
56 to as mutagens. Consequently, akin to all genetic disorders, the development of cancer is a  
57 consequence of modifications occurring within the DNA [10]. Tobacco use, exposure to  
58 radiation or chemicals, virus infections, and specific dietary and lifestyle variables can all result  
59 in these alterations, which can either be inherited or acquired [11][12].

60 Despite notable advancements in cancer research and treatment, there is still a great  
61 need for new and effective therapies that can improve patient outcomes with fewer toxic side  
62 effects. In particular, the lipid composition of the plasma membrane has been associated with  
63 the advancement and progression of cancer [12]. One promising avenue of research involves  
64 targeting PS, a molecule pivotal for proliferation and survival. PS and phosphatidylcholine  
65 (PC) are two significant phospholipids that contribute to cellular membrane structural and



66 functional properties [3]. PS, a negatively charged phospholipid naturally present on the inner  
67 layer of the cell membrane, but during apoptosis or cell activation, the asymmetric arrangement  
68 of phospholipids of the cell membrane is disrupted. With this novelty PS is flipped to the outer  
69 leaflet of the membrane, where it serves as an “eat-me” signal for phagocytic cells [6]. And,  
70 PC is abundant in the outer leaflet of the cell membrane and contributes to membrane stability  
71 and fluidity. PS, which is available in outer plasma membranes and is subjected to a variety of  
72 stimuli, also involves the progression of various diseases [13, 14], which may be found in other  
73 membranes during cell formation.

74 Basically, PS is dysregulated in different cancer types, including breast, lung, and colon  
75 cancer [15]. Now a days PS is a promising target to focus for cancer therapy due to its  
76 involvement in cancer progression and its potential as a focal point for therapeutic intervention.  
77 Understanding the role of PS in cancer cell survival and proliferation could lead to the  
78 development of innovative treatment approaches that specifically disrupt cancer cell function  
79 while sparing normal cells [16]. Recent studies suggests that the inhibition of PS synthesis or  
80 disruption of its interaction with proteins involved in cancer cell function presents an exciting  
81 avenue for novel cancer therapy [17]. Also, PS not only contributes to cancer cell survival and  
82 proliferation but also regulates tumor progression and metastasis [18][19]. This dysregulation  
83 of PS has been associated with the ability of cancer cells to evade the immune system and  
84 promote angiogenesis, the neo-genesis of blood vessels that are crucial for tumor growth and  
85 dissemination [20-22].

86 Furthermore, the relationship between PS and drug resistance in cancer has garnered  
87 significant attention. Cancer cells with elevated levels of PS have been found to exhibit  
88 increased resistance to chemotherapy and targeted therapies, posing a novelty and a  
89 considerable challenge in the clinical management of cancer [23]. In addition to targeting PS  
90 directly, there is growing interest in utilizing PS as a cancer biomarker for prognosis as well as  
91 diagnosis [24][25]. Advancements in imaging technologies and molecular profiling techniques  
92 have enabled the detection of elevated PS levels in cancerous tissues, providing valuable  
93 insights into disease progression and potential response to treatment [15, 26]. Herein we  
94 discussed, several strategies that are being explored, including the use of antibodies, small  
95 molecules, and nanoparticles. By diminishing the levels of PS within cancer cells, these  
96 molecules can hinder their proliferation and viability. Encouraging outcomes have been  
97 observed in clinical trials investigating PS-targeted therapies[26]. Furthermore, this review will  
98 address the challenges and future perspectives of PS-targeted therapies. Despite the promising  
99 potential, several hurdles remain, including the need for precise delivery systems and a better  
100 understanding of PS's role in different cancer types. By elucidating these aspects, we hope to



101 pave the way for the successful integration of PS-targeted strategies into the broader framework  
102 of cancer treatment. As research continues to unravel the complexities of PS and its  
103 interactions, it holds the promise of transforming cancer treatment paradigms, making it an  
104 essential focus for future oncological research.

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## 106 **2. Structural Organization of PS**

107 In a cell PS and PC are the crucial phospholipids that are essential components of cell  
108 membranes. These phospholipids are asymmetrically distributed on the cell membrane, with  
109 PS predominantly located on the inner surface of the cell membrane and PC predominantly  
110 situated on the outer side [27]. Cell-to-cell contacts, membrane trafficking, signal transduction,  
111 and other cellular activities are all significantly impacted by the structural arrangement of PS  
112 and PC on the cell membrane [28]. Understanding the organization and function of PS on the  
113 cell membrane is essential for developing targeted therapies for a variety of diseases, including  
114 cancer [29, 30].

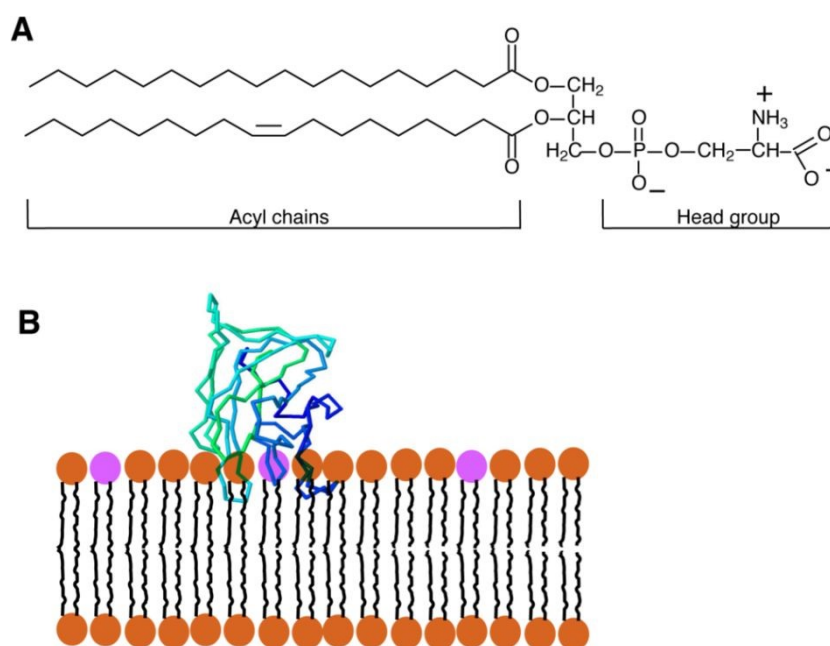
### 115 **2.1. Molecular structure of PS with PC**

116 PS is a negatively charged phospholipid composed of a serine molecule, a glycerol  
117 backbone, along with two fatty acid chains. The serine molecule is attached to the glycerol  
118 backbone by a phosphate group, and the fatty acid chains which form the hydrophobic tail [31].  
119 The hydrophilic head of PS contains a phosphate group, a serine amino acid, and a carboxyl  
120 group, which contribute to its negative charge [32-36].

121 The plasma membrane of mammals is composed of nearly 1000s of phospholipid molecules,  
122 out of which PS comprises approximately 2-10% of total phospholipids [37]. PS is an crucial  
123 phospholipid that is made up of two fatty acids attached by an ester linkage to the first and  
124 second carbons of the glycerol and serine linked by a phosphodiester linkage to the third  
125 carbon. It is a principal cell membrane component, more specifically present in the inner  
126 cytoplasmic leaflet of the membrane [38]. PC and phosphatidylethanolamine (PE) are mostly  
127 converted enzymatically by mammalian cells via a serine exchange process to produce PS by  
128 the action of 2 enzymes, i.e. S-synthase 1 (substrate PC) and PS-synthase 2 (substrate PE)  
129 generated from the endoplasmic reticulum [39]. PS is essential for apoptosis and cell signalling  
130 [40]. It's generally accepted that one of the primary mechanisms for eliminating apoptotic cells  
131 is PS exposure to the outside of the cell membrane, which sends a signal called “eat me signal”  
132 and marks the cell for destruction [41]. The chemical structure of PS confers upon it a crucial  
133 function as an essential constituent of cell membranes. It comprises glycerol backbone, fatty



134 acid chains, phosphate group, and amino acid serine. The glycerol backbone is a three-carbon  
 135 structure that provides the rigid framework for the careful arrangement of the other PS  
 136 constituents, 2 fatty acid chains are present in PS that provide fluidity and stability to the cell  
 137 membrane [42]. The glycerol backbone of PS molecule is attached to a phosphate group which  
 138 is further linked to a serine amino acid, hence giving the molecule the name  
 139 "phosphatidylserine." This linkage delivers the hydrophobic properties to the molecule, shown  
 140 in Figure. 1 [42].



141  
 142 Figure 1: (A) Diagram showing the structure of a typical glycerophospholipid, or PS, which  
 143 has both unsaturated and saturated fatty acyl chains. The head-group has one net negative  
 144 charge at physiological pH. The interaction between PS and the C2 domain of lactadherin  
 145 (LactC2; blue-green-barrel structure) in a membrane bilayer is depicted in (B). The PS head-  
 146 groups are displayed in fuchsia, whereas the remaining lipids are brown. Observe the three  
 147 "fingers" of the LactC2 structure, which are thought to reach the hydrophobic area of the  
 148 membrane bilayer and contain hydrophobic amino acids. Reprinted from [41], Copyright CC  
 149 BY 2011 MDPI.

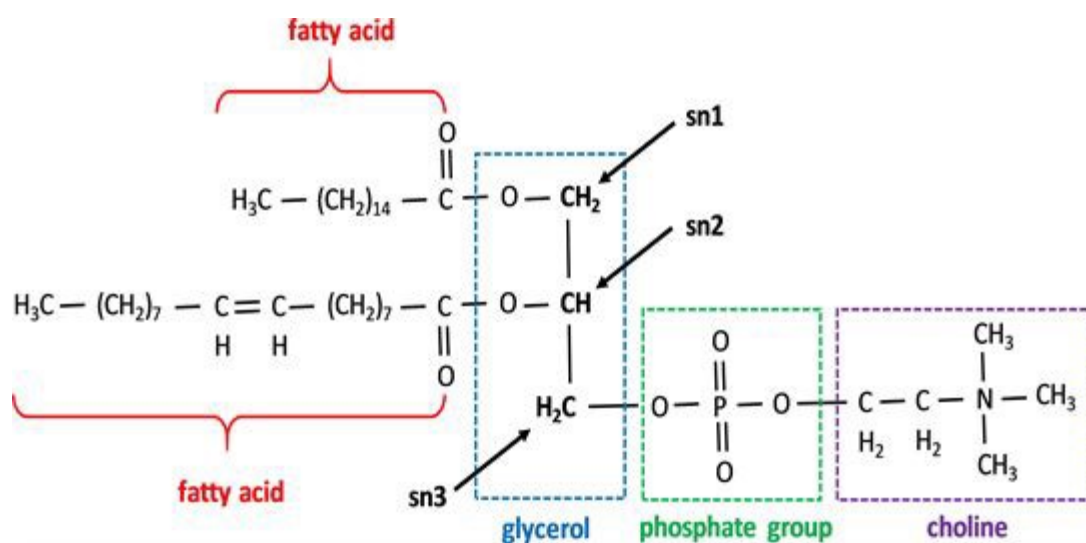
150

151 PC, on the other hand, is a neutral phospholipid composed of a choline molecule, a  
 152 glycerol backbone, along with two fatty acid chains [5]. Fatty acid chains produce the  
 153 hydrophobic tail of the choline molecule, which is joined to the glycerol backbone by a  
 154 phosphate group. The hydrophilic head of PC contains a phosphate group, a choline molecule,  
 155 and a glycerol group [32, 43]. All the phospholipids present in eukaryotic cell membranes, PC  
 156 is the most prevalent, making up 40–50% of the total [37]. It is one of the main components of  
 157 lung surfactant and cell membranes and is abundantly present on the outer surface of cell



158 membrane. It is believed that the PC transfer protein (PCTP), which belongs to the  
 159 steroidogenic acute regulatory protein-related transfer (START) superfamily, moves between  
 160 membranes inside the cell and has a crucial role in cell signalling [44]. A range of fatty acids  
 161 make up the glycerophosphoric acid and choline head group that make up this phospholipid. A  
 162 significant amount of dipalmitoylphosphatidylcholine can be found in animal lung PC [45].

163 Two methods allow mammalian species to generate PC out of which CDP-choline  
 164 pathway is most important. This pathway is also known as ‘Kennedy pathway’ as it was  
 165 described by Eugene and Kennedy [46]. Three enzymatic steps comprise the CDP-pathway  
 166 that require choline. First, choline kinase phosphorylates the choline into PC at the expense of  
 167 ATP. Second, PC using CTP in the presence of cytidylyltransferase (CT) is converted into  
 168 CDP-choline. Third, the conversion of CMP to diacylglycerol to create PC is catalyzed by 1,2-  
 169 diacylglycerol choline phosphotransferase (Figure. 2). Through three consecutive methylations  
 170 of PE by phosphatidylethanolamine N-methyltransferase (PEMT), PC can also be produced  
 171 endogenously in a second pathway [47]. The relevance of the endoplasmic reticulum (ER) in  
 172 PC production was highlighted by subcellular fractionation, which showed that PEMT and the  
 173 last enzyme in the CDP-choline pathway are both found there [48, 49].



174  
 175 Figure 2: Phospholipids showing the structure of PC. All phospholipids share the same  
 176 fundamental structure but differing fatty acids and head groups in place of choline. Sn 1, 2, and  
 177 3 positions are marked. Reprinted from [41], Copyright CC BY 2019 MDPI.

### 178 2.1.1. Distribution of PS and PC in the membrane

179 PS and PC are both found in the membrane of all cells, but their distributions are  
 180 different in different cell types and the specific membrane domain. In general, PS is more  
 181 abundant in the inner side of the cell membrane, where it constitutes up to 10-20% of the total  
 182 phospholipids [50]. This asymmetric distribution is maintained by a class of enzymes called

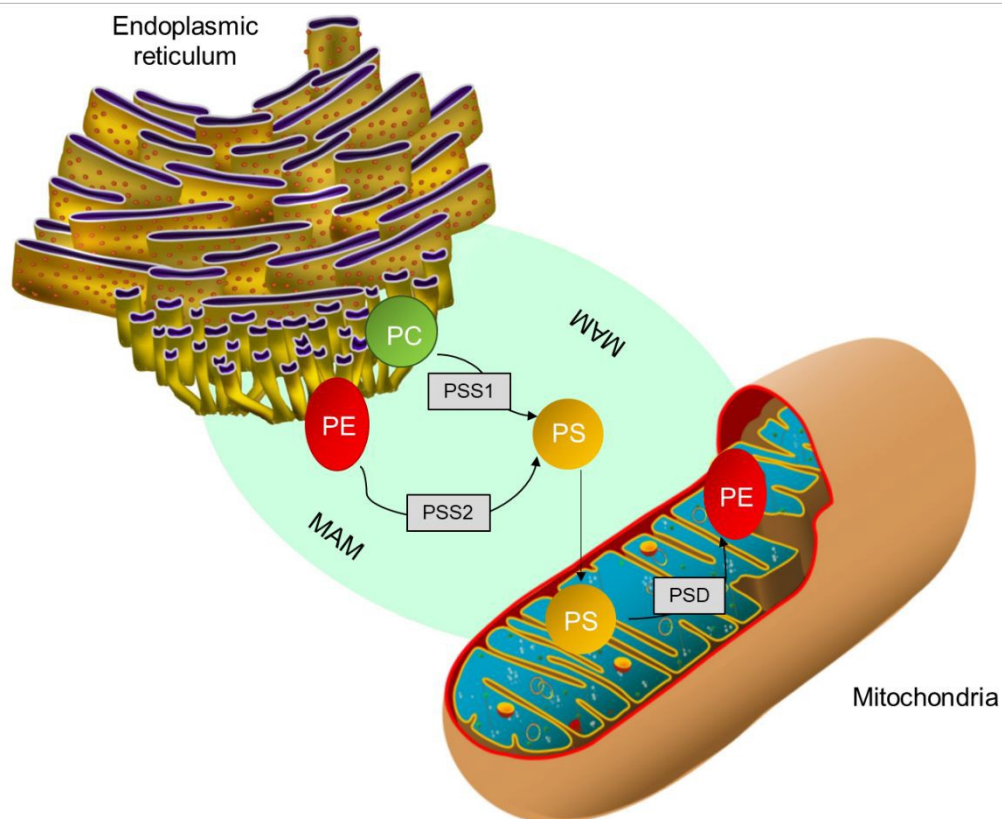


183 flippases [51], which selectively transport PS from the outer to the inner leaflet. In healthy  
184 cells, the exposure of PS on the outer leaflet is minimal [52]. Still, in apoptotic cells, PS is  
185 translocated to the outer surface, where it serves as a signal for phagocytosis by macrophages  
186 and other immune cells [53]. On the other side, PC is more abundant in the outer side of the  
187 cell membrane, constituting up to 50-60% of the total phospholipids [54]. This distribution is  
188 also maintained by flippases, which selectively transport PC from the inner to the outer surface.  
189 Furthermore, PC is present in lipid rafts [55], which are specialized membrane regions essential  
190 for cell signalling and signal transduction and rich in signalling molecules and membrane  
191 receptors [56].

192 In mammalian cells, PS is produced inside a particular area known as mitochondria-  
193 associated-membrane (MAM) [57]. Which present in the ER and is synthesized from PC or PE  
194 by phosphatidylserine synthase-1 (PSS-1) or phosphatidylserine synthase-2 (PSS-2) through a  
195 base-exchange process with serine which was shown in Figure 3. After blending due to the  
196 interaction of MAM and mitochondrial exterior layers, a part of the PS moves into the  
197 mitochondria [24, 58]. The PS present inside mitochondria releases carbon dioxide. PE is  
198 regulated by PS decarboxylase (PSD) found in prokaryotes and mitochondria of eukaryotes  
199 Figure. 3. Further, it was found that insufficiency of PSD quality resulted in underdevelopment  
200 and severe health issues in mice [24, 58]. Thus, for proper cell growth and mitochondrial  
201 functionality, the process of PE production through PS becomes very crucial. The remaining  
202 PS is sent to various organelles, such as plasma film and Golgi, as shown in Figure. 3. Most of  
203 the transportation occurs through non-spontaneous processes, such as solvent vehicle proteins  
204 or vesicles [58]. However, the number of integrated PS molecules that get into mitochondria  
205 vs. other organelles is still a mystery.







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207 Figure. 3 PS production inside MAM from PC either by PSS1 or PSS2. Further, a few PSs are  
208 transmitted to mitochondria and decarboxylated to form phosphatidylethanolamine by PS  
209 decarboxylase [58].

210

### 211 2.1.2. Interactions of PS and PC with other lipids and proteins

212 The presence of PS and PC in the membrane has important implications for their  
213 interactions with other lipids and proteins. PS has a high affinity for calcium ions, and its  
214 binding to calcium is known to regulate a variety of cellular processes, such as blood clotting,  
215 synaptic transmission, and apoptosis [59]. In addition, PS can interact with other lipids, such  
216 as cholesterol and sphingolipids [51], to form specialized membrane domains known as lipid  
217 rafts. These domains are enriched in signalling molecules and membrane receptors and are  
218 essential for signal transduction and cell signalling.

219 PC also interacts with a variety of lipids and proteins, including sphingomyelin [60], which is  
220 another abundant phospholipid in the membrane. The interaction between PC and  
221 sphingomyelin is known to form a specialized type of lipid raft, known as the liquid-ordered  
222 phase or the raft phase, which has distinct biophysical properties compared to the surrounding  
223 membrane [61]. These domains are essential for the organization of signalling molecules and  
224 membrane receptors, as well as for the regulation of membrane fluidity and permeability.



225 In addition to their interactions with lipids, PS and PC also interact with proteins in the  
226 membrane. For example, PS has been shown to interact with several proteins involved in blood  
227 clotting, including coagulation factor Va, prothrombin, and protein C [62]. These interactions  
228 are essential for the regulation of blood clotting and thrombosis. PC, on the other hand, interacts  
229 with several membrane proteins, like ion channels, transporters, and receptors. These  
230 interactions are essential for the regulation of cell signalling, ion homeostasis, and nutrient  
231 uptake.

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## 232 **2.2. Functional roles of PS and PC in the cell**

233 The distribution and interactions of PS and PC in the membrane play critical functional  
234 roles in the cell. For example, the asymmetric distribution of PS in the cell membrane is vital  
235 for the recognition and clearance of apoptotic cells by phagocytic cells [63]. The exposure of  
236 PS on the outer surface of the membrane serves as a signal for recognition and phagocytosis,  
237 which is an essential process for the removal of damaged or infected cells [5].

238 The distribution of PC in the outer surface of the cell membrane is vital for the formation and  
239 stability of lipid rafts, which are critical for cell signalling and membrane organization. The  
240 interaction between PC and sphingomyelin in the lipid raft phase is vital for the organization  
241 of signalling molecules and membrane receptors, which is critical for signal transduction and  
242 cell signalling [64]. In addition, the fluidity and permeability of the membrane are regulated by  
243 the distribution and interactions of PS and PC with other lipids and proteins, which is essential  
244 for the regulation of cell function and adaptation to changing environmental conditions [50].

245 In many different biological processes, PS or PC, is essential for function. It is a component of  
246 the cell membrane and has a role in endocytic internalization, intracellular vesicle movement,  
247 and the establishment of cell polarity. Furthermore, PS plays a vital role in preserving the  
248 structural and functional integrity of cell membranes [65]. Additionally, PS exposure is  
249 connected to phospholipid externalization during biological processes such as apoptosis [33,  
250 66]. In terms of brain function, PS and DHA are essential for the structure and operation of the  
251 brain [67]. Furthermore, the synthesis of PS by PS synthase is pivotal for the growth and  
252 maintenance of neurons [68]. PS plays a function in neurotoxicity and Alzheimer's disease  
253 because it is widely distributed in neuronal membranes and is exposed on the cell surface when  
254 oxidative damage occurs [69]

255 Moreover, PS modifies the secondary structure of protein aggregates and impacts the rates at  
256 which proteins form, especially when amyloidogenic proteins are present. Furthermore, PS  
257 plays a role in insulin production, insulin signaling transduction, and the development of  
258 problems related to diabetes. For instance, PS supplementation has demonstrated promise in



259 preventing unfavorable ventricular remodeling and decreasing the size of myocardial infarcts  
260 [70], indicating a possible role in cardiac health. In tumor microenvironments, PS has also been  
261 linked to the control of T cell responses, suggesting that PS may be a valuable target for cancer  
262 immunotherapy. It's critical to know PS's functioning properties in order to comprehend  
263 different disorders and create effective treatment plans [22, 33, 71, 72]. PS is crucial for more  
264 than just cell activities; it may also have therapeutic uses.

265 Regardless of the evident potential of PS-targeted therapies, notable challenges and constraints  
266 exist that necessitate attention. For instance, certain tumors may exhibit low expression levels  
267 of PS, rendering them less responsive to these treatments, as shown in Table 2 [15].  
268 Furthermore, these therapies may exert off-target effects that have the potential to induce  
269 undesired adverse reactions. Nevertheless, there is a basis for optimism regarding the future of  
270 PS-targeted therapies in the realm of cancer treatment. An intriguing prospect involves the  
271 combination of PS-targeted therapies with immunotherapy [15]. PS and immune cell exposure  
272 in tumor microenvironments give rise to immunosuppression and advancement of tumor  
273 development. Therefore, for survival of cell proliferation, growth and symptoms linked to  
274 cancer, the PS position on the membrane is vital [38, 73].

275 Understanding the intricate interaction of PS within the field of cancer biology and its  
276 repercussions for the resistance to treatment is of utmost importance for the advancement of  
277 more efficient therapeutic approaches. Through the specific targeting of PS and the pathways  
278 associated with it, innovative treatment methods have the potential to enhance the effectiveness  
279 of current therapies and surmount drug resistance in cancer.

### 280 3. PS in cancer treatment

#### 281 3.1 Exposure of PS to Immune Suppression

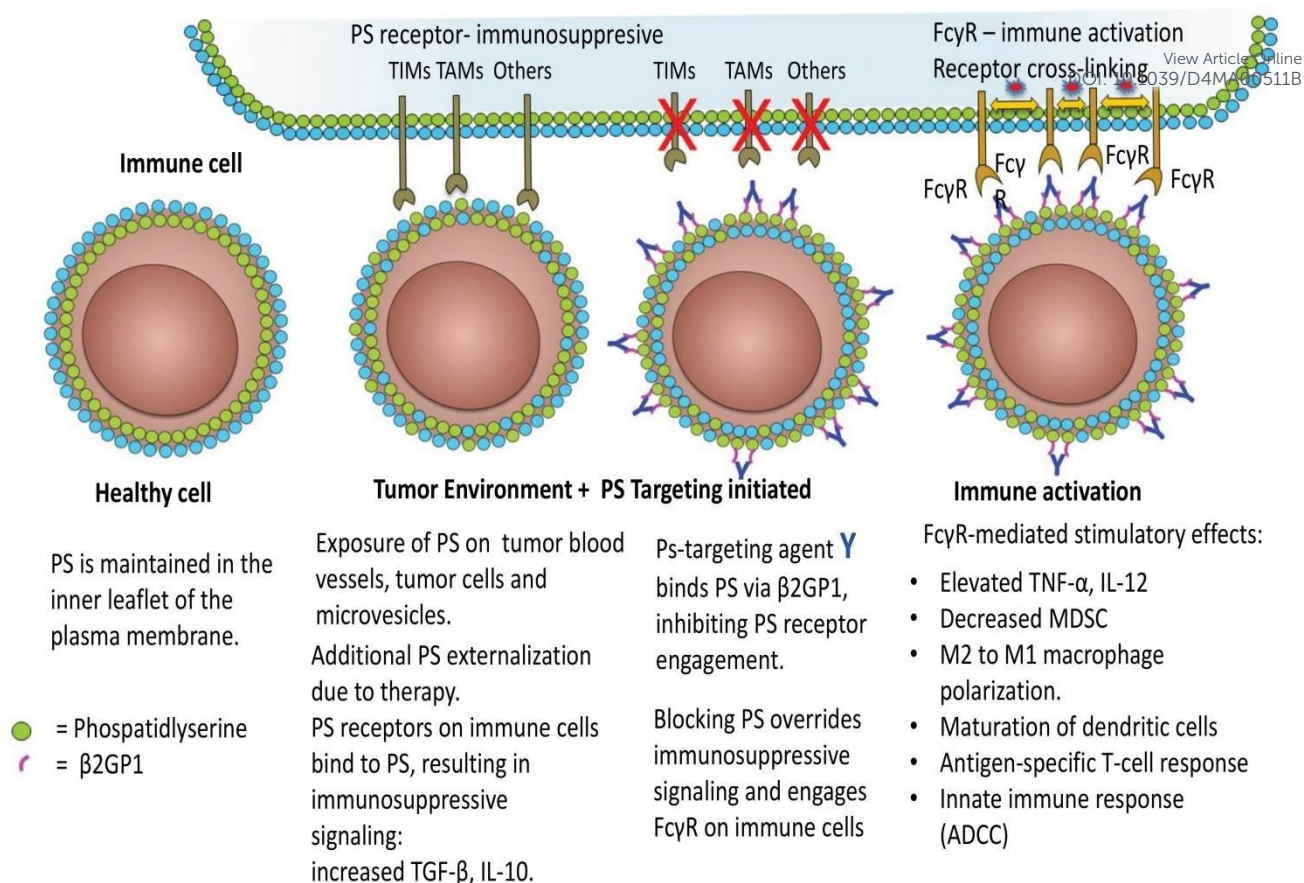
282 Immune suppression and inflammation provide favourable conditions for tumor  
283 development by impairing the immune system's capacity to identify and eliminate cancer cells  
284 [74-76]. Tumor cells can escape from immune system detection by interfering with the stage  
285 of T-cell activation [77]. In the tumor microenvironment, PS is abnormally exposed and is  
286 essential to produce immunosuppressive signals that obstruct the development of both systemic  
287 and local antitumor immune responses [29, 76, 78]. PS is exposed to the outer membrane within  
288 the tumor microenvironment due to a variety of metabolic processes linked to apoptosis.  
289 Activation of caspases, production of reactive oxygen species, and cell activation-induced  
290 calcium ion inflow are some of these processes [79]. Significantly, PS exposure impairs both  
291 innate and adaptive immune responses, enabling tumor cells to avoid immune detection [80].  
292 Interestingly, PS exposure is known to be a point of control that may be addressed



293 pharmacologically and functions as an early indicator or precursor of the several  
294 immunosuppressive signals that follow [81]. The primary cause of PS exposure in the tumor  
295 microenvironment are necrotic tissue and apoptotic cells brought on by pathological states or  
296 treatment. Nevertheless, it has also been seen in living endothelial cells and extracellular  
297 vesicles derived from leukocytes, stroma, and tumors. Tyro, Axl, and Merck (TAM receptor  
298 tyrosine kinases) are among the several PS receptors that are particularly crucial for the  
299 detection of PS and the induction of immune suppression in the tumor microenvironment [82].  
300 Tcell/transmembrane, immunoglobulin, and mucin (TIM) are additional PS receptors [83].  
301 Tim-1, Tim-3, and Tim-4 are linked to receptor-mediated immunosuppression of Th1 cells,  
302 activation of Th2 cells—a subset of T helper cells important in humoral immunity—and the  
303 uptake of apoptotic cells by macrophages and dendritic cells, respectively. The receptor  
304 tyrosine kinases (RTKs) that make up the TAM gene family are expressed on leukocytes and  
305 different kinds of tumours. Protein S or Gas6 are bridge proteins that have  $\gamma$ -carboxylated  
306 domains that allow TAM RTKs to connect to PS. As the TAM receptor is engaged in  
307 interaction with the receptor-binding domain, the exposed PS is directly bound by the  $\gamma$ -  
308 carboxylated GLA domain of Gas6 and Protein S. Therefore, when TAMRTKs on  
309 macrophages are activated, target cells exposed to PS are phagocytosed, resulting in  
310 macrophage polarization, which acts as a pro-inflammatory signal, changes the phenotype of  
311 the macrophages from "M1" to "M2," which has pro-tumor activity, and allows for the  
312 production of interleukin-10 and TGF- $\beta$  [84]. TAM RTKs activation on tumor cells is  
313 associated with resistance to chemotherapy and the ability of tumor cells to undergo epithelial  
314 plasticity. Preclinical studies show decreased tumor growth and metastasis when the vitamin K-  
315 dependent  $\gamma$ -carboxylation of Gas6 is inhibited, preventing Axl from being activated on tumor  
316 cells [84]. Additionally, PS-TAM RTK binding on tumor cells also escalates the expression of  
317 programmed death-ligand 1 (PD-L1). Therefore, blocking the activation of TIM and TAM-  
318 RTK pathways by PS can enhance immune responses against cancer, as shown in Figure. 4  
319 [85]. Furthermore, a study revealed that radiation therapy also elevates PS expressions in B16  
320 melanoma in mice bodies. The extrinsic plasma membrane surface of tumor cells, PS, hinders  
321 the immunological response to receptors found in T cells and others [85, 86]. Inhibition of  
322 dendritic cell antigen representation and induction of regulatory T-cell limits T-cell activation  
323 by producing immunosuppressive cytokines such as IL 10 or TGF-  $\beta$ .

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324 Figure 4. Immune cells are activated in the tumor microenvironment by PS-targeting  
 325 antibodies interacting with exposed PS. As the article explains, oxidative stress and an  
 326 immature tumor vasculature work together to cause PS to show marked dysregulation in the  
 327 tumor microenvironment. This imbalance is further exacerbated by the release of PS-positive  
 328 tumor exosomes and the increased apoptotic index of tumours that are actively growing.  
 329 Antibodies that specifically target PS are thought to be able to bind to externally exposed PS  
 330 molecules with selectivity and interfere with PS's ability to regulate tumor microenvironmental  
 331 functions by preventing PS from connecting to PS receptors and by blocking Fc $\gamma$ -mediated  
 332 ADCC (antibody-dependent cell-mediated cytotoxicity). Consequently, immunogenic signals  
 333 are triggered in the tumor microenvironment. Reprinted from [85], Copyright CC BY 2018  
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335 Moreover, the binding of PS to T cell receptors blocks T cells that are activated by  
 336 GPR174 (G-protein coupled receptor 174 is a protein that in humans is encoded by the GPR174  
 337 gene). This G-protein coupling receptor shortage T-reg cells can induce macrophage  
 338 polarization and upsurge IL-10 expression [24]. When PS encounters macro-vesicles by PS,  
 339 phagocytes clear apoptotic cells, preventing undesired inflammatory reactions and  
 340 equilibrating an anti-inflammatory state in the tumor's microenvironment, as shown in Figure.  
 341 5. Exposure of PS from tumor samples in patients was demonstrated as suppressing the

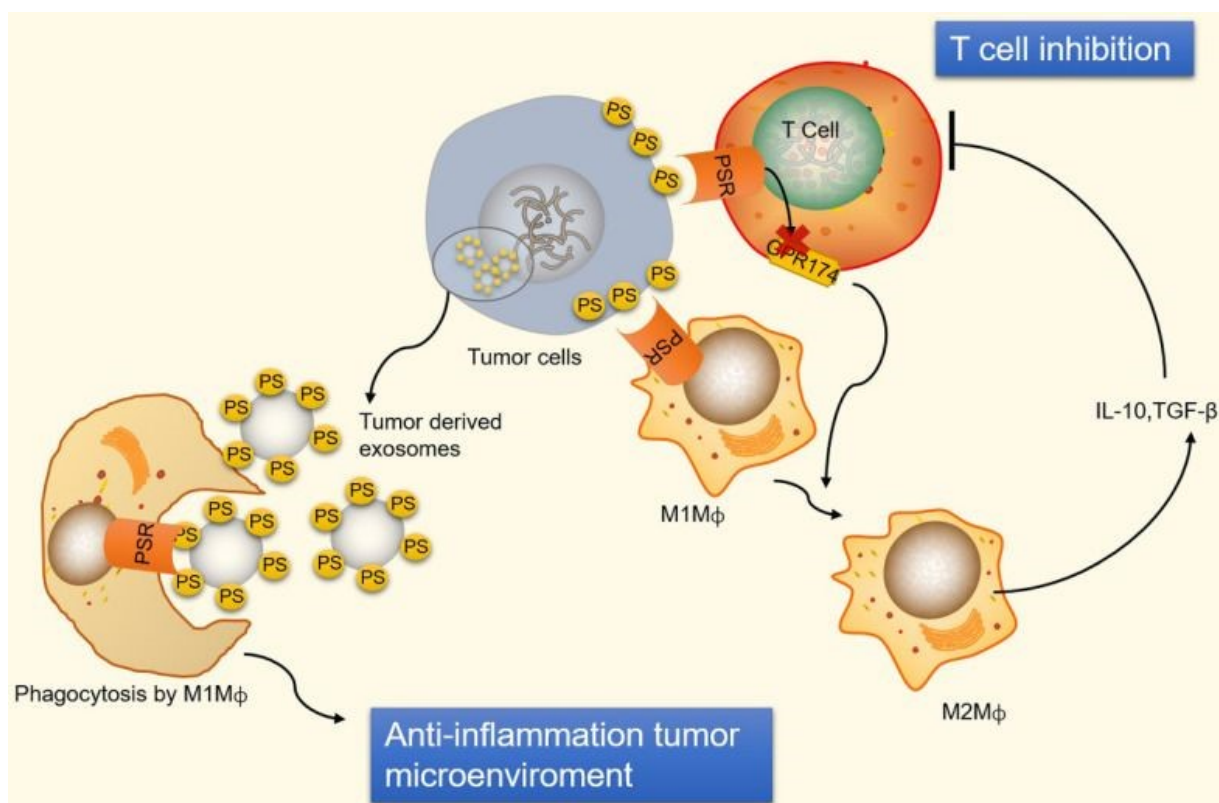


342 activation of T-cell reactions [24] in micro-vesicles (exosomes). However, PS exposure in  
 343 tumor cells also promotes anti-tumor effects through the mediation of long-term inflammation.

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345



346

347 Figure 5. Immunological suppression occurs in tumour cells and vesicles because of PS  
 348 exposure. Immunological suppression occurs when PS is given to cancer cells because it ligates  
 349 to receptors on T cells and macrophages. When PS binds to PSR on macrophages, M2-like  
 350 macrophages develop and start to release TGF- and IL-10, two anti-inflammatory cytokines.  
 351 TGF- and IL-10 are cytokines that inhibit the immune system and stop T cells from activating .  
 352 Reprinted from [24], Copyright 2020 Theragnostic.

353 As per the recent study, it has been observed that PS exposure in the body, in a condition  
 354 with a large number of cells, is essential to bind IFN $\gamma$  and IL-12 for the conversion of transient  
 355 cytokine stimuli towards a long-lasting inflammation as a result to reducing  
 356 immunosuppressive functions [87]. In conclusion, the immune response of PS and its  
 357 immunosuppressive consequences is an intricate process in the tumor microenvironment. This  
 358 mechanism seems to be a conserved evolutionary strategy among higher metazoans to  
 359 safeguard against autoimmune complications that may arise during the regular disposal of  
 360 dying host cells [87]. While the development of agents that target PS receptors is progressing  
 361 through various stages of pre-clinical and clinical investigation, advanced-stage clinical trials



362 are currently underway to evaluate the efficacy of the PS-targeting antibody bavituximab in  
363 multiple oncology indications.

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### 364 3.2 PS in Biomarkers for Imaging tool

365 The discovery and characterization of biomarkers are essential to the advancement of cancer  
366 research since it enhance the effectiveness of therapies, diagnosis, and screening. PS has  
367 demonstrated potential as a biomarker for the identification and imaging of several forms of  
368 cancer. Studies show that PS is present on the interface of cancer cells. This process is  
369 controlled by calcium-dependent flippases and scramblases, which are enzymes that catalyze  
370 the transfer of lipid molecules. The former moves lipids towards the cytoplasm, while the latter  
371 moves phospholipids ATP-independently between the inner and outer leaflets. For this reason,  
372 PS labelling techniques are useful for enabling tumor visualization [27]. Cancer cells exhibiting  
373 a lower presence of PS on their surface seem to display a heightened susceptibility towards  
374 both irradiation and chemotherapeutic agents such as gemcitabine (Gemzar)/nab-paclitaxel  
375 [88]. On the other hand, cancer cells with higher PS levels on their surface are more susceptible  
376 to PS-targeting anticancer therapies, such as saposin C encapsulated in a dioleoyl PS  
377 nanovesicle (SapC-DOPS) [89]. Using different strategies, it has been demonstrated that cancer  
378 cells that are targeting PS have been imaged [90, 91]. In one study, researchers used a  
379 compound called Annexin V-Cy to image gliosarcoma tumors in mice. When pro-apoptotic  
380 drugs were administered to the tumors, they observed that the fluorescent signal in the tumors  
381 increased by three times. Zhao et al., used PGN635, a new monoclonal antibody that binds  
382 exclusively to PS, to perform in vivo optical imaging of exposed PS. The F(ab')<sub>2</sub> fragment  
383 of PGN635 was linked to the near-infrared (NIR) dye IRDye800CW. After injecting 800CW-  
384 PGN635 into mice with subcutaneous or orthotopic U87 glioma xenografts, either treated with  
385 radiation or not, in vivo dynamic NIR imaging was performed. Following the injection of  
386 800CW-PGN635 into non-irradiated subcutaneous U87 gliomas, the resulting NIR optical  
387 imaging showed a clear contrast in the tumors [92]. Another imaging technique called Positron  
388 emission tomography (PET) is employed for radioactive tracers to visualize PS. In other  
389 investigation, researchers utilized PGN635 - <sup>89</sup>Zr (Zirconium-89) to visualize tumors in both  
390 mice and humans. They discovered that the tumors exhibited elevated levels of the radioactive  
391 tracer, thereby enabling their visualization through PET. Another method for visualizing PS is  
392 to use radio waves and magnetic fields, called magnetic resonance imaging (MRI). In a  
393 subsequent study, PGN635, along with superparamagnetic iron oxide nanoparticles (SPIO),  
394 was used to visualize tumors (breast cancer) in mice wherein the researchers noticed that the  
395 tumors displayed a reduction in signal intensity on MRI, thereby facilitating their detection. A  
396 Nano-probe known as PGN-IOL / DiR, which is made up of liposomal that binds to PS, and



397 enters cells, has a good effect on mice breast cancers of the MDA-MB 231 [88]. Similarly, the  
398 use of green indocyanine bonded to PS antibodies in three negative breast cancer cells enabled  
399 tracking and mapping apoptosis [89] and facilitated an effective therapy strategy. PS species  
400 may also be biomarkers for the diagnosis of malignancy. The 36 most frequent lipid types were  
401 evaluated in one clinical investigation of 15 patients with prostatic cancer and 13 healthy  
402 controls. The result demonstrated that a specific species "PS (18: 1/18: 1)", appeared to have  
403 significant implications among normal patients and prostate cancer; the two categories, % of  
404 sensitivity and differed in the combinations PS (18: 1/18; 1), lactosylceramide (18: 1/16: 0) and  
405 PS (18:1/18: 2) [90]. A new study in patients with lung cancer evaluated lipids in the aerosols  
406 between cancer and normal cells. In cancerous aerosols, the overexpose of PS, was detected  
407 with declining PCs [91], which shows PS as a viable biomarker for lung cancer in combination  
408 with other indices. PS species can also relate to the growth and progression of cancer. A method  
409 that combines PS and SapC, an endogenous sphingolipid activator protein, can be used to track  
410 several cancer cell lines. At an acidic pH, SapC has a considerable binding affinity for PS and  
411 is essential for the activation of lysosomal enzymes and the production of sphingosine and  
412 ceramide from the breakdown of sphingolipids. Because of the release of lactate from  
413 anaerobic glycolysis, tumors are known to have high levels of PS on their cell surface and to  
414 have a lower extracellular pH in comparison to normal tissues. [91]. As a result, the  
415 combination of SapC and PS provides a beneficial and selective technique for targeted tumor  
416 imaging and treatment. An illustration of the various SapC-imaging modalities is presented in  
417 Figure 6. The PS-targeting SapC-DOPS nanocarrier technology has been effectively applied to  
418 single-photon emission computed tomography (SPECT), MRI, and optical cancer cell imaging  
419 [93]. Chu et al. [94] used CellVue Maroon (CVM)-tagged SapC-DOPS nanovesicles, which  
420 contain the far-red fluorophore CVM, to show how to identify brain tumors and arthritic joints  
421 in mice. MRI containing iron oxide particles enclosed in SapC-DOPS nanocarriers was utilized  
422 to image neuroblastoma selectively. Winter et al., used paramagnetic gadolinium chelates,  
423 namely gadolinium-DTPA-bis(stearylamide) (Gd-DTPA-BSA)-loaded SapC-DOPS vesicles,  
424 as a targeted contrast agent for glioblastoma multiforme tumor imaging [95]. Figure 7 presents  
425 the inferred findings from the Winter et al. experiment, which depicts the tumor cells' T1 maps  
426 both before and after a 10-hour injection of Gd-DTPA-BSA/SapC-DOPS vehicles. In  
427 summary, the distinctive characteristics of polystyrene offer significant benefits in the realm  
428 of imaging applications, as PS-targeting techniques allow for the specific visualization of  
429 tumors. Undoubtedly, these techniques will have a significant impact on upcoming diagnostic  
430 applications.

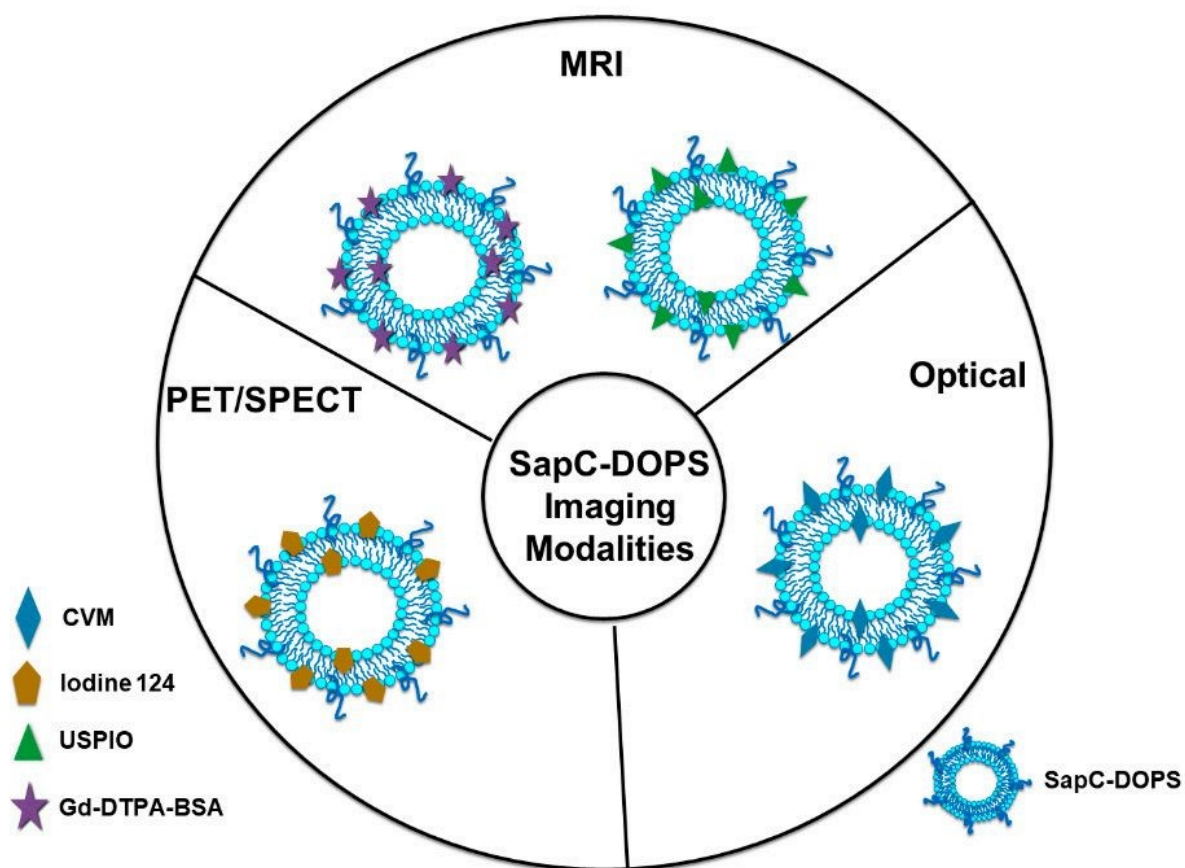
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431 With the finding of anxA5, a PS-binding protein [96] results in the invention of the  
 432 anxA5 affinity test for evaluating apoptosis by focusing on PS that is exposed to the surface  
 433 [97, 98]. With the exception of the myoblast and the megakaryoblast, anxA5 interacts with  
 434 dying cells rather than surviving ones throughout the body's diverse environment, according to  
 435 the study. In animal vitro models, some researchers have expanded the range of in vivo  
 436 applications of the anxA5 affinity method [99-101]. Because the AnxA5 is associated with  
 437 apoptosis, macrophage infiltration, and the release of red blood cells through intraplate  
 438 bleeding, it has recently been found to be a helpful marker for differentiating between stable  
 439 and unstable atherosclerotic plaques in patients [102]. Recent research suggests that a PS-  
 440 binding antibody can be used for molecular imaging of the vascular endothelial cells in solid  
 441 tumors [103].

442



443

444 Figure 6: Schematic diagram showing the many imaging modalities used using saposin C-  
 445 dioleoyl phosphatidylserine (SapC-DOPS)-based modalities to visualize tumors. The far-red  
 446 fluorophore CVM can be attached to the SapC-DOPS nanovesicles to provide optical imaging  
 447 for both in vivo and in vitro research. The use of gadolinium chelates, such as ultrasmall  
 448 superparamagnetic iron oxide (USPIO) or gadolinium-DTPA-bis (stearyl amide) (Gd-  
 449 DTAPBSA), as MRI contrast agents are necessary to enable in vivo MRI. Moreover, SapC-

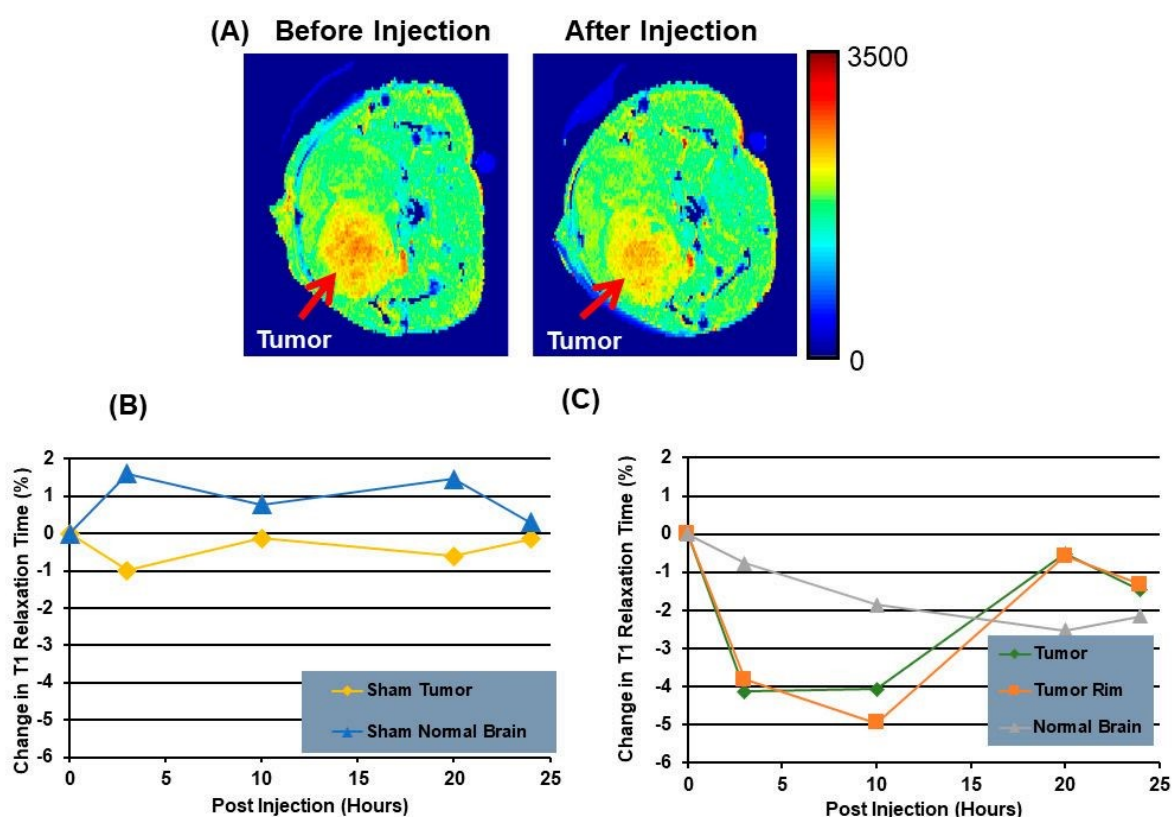


450 DOPS combined with an iodine-124 contrast agent is used for in vivo PET/SPECT imaging.

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453

454 Figure 7: A mouse model of brain cancer is used to investigate the use of saposin C-dioleoyl  
 455 phosphatidylserine (SapC-DOPS) as a carrier for contrast agents in MRI. First, a mouse with a  
 456 glioma undergoes a high-resolution MRI (A). Finally, after injecting Gd-DTPA-BSA/SapC-  
 457 DOPS vesicles into the tumor rim cells, and normal brain, the percentage change in T1 is  
 458 evaluated (B, C). Reprinted from [18], Copyright CC BY 2022 MDPI.

### 459 3.3 PS in Liposomal Carriers

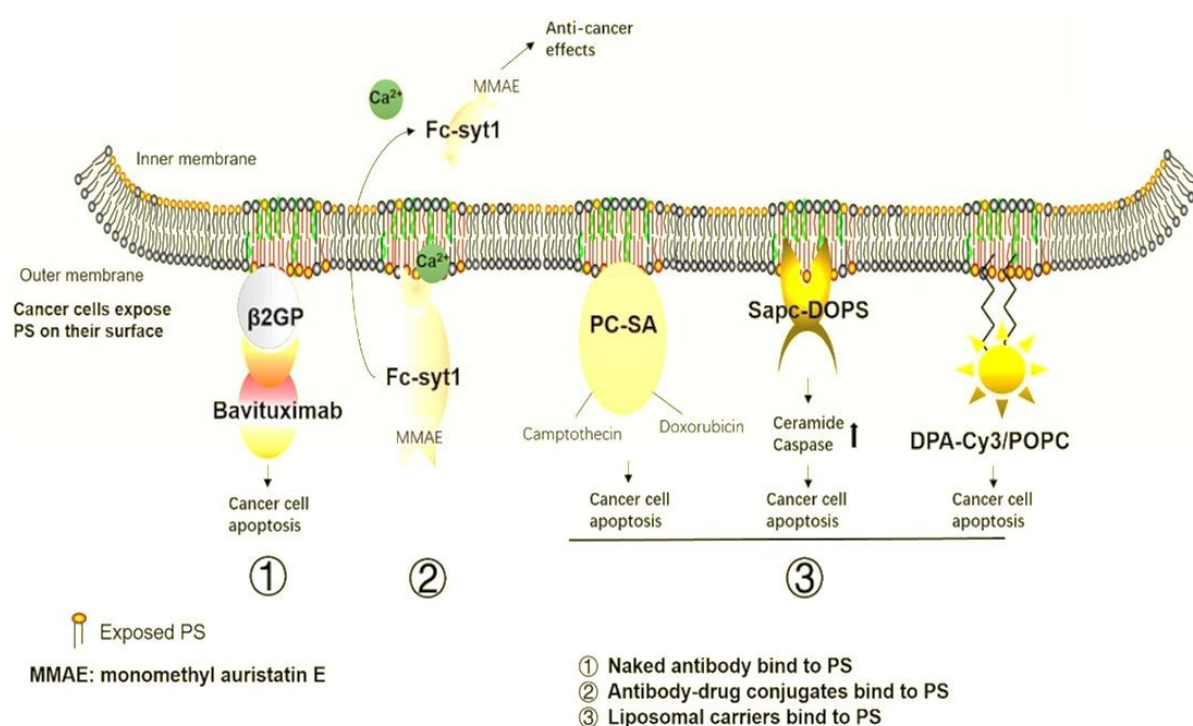
460 PS is a crucial anti-tumor mechanism in several liposomal carriers. These liposomal  
 461 transporters stick with PS in the tumor microenvironment and release anti-tumor sequels  
 462 through metabolism or synergistic drug effects.. SapC-DOPS interacts primarily with cancer  
 463 cells that contain PS and causes apoptosis by causing ceramide accumulation and caspase  
 464 activation. Research organizations have used SapC-DOPS to treat several cancers, such as  
 465 brain cell tumors [104] and cancer cell lines from the skin [105], as well as cancer cells from  
 466 the pancreas [106]. SapC-DOPS was found to be beneficial for a variety of metastatic tumors,  
 467 according to the findings. Following that, the same research discovered that cells with high top  
 468 PS levels have more survival probability under radiation therapy and that this was inversely



469 related to themselves. In response to SapC-DOPS therapies, this may have been employed in  
 470 combination treatment with significant exposure to tumor cells during radiation therapies, as  
 471 shown in Figure. 8 [107].

472 Furthermore, in cancer cells and tumors, PC-SA is a liposomal carrier with a cationic property.  
 473 PC-SA was found to be more effective when combined with typical tumor treatments in a  
 474 preclinical study, and it also had anti-cancer benefits when used alone. The cancer medicines  
 475 that are camptothecin with doxorubicin encapsulated in PC-SA type liposomes were used in  
 476 this work to decrease tumor growth as PC-SA raises the half-life of anti-tumour drugs to carry  
 477 a long-range in cancer treatment [107].

478



479

480 Figure 8: Cancer therapies involving PS. The medications aim to target PS in cancer treatment.  
 481 1) Naked antibodies bind to PS. 2) Antibody-drug conjugates bind to the PS. 3) Liposomal  
 482 carriers adhere to PS. Reprinted from [24], Copyright CC BY 2020 Theragnostic.

### 483 3.4 PS in Targeted Drug Delivery

484 PS is expressed on the surface of dead cells and alive cancer cells [108, 109], as well  
 485 as on the endothelial cells of blood vessels [110]. This has led to recent speculations that PS is  
 486 a viable drug-supply target. PS as a medication delivery target is further supported by clinical  
 487 experience with molecular oncological imaging of PS (TDD). PS selection using 3G4  
 488 monoclonal anticorps has recently been shown to improve cytostatic treatment efficacy in  
 489 mouse cancer models [111, 112]. Surprisingly, 3G4 was designed to identify the plasma PS-



490 binding protein beta2-glycoprotein 1 rather than PS. This demonstrates that when PS is  
 491 delivered to the membrane in-vivo, it is immediately opsonized by a variety of endogenous PS-  
 492 binding proteins, such as beta2 glycoprotein 1. In addition to AnxA5, it is part of the same PS-  
 493 binding protein family as AnxA5. AnxA5 competes effectively with other PS-binding proteins  
 494 for PS-bound locations in vivo in humans, according to the molecular imaging experience with  
 495 the protein. This has been confirmed in vitro system using isolated proteins and PS binding  
 496 assays [113]. As a result, AnxA5 is a potential therapeutic option for treating PS in-vivo.

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### 497 3.5 PS in Molecular Imaging Target

498 With the finding of anxA5, a PS-binding protein [96] results in the invention of the  
 499 anxA5 affinity test for evaluating apoptosis by focusing on PS that is exposed to the surface  
 500 [97, 98]. Apart from the myoblast and the megakaryoblast, anxA5 interacts with dying cells  
 501 rather than surviving ones throughout the body's diverse environment, according to the study.  
 502 In animal vitro models, some researchers have expanded the range of in vivo applications of  
 503 the anxA5 affinity method [99-101]. Because the AnxA5 is associated with apoptosis,  
 504 macrophage infiltration, and the release of red blood cells through intraplate bleeding, it has  
 505 recently been found to be a helpful marker for differentiating between stable and unstable  
 506 atherosclerotic plaques in patients [102]. Recent research suggests that a PS-binding antibody  
 507 can be used for molecular imaging of the vascular endothelial cells in solid tumors, as shown  
 508 in Table No. 1 [103].

509 **Table 1.** PS-targeting imaging strategies and therapeutics, along with their findings.

Strategy/Approach	Description	Findings and Applications
PS-Targeting Imaging Strategies		
Annexin A5 (anxA5) Affinity Test	Utilizes the high affinity of Annexin A5 for PS to measure apoptosis.	Effective in identifying apoptotic cells in various cancer types. Widely used in molecular imaging for cancer diagnosis.
Radiolabeled PS Probes	PS-binding proteins tagged with radioactive isotopes.	Enables non-invasive imaging of tumor cells. Provides clear visualization of apoptotic cells in preclinical and clinical settings.

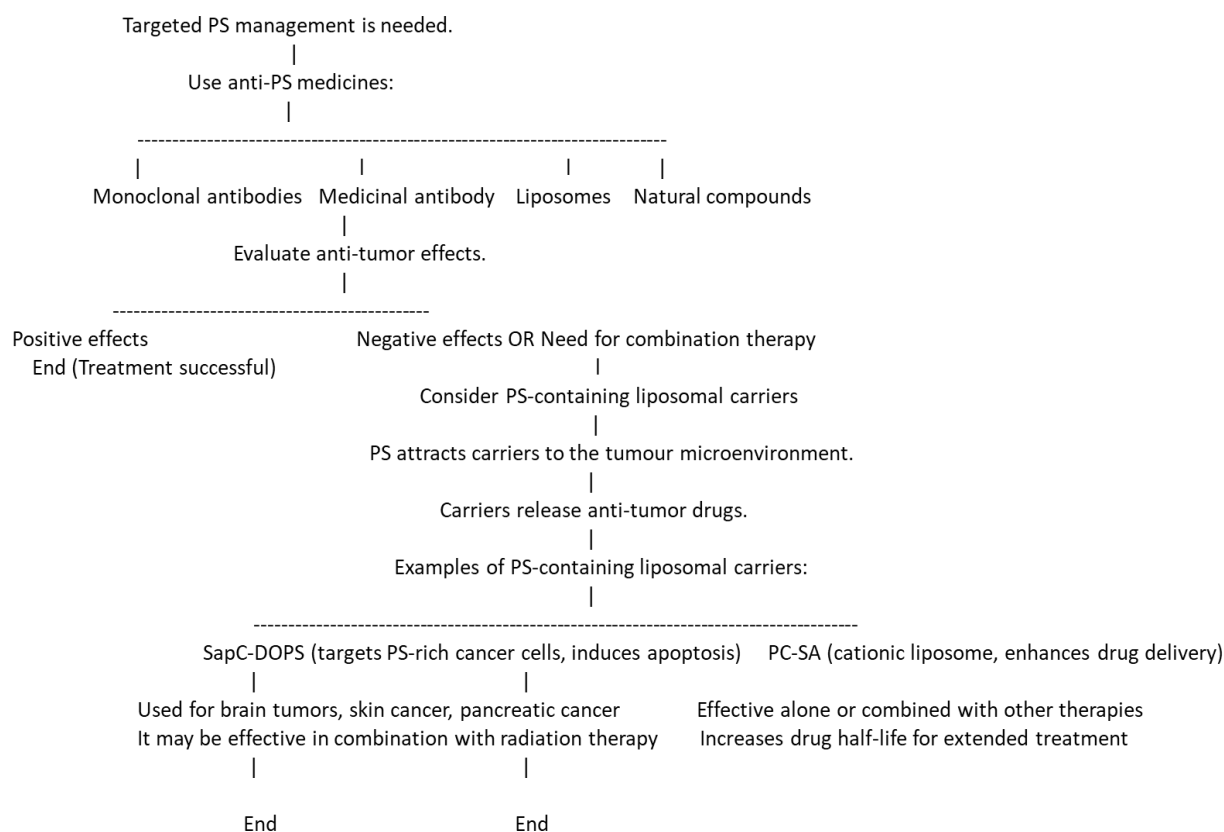


PS-Targeting Liposomes	Liposomes designed to bind to PS-exposed cancer cells.	Enhances the delivery of imaging agents to tumor sites, improving the accuracy of cancer detection.
Optical Imaging with PS-Binding Dyes	Dyes that specifically bind to PS and emit fluorescence.	Allows real-time imaging of tumor progression and response to therapy. Useful in intraoperative imaging to guide surgical resection of tumors.
PS-Targeting Therapeutics		
PS-Targeting Antibodies	Antibodies designed to bind specifically to PS-exposed cells.	Effective in delivering cytotoxic agents directly to tumor cells, reducing off-target effects. Examples include bavituximab.
Liposomal Drug Delivery Systems	Liposomes encapsulating chemotherapeutic drugs that target PS.	Improves the pharmacokinetics and biodistribution of drugs, enhancing their therapeutic efficacy.
Antibody-Drug Conjugates (ADCs)	Antibodies linked to cytotoxic drugs targeting PS-exposed cells.	Shows promising tumor-killing effects in preclinical models. Potential for reduced side effects compared to conventional chemotherapy.
Natural Plant Extracts	Extracts from plants that exhibit anti-cancer properties targeting PS.	Demonstrates potential in inducing apoptosis in cancer cells. Offers a natural and potentially less toxic alternative to synthetic drugs.
PS-Targeting Nanoparticles	Nanoparticles functionalized to bind PS on tumor cells.	Facilitates targeted drug delivery and reduces systemic toxicity. Shows potential in improving the efficacy of cancer treatments.

510

511 **3.6 PS in Antibody-drug conjugates**

512 Many conjugate antibody drugs are part of developing targeting medicines which use  
 513 PS. These drugs are associated with PS-targeting immunizer antibodies, which are cytotoxic  
 514 medicines that produce anti-tumor effects, as shown in Figure. 9 Some immunizer antibody-  
 515 drug conjugates medication forms have been proven in trials to have good tumor-suppressive  
 516 activity to "bare" antibodies available on it, for example, a PS-focusing agent made by using  
 517 PS binding particles, which will be a human Fc component that is C2A identified by IgG1. In  
 518 the breast and prostate cancer models of the mouse, the noted usage of an Fc-Syt1 synthesized  
 519 to monomethyl auristatin E, which acts as a cytotoxic chemical, had significant anticancer  
 520 effects [114]. According to some experts, the PS peptide is transferred to pH-sensitive micelles  
 521 (PEGPDLLA) and then used as a chemotherapeutic drug paclitaxel (PTX) in those micelles.  
 522 These pH-sensitive micelles are designed to address a corrosive set-off drug discharge  
 523 framework which is appropriate for an acidic environment with a tumor. Also vivo research  
 524 concluded that the produced agents generated cytotoxicity and tumor cell uptake, as well as  
 525 aggregation of tumors [115]. In addition, fusion proteins containing L-methionase linked to  
 526 human Annexin-V have been discovered. In comparison to L-methionine with no fusion  
 527 protein present, an antibody PS has the most significant effect on tumor cell killing [116].  
 528 Furthermore, the fusion protein does not affect normal cells, indicating that technology has the  
 529 potential for the development of novel drugs.



530

531 Figure 9: PS management in details for end-to-end using manners.



### 532 3.7 PS with CD47 as a therapy for cancer

533 PS exposure to phospholipid bilayer has the potential to induce phagocytic signs, which  
 534 can be distinguished by immune cells like macrophages. In contrast, at the same time, CD47  
 535 articulation on the cells can repress the process of phagocytosis. The CD47 is a vital immune  
 536 suppressive signal used by different tumor cells [117]. The CD47 can act as a ligand for a  
 537 category of glycoprotein known as signal regulatory protein- $\alpha$  or SIRP $\alpha$ . Upon binding of the  
 538 CD47, the SIRP signaling cascade can be activated, which in turn inhibits phagocytosis [81]  
 539 and allows tumor cells to escape from surveillance of immune cells like macrophages and T  
 540 cells [118]. However, in contrast, CD47 ligation induces articulation of PS in erythrocytes as  
 541 part of a demise pathway, and CD47 is suggested to impact PS exposure [119]. Again,  
 542 knocking down the CDC50A, which is a subunit of ATP11C involved in the flipping of PS,  
 543 can cause the tumor-associated macrophages to improve the anti-CD47 bar's activity to limit  
 544 tumor growth [120]. As we have mentioned previously, the knockdown of CDC50A, as a  
 545 result, increases the exposure of PS in the immortalized category T cells known as Jurkat cells.  
 546 Hence, it is supposed to impact T cell function. As a result, the CD47 barrier prevents  
 547 phagocytosis; nonetheless, PS openness is used to target tumor cells associated with  
 548 macrophage clearance work. When rituximab, an anti-CD20 antibody, is used with anti-CD47  
 549 medications, an anti-CD47 inhibitor, clinical trials have demonstrated some anti-cancer activity  
 550 with negligible adverse effects in patients with aggressive and indolent lymphoma [121].

551 **Table 2.** PS is in clinical use with the appropriate clinical significance role.

Characteristics	Clinical significance of PS	Diseases.	References
Biomarker	Increased PS expressions	B16 melanoma	[122]
Biomarker	Exosomal phosphatidylserine (PS) 18:1/18:1	prostate cancer	[90]
Biomarker	Presence of IgM anti-PS-prothrombin complex.	Cancer-associated vasculitis.	[27]
Biomarker	Presence of IgA anti-PS-prothrombin complex.	Henoch-Schonlein purpura	[123]
Imaging tool	Liposomal PGN-IOL / DiR, that binds to PS.	Breast cancer	[88]
Imaging tool	Indocyanine green, tagged PS antibodies	Triple-negative breast cancer	[89]
Immunosuppression	phosphatidylserine-micro vesicles induce TGF- $\beta$	Melanoma	[29]



	expression and suppression of macrophages		View Article Online DOI: 10.1039/D4MA00511B
Tumoricidal target	SapC-DOPS Nano vesicles-phosphatidylserine-targeting agent	Neuroblastoma	[30, 104]
Tumoricidal target	SapC-DOPS Nano vesicles-phosphatidylserine-targeting agent	Skin cancer	[105]
Tumoricidal target	SapC-DOPS Nano vesicles-phosphatidylserine-targeting agent	Pancreatic cancer	[93]
Antitumor effect	3G4 monoclonal against PS	Breast cancer	[111]
Antitumor effect			
	PS externalizations and DNA fragmentation by plant products (Chalepin)	Breast cancer	[32, 124]
Antibody-based killing	Rituximab mediated killing of CD 20 positive cells.	CD20-positive tumour cells	[43, 121]

552

#### 553 4. Functions of PS in Cancer

554 In the discipline of immunology, the goal of treatment is to pinpoint the signals that tell  
555 cancerous cells apart from non-cancerous ones: ‘eat me’ and ‘don't eat me’. The uneven  
556 distribution in eukaryotic cells results in the presence of unfavourable lipids like PS and PE on  
557 the cytoplasmic side of the cell membrane and positive phospholipids like PC and  
558 sphinomyline on the outer side, sustainably mediated by the controlled activity of ATP-  
559 dependent and -independent enzymes referred to as scramblases, floppases, and flippases. In a  
560 tumor environment, due to various pathways, the PS is exposed to external surfaces, which  
561 results in an ‘eat me’ signal, leading the cell towards apoptosis [43, 121]. Due to PS exposure's  
562 immunosuppressive qualities, tumor cells are better able to elude immune surveillance by  
563 weakening both innate and adaptive immune responses. The TAM gene families, as well as T  
564 cell/transmembrane, immunoglobulin, and mucin (TIM)44, are the PS receptors in the tumour  
565 microenvironment [82].

#### 566 4.1. Apoptotic function of PS





567 Every day, millions of cells constantly die and regenerate in our bodies. The dead cells  
568 are then eliminated by the action of PS, which initiates a process known as apoptosis that causes  
569 the dead cells to be phagocytosed [98, 125]. The apoptosis pathway takes place in 2 different  
570 ways, i.e. intrinsic pathway, which takes place inside the mitochondria, and extrinsic pathway,  
571 via numerous apoptotic factors. The intrinsic pathway is initiated by the activation of  
572 cytochrome C, which further activates the caspase 3/7 liberated from mitochondria. In contrast,  
573 in the extrinsic pathway, apoptotic factors like TNF and Fas ligand (FasL) activate the caspase  
574 3 and promote cell apoptosis. Exposure of the cell membrane PS serves as a clear signal for the  
575 recruitment of macrophages and the engulfment of cells, indicating the initiation of apoptosis.  
576 Studies on mice and humans have shown that another protein called Xk-related protein 8  
577 (Xkr8) also known as scramblase, if hypermethylated then becomes incompetent to expose PS  
578 during apoptosis as its expression gets suppressed. Moreover, other proteins like basigin (BSG)  
579 and neuropilin (NPTN), belonging to the Ig superfamily, and ATPase Phospholipid  
580 Transporting 11C(ATP11C), a significant flippase protein-producing gene is necessary for  
581 flipping of PS to the outer leaflet of the membrane. Any mutation can lead to no apoptosis and  
582 is not taken up by macrophages. Thus, it concludes that both flippase and scramblase activities  
583 are necessary for PS exposure and performing programmed cell death [23, 100, 126]. Further,  
584 the non-apoptotic functions of PS include blood coagulation, myoblast fusion and T  
585 lymphocyte activation.

#### 586 4.2. PS with TIM and TAM receptor function

587 Two receptor families mediate the phagocytic clearance of apoptotic cells, TIM and  
588 TAM proteins, which are dependent on PS (PtdSer). Tim receptors are a type of membrane  
589 receptor that recognizes PS signals and exerts immune suppression [127-129]. Three receptors  
590 are being encoded by TIM gene on human chromosome i.e. TIM1, TIM3 and TIM4, out of  
591 which TIM 3 is highly studied in the immune-cancer biology area. Tim 3 is expressed on  
592 dendritic cells, CD8<sup>+</sup> T cells, antigen-presenting cells, and other immune cells such as  
593 monocytes and natural killer cells. It promotes the phagocytosis of apoptotic bodies, preserves  
594 immunological tolerance, and triggers inflammatory reactions. It is well-established that  
595 inhibiting TIM-3 can stop tumor development [130]. Furthermore, the mast cells, b cells and  
596 cd4<sup>+</sup> t cells express TIM-1 receptor which also acts as a binding site for ebola virus. Dendritic  
597 cells and macrophages have significant levels of TIM-4 expression. Tim-4 is involved in the  
598 inflammatory reactions of the body [131, 132].

599 According to recent research, TAM functions as an oncogene that expresses itself  
600 inappropriately in malignant cells and is essential for the migration of apoptotic bodies as well  
601 as the growth and survival of cells. The TAM encodes three tam receptors:



602 Tyro3, AXL, and MerTK are proteins known to bind specifically to vitamin K-dependent  
 603 protein S, with Tyro3 and MerTK showing an exclusive affinity for it, trigger in T cells with  
 604 exposure to interleukin-4, while growth arrest protein 6 (Gas6) is another ligand that interacts  
 605 to tam receptors respectively. By binding to ligands, the team receptors are activated via the  
 606 RTK pathway, which in turn causes the kinetic domains to become auto-phosphorylated,  
 607 triggering signaling cascades and gene regulation [133].

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### 608 4.3. Molecular role of PS in cancer therapy

609 Fracturing PS and its receptors is one possible avenue for cancer treatment. Clinical  
 610 efforts are conducted using two methods to inactivate PS and increase antitumor activity:  
 611 interrupting PS in tumor cells and aiming at PS receptors to interfere with receptor signaling.  
 612 PS and its anti-cancer effects can be bound by a variety of agents, such as liposomal vesicles  
 613 (SapC-DOPS), monoclonal antibodies (bavituximab), and antibody conjugated with medicines  
 614 (Monomethyl auristatin E coupled with Fc-Syt1, Annexin-V coupled with L-methionase) [30,  
 615 88]. These agents work by forcing cancer cells to undergo apoptosis and controlling the body's  
 616 immune system. These innovative treatments are revolutionizing the way that cancer is treated  
 617 and offering hope for the creation of new drugs. The PS receptors can be the target of a wide  
 618 variety of compounds. Some of the compounds that can bind to and block all three of the TAM  
 619 receptors are sitravatinib, LDC1267, and RXDX-106. R428 (BGB324), TP0903, KIT, FLT3,  
 620 VEGFR, PDGFR, BMS777607, NPS-1034, and DP3975 are a few receptors for axl. The  
 621 MerTK inhibitors are UNC2025, UNC3133, ONO747, and MRX-2843. In addition, Pfizer-11  
 622 and 12, KRCT-7j, GL21.T, 20G7-D9, and AXL-107-MMAE are among the Tyro3 prohibitors.  
 623 These are strong protein- or c small molecule inhibitors that work by blocking TAM receptors,  
 624 which limit the spread and invasion of metastatic cells in the cancer microenvironment and  
 625 boost the innate and adaptive immune cells' activity [105, 121, 134]. Due to their anti-tumor  
 626 properties, these compounds have created new therapeutic opportunities. They can be utilized  
 627 to treat malignancies of the breast, oral, cervix, lung, and pancreas in addition to lymphocyte  
 628 leukemia (Table 3 and Table 4).

629 **Table 3.** Small chemical inhibitors of several kinases to target TAM [30, 88, 105, 121].

Agents	Primary target (IC50 value)	TYRO3 IC50	MER IC50	AXL IC50	Development stage	Primary indication



Bosutinib	BCR/ABL			0.6 nM	Approved	Chronic myelogenous leukemia
Cabozantinib	MET VEGFR2			294	Approved	Medullary thyroid cancer renal cell carcinoma hepatocellular carcinoma
Crizotinib	c-MET (8 nM) ALK (20 nM)	>10,000		7 nM	Approved	Non small cell lung cancer
LDC1267	AXL, MER, TYRO3	8 nM	<5 nM	29 nM	Preclinical	Boosting NK cell activity in tumor microenvironment
RXDX-106	AXL, MER, TYRO3, MET	19 nM	29 nM	7 nM	Termination of Phase 1	Immune activation in tumor microenvironment
Sitravatinib (MGCD516)	VEGF, c-MET, AXL, MER, TYRO3		2 nM	1.5 nM	Phase 2	A potent multi-kinase inhibitor in different models of sarcoma

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630

631 **Table 4.** TAM-targeting selective small molecule inhibitors.

Agents	Primary target (IC50 value)	TYRO3 IC50	MER IC50	AXL IC50	Development stage	Primary indication
Small molecule inhibitors for targeting MER						
MRX-2843	MER, FLT3	17 nM	1.3 nM	15 nM	To overcome resistance	Phase 1



					conferring FLT3 mutations in AML	View Article Online DOI: 10.1039/D4MA00511B
UNC-2025	MER, FLT3(0.80 nM)	17 nM	0.74 nM	14 nM	Leukemia	Preclinical
UNC-3133	MER	31 nM	3.0 nM	17 nM	Cancer (FLT IC50 = 6.8 nM), virus infection	Preclinical
ONO-7475	AXL, MER, FLT3		0.4 nM	2.2 nM	AML	Phase 1
Small molecule inhibitors for targeting AXL						
R428 (BGB324)	AXL			14 nM	Cancer	Phase 2
TP-0903	AXL			19 nM	Chronic lymphocytic leukemia	Phase ½
BMS-777607	AXL, MET (3.9 nM)	4.3 nM	14 nM	1.1 nM	Met-dependent gastric carcinoma	Phase 2 completed
NPS1034	AXL, MET(0.80 nM)			48 nM	EGFR-resistant lung cancer cells due to MET or AXL acitivity	Preclinical
Small molecule inhibitors for targeting TYRO3						
Pfizer compound 11	TYRO3	0.52	<10 fold than TYRO3		Thrombosis	Preclinical



Pfizer compound 12	TYRO3	0.27	<10 fold than TYRO3	10–100 fold than TYRO3	Thrombosis	Preclinical <small>View Article Online DOI: 10.1039/D4MA00511B</small>
Pfizer compound 19	TYRO3	0.010	>100 fold than TYRO3	>100 fold than TYRO3	Thrombosis	Preclinical
Pfizer compound 21	TYRO3	0.0007	10–100 fold than TYRO3	>100 fold than TYRO3	Thrombosis	Preclinical
KRCT-6j	TYRO3	0.028	12	>10	Cancer	Preclinical

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Recent research indicates that PS plays a role in the advancement of tumors. PS is externalized to the outer plasma membrane in different cancer cells; this process is controlled by calcium-dependent membrane proteins like flippases and scramblases. The development of tumors and resistance to chemotherapy and radiation therapy have been connected to this externalization of PS. On the other hand, PS's possible use in cancer treatment has also been investigated. According to a preclinical investigation, PS-targeting medications exhibit anticancer properties both on their own and in combination with conventional antitumor medications, where they exhibit greater potency [132, 134, 135]. The mechanisms of PS in cancer therapy are complex and multifaceted. PS-targeting agents have been shown to enhance. Furthermore, it has been discovered that administering an antibody that targets PS improves overall survival and amplifies the anti-tumor efficacy of tumor-directed radiation therapy. PS-targeting agents have also been explored as a potential cancer immunotherapy strategy, with one study showing that blocking PS externalization can restore pathogen and tumor immunity [33, 34, 136, 137]. Furthermore, PS synthase has been recognized as a promising therapeutic target for regulating cellular homeostasis in cancer cells.

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Numerous clinical investigations have been carried out to explore the possibilities of PS-targeting medications in the management of cancer. Patients with HER2-negative breast cancer were treated with the PS-targeting combination of paclitaxel and bavituximab in a phase I



651 clinical study [18, 108]. It has been demonstrated that SapC-DOPS can efficiently target and  
652 eradicate several cancer types, including skin, lung, brain, and pancreatic cancer. Clinical trials  
653 have also shown that treatment with an antibody that targets PS improves overall survival and  
654 increases the effectiveness of tumor-directed radiation therapy. These findings imply that PS-  
655 targeting drugs may represent a fresh and exciting development in cancer treatment.

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#### 656 4.4 PS and PC in host immunity

657 PS and PC are pivotal in host immunity. A significant part of the cell membrane that  
658 separates the intracellular and extracellular environments is made up of phospholipids. In  
659 addition to their structural role, phospholipids also have signalling functions, including the  
660 modulation of immune responses [126, 138, 139].

661 PS is vital in the identification and elimination of apoptotic cells for the purpose of  
662 preserving immunological tolerance and averting autoimmunity. PS is also involved in  
663 activating immune cells, including natural killer cells and macrophages, to protect the body  
664 against bacterial and viral infections [140, 141]. Exposure to PS causes pro-inflammatory  
665 cytokines to be downregulated and anti-inflammatory cytokines to be released, which resolves  
666 inflammation. In addition to its role in apoptosis, PS also plays a role in the activation of  
667 immune cells. PS can activate natural killer (NK) cells, which are a type of lymphocyte that  
668 can kill virus-infected or tumor cells without prior sensitization [142-144]. PS also enhances  
669 the phagocytic activity of macrophages and stimulates the production of reactive oxygen  
670 species (ROS), which can kill bacteria and other pathogens.

671 Research has shown that PS supplementation can enhance the immune response in individuals  
672 with weakened immune systems [105, 131]. In a study involving elderly individuals, PS  
673 supplementation was found to improve the function of immune cells and reduce the incidence  
674 of infections. Other studies have shown that PS supplementation can boost the immune  
675 response to vaccinations and increase antibody production, which is essential for long-term  
676 protection against infectious diseases [145-147].

677 On the other hand, PC is the most abundant phospholipid in cell membranes and is also  
678 a significant component of lipoproteins, which transport lipids in the bloodstream. PC has been  
679 shown to modulate immune responses in several ways [42]. For instance, PC can prevent the  
680 synthesis of pro-inflammatory cytokines, including interleukin-1 beta (IL-1 $\beta$ ) and tumor  
681 necrosis factor-alpha (TNF- $\alpha$ ) [42]. Additionally, PC can increase the synthesis of cytokines  
682 that reduce inflammation, like transforming growth factor beta (TGF- $\beta$ ) and interleukin-10 (IL-  
683 10) [148]. PC also affects lymphocytes through immunomodulatory means. PC can inhibit the  
684 activation of T cells, which are involved in cell-mediated immunity. PC can also enhance the



685 proliferation of B cells, which produce antibodies in the body [149]. PC has also been shown  
686 to improve the activity of the complement system, which is a group of proteins that play a  
687 crucial role in the immune response. The complement system helps to identify and eliminate  
688 pathogens, and deficiencies in this system can lead to increased susceptibility to infections.  
689 Furthermore, PC can modulate the function of dendritic cells, which are specialized antigen-  
690 presenting cells that initiate adaptive immune responses [148, 150].

691 Research has shown that PC supplementation can enhance the function of system  
692 immune cells - T cells and natural killer cells. PC can also reduce inflammation, which is a  
693 crucial aspect of the immune response [93, 104]. In a study involving patients with ulcerative  
694 colitis, PC supplementation was found to reduce inflammation and improve symptoms. Further  
695 research is required to determine the optimal doses and duration of supplementation for  
696 different populations. PS and PC also play a crucial role in cancer immunity. Cancer cells can  
697 evade the immune system by expressing surface molecules that suppress immune cell  
698 activation and induce apoptosis of immune cells [105]. PS and PC can modulate immune  
699 responses to cancer cells by regulating the activation and proliferation of immune cells and by  
700 promoting the phagocytosis of cancer cells.

701 PS is also flipped out on the surface of cancer cells, which act as an "eat-me" signal that  
702 is recognized by immune system cells like phagocytic cells, such as macrophages and dendritic  
703 cells [140, 141]. PS activates NK cells, which recognize and kill cancer cells with low amounts  
704 of major histocompatibility complex (MHC) class I molecules, which are necessary for antigen  
705 presentation to T cells. PS also has immunomodulatory effects on dendritic cells, which are  
706 specialized antigen-presenting (sAPC) cells that are used to initiate adaptive immune responses  
707 [151]. PS can enhance the antigen-presenting capacity of dendritic cells and promote the  
708 differentiation of r Th cells towards a Th1 phenotype. Th1 cells produce cytokines like IFN- $\gamma$ ,  
709 which activate macrophages and improve their ability to kill cancer cells.

710 PC can also modulate the function of T cells, which are involved in cell-mediated  
711 immunity. PC can inhibit T cells' activation and proliferation, reducing the immune response  
712 to cancer cells. However, PC can also enhance the differentiation of regulatory T cells ( $T_{\text{regs}}$ ),  
713 which can impair the effector T cells' activation and proliferation of.  $T_{\text{regs}}$  are essential for  
714 maintaining immune tolerance, but they can also nurture cancer growth by suppressing the  
715 immune response to regulate cancer cell proliferation [152]. However, the role of PC in case  
716 of cancer immunity is complex and context-dependent, and it needs further research to  
717 understand the mechanisms of action of PC in cancer immunity.

#### 718 **4.5 Signaling cascade influenced by PS and PC**



719 PS and PC play critical roles in regulating cell signalling pathways in cancer. Aberrant  
720 signalling cascades are a hallmark of cancer, and targeting these pathways is a promising  
721 approach for cancer therapy. PS and PC have been shown to influence various dysregulated  
722 signalling cascades in cancer cells, making them attractive targets for targeted cancer therapy.

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723 PS and PC influence the PI3K/AKT/mTOR cell signaling pathway. These cell  
724 signalings, which regulate cell growth, survival, and metabolism, are frequently dysregulated  
725 in cancer cells [153]. PS can initiate the PI3K/AKT pathway by promoting the recruitment of  
726 AKT to the plasma membrane. The AKT activation in turn phosphorylates and activates the  
727 downstream targets, including mTOR, which promotes cell growth and proliferation. PC has  
728 also been shown to influence the PI3K/AKT/mTOR pathway [153]. Studies have shown that  
729 PC can activate AKT by promoting the activation of phosphoinositide-dependent kinase-1  
730 (PDK1), which is an upstream regulator of AKT. PC has also been shown to regulate mTOR  
731 activity by modulating the expression of mTOR regulatory proteins.

732 Targeting the PI3K/AKT/mTOR signaling pathway has been the focus of many cancer drug  
733 development efforts [153]. Several medications targeting this system, including PI3K  
734 inhibitors, AKT inhibitors, and mTOR inhibitors, are now under clinical studies. However, the  
735 efficiency of these drug molecules has limitations like off-target effects and drug resistance  
736 [154]. Targeting PS and PC may provide a novel approach for modulating this pathway in  
737 cancer cells.

738 Another signaling cascade that PS and PC influence is the JAK/STAT pathway [155]. This  
739 pathway plays a role in controlling cell growth, differentiation, and immune function, and it is  
740 often dysregulated in cancer cells [156]. PS can activate the JAK/STAT signalling pathway by  
741 inducing Janus kinase (JAK) followed by phosphorylation and activation of signal transducer  
742 and activator of transcription (STAT) proteins. PC also modulates the JAK/STAT pathway by  
743 activating STAT proteins and promoting their phosphorylation and nuclear translocation [156].  
744 PC has also been shown to regulate the expression of JAK proteins, which may impact the  
745 activation of this pathway. Targeting the signalling pathways of JAK/STAT has been the focus  
746 of many cancer drug development efforts. Several drugs targeting this pathway, including  
747 inhibitors of both JAK and STAT, are recently used in clinical trials [155]. However, the  
748 efficacy of these inhibitors are often limited by off-target effects and possibly show drug  
749 resistance. Targeting PS and PC may provide a novel approach for modulating this pathway in  
750 cancer cells.

751 In addition to both the JAK/STAT and PI3K/AKT/mTOR signaling pathways, PS and PC have  
752 been shown to influence other signaling cascades that are dysregulated in cancer cells [155].





753 For example, PS has been shown to turn on the MAPK/ERK signalling pathway, which is the  
754 key regulator pathway for both cell growth as well as cell differentiation. Again, PC has been  
755 reported to regulate developmental pathways like Wnt/ $\beta$ -catenin pathway, which plays  
756 important role and is also involved in the regulation of cell differentiation and cell proliferation.

757 Targeting PS and PC for cancer therapy has several advantages over traditional  
758 approaches that target specific proteins or pathways [134, 157, 158]. First, PS and PC are  
759 ubiquitous components of cell membranes, making them essential for cell survival. Therefore,  
760 targeting these phospholipids may have fewer off-target effects than drugs that target specific  
761 proteins or pathways. Second, PS and PC are highly expressed in cancer cells, making them  
762 attractive targets for cancer-specific therapies [134, 144, 159]. Targeting PS and PC may  
763 overcome drug resistance mechanisms that often arise with targeted therapies, as cancer cells  
764 may develop resistance to a specific protein or pathway but are unlikely to be able to develop  
765 resistance to fundamental components of cell membranes.

766 Several strategies for targeting PS and PC for cancer therapy have been developed. One  
767 approach is to use agents that specifically bind to these phospholipids and initiate apoptotic  
768 pathways in cancer cells [111, 112]. For example, some monoclonal antibody like bavituximab,  
769 that binds to PS and has been shown to prompt apoptosis in case of cancer cells by activating  
770 the immune system. Another approach is to use lipid-based delivery of drugs using  
771 nanoparticles to cancer cells [33, 134, 160]. These engineered small particles used to target PS  
772 and PC on the surface of cancer cells, allowing for selective delivery of drugs. Current era of  
773 anti-cancer drugs with liposomal formulations such as doxorubicin and paclitaxel, have  
774 developed that target PS on the surface of cancer cells and have depicted promising results in  
775 case of preclinical studies [32, 159, 161]. Finally, targeting the enzymes that synthesize PS and  
776 PC may be a promising approach for cancer therapy. For example, the enzyme PSS is  
777 responsible for the synthesis of PS, and inhibitors of PS have been reported to instigate  
778 apoptotic events in cancer cells. Similarly, the enzyme choline kinase (CK), which is  
779 responsible for the synthesis of PC, is often overexpressed in cancer cells, and inhibitors of CK  
780 have manifested potential results in preclinical studies. These approaches have been shown to  
781 have promising results in case of preclinical studies and it can offer a new avenue for  
782 developing cancer therapies.

## 783 6. Future Perspective and challenges

784 PS has emerged as a pivotal molecule with transformative potential in the landscape of  
785 cancer therapies. As we delve deeper into understanding its intricate molecular functions and



786 dynamic role in cancer progression, several emerging trends and future directions come to light.

787 These are;

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788 • Role of PS in Cancer Progression:

789 One of the critical areas of focus in the future will be unravelling the precise role of PS in  
790 driving cancer progression. Like its involvement in crucial cellular processes such as  
791 proliferation, apoptosis evasion, and metastasis formation. Understanding these mechanisms  
792 will provide valuable insights into designing targeted therapies that specifically disrupt PS-  
793 mediated pathways.

794 • Dynamic Interplay within the Cancer Microenvironment:

795 The cancer microenvironment takes on a vital role in tumor development and response to  
796 treatment. Investigating how PS actively contributes to the complex interplay within this  
797 microenvironment will be a crucial area of research. Its interactions with immune cells, stromal  
798 cells, and signaling molecules offer new avenues for innovative therapeutic strategies that  
799 leverage these interactions.

800 • Harnessing PS for Targeted Cancer Therapies:

801 Future research will focus on developing novel methodologies and approaches that harness the  
802 potential of PS for targeted cancer therapies. Exploring PS-targeted drug delivery systems,  
803 immunotherapies targeting PS-expressing cells, and combination therapies that synergize with  
804 PS-mediated pathways to intensify treatment efficacy as well as impair side effects.

805 • Age-Dependent Implications of novel PS in Cancer:

806 Understanding the age-dependent implications of PS in cancer will be crucial for personalized  
807 treatment strategies. Senescence-related changes in PS expression and function may impact  
808 treatment response and toxicity profiles. Tailoring therapies based on age-specific  
809 considerations will be an essential aspect of future cancer care.

810 • Interactions with Mast Cells and Microglia:

811 The intricate crosstalk between PS, mast cells, and microglia presents a promising avenue for  
812 therapeutic intervention. Investigating how PS influences immune cell behavior within the  
813 tumor microenvironment can lead to the development of immunomodulatory strategies that  
814 enhance anti-tumor immune responses.

815 • Future Horizons for Next-Generation Cancer Therapeutics:



816 PS holds tremendous potential as a cornerstone for next-generation cancer therapeutics.  
817 Continued research efforts aimed at elucidating its multifaceted roles, exploring innovative  
818 targeting approaches, integrating age-specific and microenvironmental considerations will  
819 pave a new direction for more effective and personalized treatments for cancer.

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820 Despite the promising advancements, several challenges remain. The precise delivery  
821 of PS-targeted therapies, the heterogeneity of PS expression across different cancer types, and  
822 the need for a deeper understanding of PS's role in various cellular contexts are critical areas  
823 that require further investigation. Additionally, integrating PS-targeted therapies with existing  
824 treatment modalities to maximize therapeutic efficacy and minimize adverse effects presents a  
825 complex yet rewarding challenge.

826 Looking forward, the future of PS in cancer therapy is bright. With ongoing research  
827 and technological advancements, the potential for PS-targeted therapies to revolutionize cancer  
828 treatment is immense. By continuing to unravel the complexities of PS and its interactions  
829 within the tumor microenvironment, researchers can develop more effective, targeted, and  
830 personalized cancer treatments. Overall, phosphatidylserine stands at the cusp of ushering in  
831 a new paradigm in cancer therapy. Its unique properties and significant role in cancer biology  
832 offer a promising avenue for developing novel therapeutic strategies. As our understanding of  
833 PS deepens, so too does the potential for groundbreaking advancements in the fight against  
834 cancer, paving the way for a future where cancer therapies are more effective, less invasive,  
835 and tailored to the individual needs of patients.

## 836 7. Conclusion

837 In tumor microenvironments, PS represents a transformative frontier in the realm of cancer  
838 therapies, offering unprecedented insights and opportunities for innovative treatments. As this  
839 review has illustrated, PS is not just a structural component of cell membranes but a pivotal  
840 player in the intricate communication networks within and between cells, particularly in the  
841 context of cancer. The externalization of PS on cancer cells and apoptotic cells reveals its  
842 crucial role in immune evasion and tumor progression, making it an attractive target for  
843 therapeutic intervention. The diverse biological functions of PS, from modulating immune  
844 responses to influencing cellular signaling pathways, underscore its potential as a multifaceted  
845 therapeutic target. Current research efforts, including the development of PS-targeting  
846 antibodies and PS-binding proteins, have shown promise in disrupting these processes, thereby  
847 enhancing the immune system's ability to recognize and eliminate cancer cells. Beyond these  
848 applications, PS is also used in antibody-drug conjugates, where it acts as a cytotoxic agent in  
849 PS-targeting antibodies to exert tumor-killing effects. These strategies, while still in the



850 experimental stages, herald a new era of precision medicine where treatments can be tailored  
851 to exploit the unique characteristics of cancer cells. Additional research is needed on cells and  
852 agents that detect PS exposure levels, together with other cancer medicines.

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## 859 Consent for publication

860 All authors have read and approved the final manuscript.

## 861 Competing interests

862 The authors declare that there are no potential competing interests.

## 863 8. References

- 864 1. Garraway, L.A. and E.S. Lander, *Lessons from the cancer genome*. Cell, 2013. **153**(1): p. 17-37.
- 865 2. Britt, K.L., J. Cuzick, and K.-A. Phillips, *Key steps for effective breast cancer prevention*. Nature  
866 Reviews Cancer, 2020. **20**(8): p. 417-436.
- 867 3. Matthews, H.K., C. Bertoli, and R.A. de Bruin, *Cell cycle control in cancer*. Nature Reviews  
868 Molecular Cell Biology, 2022. **23**(1): p. 74-88.
- 869 4. Patel, D., et al., *Equality, diversity, and inclusion in oncology clinical trials: an audit of essential  
870 documents and data collection against INCLUDE under-served groups in a UK academic trial  
871 setting*. BMC Medical Ethics, 2023. **24**(1): p. 105.
- 872 5. Birge, R., et al., *Phosphatidylserine is a global immunosuppressive signal in efferocytosis,  
873 infectious disease, and cancer*. Cell Death & Differentiation, 2016. **23**(6): p. 962-978.
- 874 6. Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality  
875 worldwide for 36 cancers in 185 countries*. CA: a cancer journal for clinicians, 2018. **68**(6): p.  
876 394-424.
- 877 7. Ene, C.I. and S.D. Ferguson, *Surgical management of brain metastasis: challenges and  
878 nuances*. Frontiers in oncology, 2022. **12**: p. 847110.
- 879 8. Gandhi, L., et al., *Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer*.  
880 New England journal of medicine, 2018. **378**(22): p. 2078-2092.
- 881 9. Takeyama, H., et al., *Impact of surgical treatment after sorafenib therapy for advanced  
882 hepatocellular carcinoma*. Surgery Today, 2018. **48**: p. 431-438.
- 883 10. Basu, A.K., *DNA Damage, Mutagenesis and Cancer*. Int J Mol Sci, 2018. **19**(4).
- 884 11. Novikov, N.M., et al., *Mutational drivers of cancer cell migration and invasion*. British journal  
885 of cancer, 2021. **124**(1): p. 102-114.
- 886 12. Laconi, E., F. Marongiu, and J. DeGregori, *Cancer as a disease of old age: changing mutational  
887 and microenvironmental landscapes*. British journal of cancer, 2020. **122**(7): p. 943-952.
- 888 13. Ale, A., et al., *Cardioprotective C-kit+ bone marrow cells attenuate apoptosis after acute  
889 myocardial infarction in mice-in-vivo assessment with fluorescence molecular imaging*.  
890 Theranostics, 2013. **3**(11): p. 903.



- 891 14. Chen, J., et al., *Dual-targeting theranostic system with mimicking apoptosis to promote*  
892 *myocardial infarction repair via modulation of macrophages*. *Theranostics*, 2017. **7**(17): p.  
893 4149. View Article Online  
DOI: 10.1039/D4MA00511B
- 894 15. Chang, W., et al., *Targeting phosphatidylserine for Cancer therapy: prospects and challenges*.  
895 *Theranostics*, 2020. **10**(20): p. 9214-9229.
- 896 16. Debela, D.T., et al., *New approaches and procedures for cancer treatment: Current*  
897 *perspectives*. *SAGE open medicine*, 2021. **9**: p. 20503121211034366.
- 898 17. Thapa, N., et al., *Discovery of a phosphatidylserine-recognizing peptide and its utility in*  
899 *molecular imaging of tumour apoptosis*. *J Cell Mol Med*, 2008. **12**(5a): p. 1649-60.
- 900 18. Kaynak, A., et al., *Phosphatidylserine: The unique dual-role biomarker for cancer imaging and*  
901 *therapy*. *Cancers*, 2022. **14**(10): p. 2536.
- 902 19. Jing, H., et al., *Microparticle phosphatidylserine mediates coagulation: involvement in tumor*  
903 *progression and metastasis*. *Cancers*, 2023. **15**(7): p. 1957.
- 904 20. Vallabhapurapu, S.D., et al., *Variation in human cancer cell external phosphatidylserine is*  
905 *regulated by flippase activity and intracellular calcium*. *Oncotarget*, 2015. **6**(33): p. 34375-88.
- 906 21. Mahapatra, M., et al., *Role of Biosurfactants in Heavy Metal Removal and Mineral Flotation,*  
907 *in Biotechnological Innovations in the Mineral-Metal Industry*. 2024, Springer. p. 141-150.
- 908 22. Naser, S.S., et al., *Posterity of nanoscience as lipid nanosystems for Alzheimer's disease*  
909 *regression*. *Materials Today Bio*, 2023: p. 100701.
- 910 23. Riedl, S., et al., *In search of a novel target - phosphatidylserine exposed by non-apoptotic tumor*  
911 *cells and metastases of malignancies with poor treatment efficacy*. *Biochim Biophys Acta*,  
912 2011. **1808**(11): p. 2638-45.
- 913 24. Chang, W., et al., *Targeting phosphatidylserine for Cancer therapy: prospects and challenges*.  
914 *Theranostics*, 2020. **10**(20): p. 9214.
- 915 25. Perez, G.I., et al., *Phosphatidylserine-Exposing Annexin A1-Positive Extracellular Vesicles:*  
916 *Potential Cancer Biomarkers*. *Vaccines*, 2023. **11**(3): p. 639.
- 917 26. Kaynak, A., et al., *Phosphatidylserine: The Unique Dual-Role Biomarker for Cancer Imaging and*  
918 *Therapy*. *Cancers (Basel)*, 2022. **14**(10).
- 919 27. Kawakami, T., S. Takeuchi, and Y. Soma, *Elevated levels of serum IgM anti-phosphatidylserine-*  
920 *prothrombin complex antibodies in patients with cancer-associated vasculitis*. *International*  
921 *journal of dermatology*, 2017. **56**(10): p. e203-e204.
- 922 28. Sakuragi, T. and S. Nagata, *Regulation of phospholipid distribution in the lipid bilayer by*  
923 *flippases and scramblases*. *Nature Reviews Molecular Cell Biology*, 2023. **24**(8): p. 576-596.
- 924 29. Lima, L.G., et al., *Tumor-derived microvesicles modulate the establishment of metastatic*  
925 *melanoma in a phosphatidylserine-dependent manner*. *Cancer letters*, 2009. **283**(2): p. 168-  
926 175.
- 927 30. Qi, X., et al., *Cancer-selective targeting and cytotoxicity by liposomal-coupled lysosomal*  
928 *saposin C protein*. *Clinical Cancer Research*, 2009. **15**(18): p. 5840-5851.
- 929 31. Da Poian, A.T., et al., *The Families of Biological Molecules*. *Integrative Human Biochemistry: A*  
930 *Textbook for Medical Biochemistry*, 2021: p. 89-185.
- 931 32. Fakai, M.I., S.N. Abd Malek, and S.A. Karsani, *Induction of apoptosis by chalepin through*  
932 *phosphatidylserine externalisations and DNA fragmentation in breast cancer cells (MCF7)*. *Life*  
933 *sciences*, 2019. **220**: p. 186-193.
- 934 33. Bandyopadhyay, A., et al., *Ligand-based active targeting strategies for cancer theranostics*.  
935 *Naunyn-Schmiedeberg's Archives of Pharmacology*, 2023.
- 936 34. Preetam, S., *Nano Revolution: Pioneering the Future of Water Reclamation with*  
937 *Micro/Nanorobots*. *Nanoscale Advances*, 2024.
- 938 35. Preetam, S., et al., *Therapeutic potential of Lipid Nanosystems for the treatment of Parkinson's*  
939 *disease: an updated review*. *Ageing Research Reviews*, 2023: p. 101965.
- 940 36. Preetam, S., et al., *Functionalized exosomes for cancer therapy*, in *Functionalized*  
941 *Nanomaterials for Cancer Research*. 2024, Elsevier. p. 167-180.
- 942 37. Vance, J.E. and R. Steenbergen, *Metabolism and functions of phosphatidylserine*. *Progress in*  
943 *lipid research*, 2005. **44**(4): p. 207-234.



- 944 38. Vance, J.E. and G. Tasseva, *Formation and function of phosphatidylserine and*  
 945 *phosphatidylethanolamine in mammalian cells*. *Biochimica et Biophysica Acta (BBA)-*  
 946 *Molecular and Cell Biology of Lipids*, 2013. **1831**(3): p. 543-554. View Article Online  
DOI: 10.1039/D4MA00511B
- 947 39. Schutters, K. and C. Reutelingsperger, *Phosphatidylserine targeting for diagnosis and*  
 948 *treatment of human diseases*. *Apoptosis*, 2010. **15**: p. 1072-1082.
- 949 40. Naeini, M.B., et al., *The role of phosphatidylserine recognition receptors in multiple biological*  
 950 *functions*. *Cellular & molecular biology letters*, 2020. **25**: p. 1-17.
- 951 41. Ribeiro, H., et al., *Apoptosis and (in) pain—potential clinical implications*. *Biomedicines*, 2022.  
 952 **10**(6): p. 1255.
- 953 42. Kay, J.G. and S. Grinstein, *Sensing phosphatidylserine in cellular membranes*. *Sensors*, 2011.  
 954 **11**(2): p. 1744-1755.
- 955 43. Maloney, D.G., B. Smith, and A. Rose. *Rituximab: mechanism of action and resistance*. in  
 956 *Seminars in oncology*. 2002. Elsevier.
- 957 44. Wirtz, K., *Phospholipid transfer proteins*. *Annual review of biochemistry*, 1991. **60**(1): p. 73-99.
- 958 45. Christie, W., *Phosphatidylcholine and related lipids: structure, occurrence, biochemistry and*  
 959 *analysis*. *Lipidlibrary*. AOCS, 2010.
- 960 46. Kennedy, E.P. and S.B. Weiss, *The function of cytidine coenzymes in the biosynthesis of*  
 961 *phospholipides*. *Journal of Biological Chemistry*, 1956. **222**(1): p. 193-214.
- 962 47. Vance, D.E. and J.E. Vance, *Biochemistry of lipids, lipoproteins and membranes*. Vol. 36. 2002:  
 963 Elsevier.
- 964 48. Vance, J.E., *Phospholipid synthesis in a membrane fraction associated with mitochondria*.  
 965 *Journal of Biological Chemistry*, 1990. **265**(13): p. 7248-7256.
- 966 49. Shields, D.J., et al., *Membrane topography of human phosphatidylethanolamine N-*  
 967 *methyltransferase*. *Journal of Biological Chemistry*, 2003. **278**(5): p. 2956-2962.
- 968 50. Szlasa, W., et al., *Lipid composition of the cancer cell membrane*. *Journal of bioenergetics and*  
 969 *biomembranes*, 2020. **52**: p. 321-342.
- 970 51. Lenoir, G., et al., *Transport pathways that contribute to the cellular distribution of*  
 971 *phosphatidylserine*. *Frontiers in cell and developmental biology*, 2021. **9**: p. 737907.
- 972 52. Shin, H.-W. and H. Takatsu, *Phosphatidylserine exposure in living cells*. *Critical reviews in*  
 973 *biochemistry and molecular biology*, 2020. **55**(2): p. 166-178.
- 974 53. Wang, W., et al., *Mobilizing phospholipids on tumor plasma membrane implicates*  
 975 *phosphatidylserine externalization blockade for cancer immunotherapy*. *Cell reports*, 2022.  
 976 **41**(5).
- 977 54. Ventura, R., I. Martínez-Ruiz, and M.I. Hernández-Alvarez, *Phospholipid membrane transport*  
 978 *and associated diseases*. *Biomedicines*, 2022. **10**(5): p. 1201.
- 979 55. Skotland, T., S. Kavaliauskiene, and K. Sandvig, *The role of lipid species in membranes and*  
 980 *cancer-related changes*. *Cancer and Metastasis Reviews*, 2020. **39**(2): p. 343-360.
- 981 56. Li, B., et al., *Lipid raft involvement in signal transduction in cancer cell survival, cell death and*  
 982 *metastasis*. *Cell proliferation*, 2022. **55**(1): p. e13167.
- 983 57. Cockcroft, S., *Mammalian lipids: structure, synthesis and function*. *Essays in biochemistry*,  
 984 2021. **65**(5): p. 813-845.
- 985 58. Xiao, D. and W. Chang, *Phosphatidylserine in diabetes research*. *Molecular Pharmaceutics*,  
 986 2022. **20**(1): p. 82-89.
- 987 59. Wang, J., et al., *The role of phosphatidylserine on the membrane in immunity and blood*  
 988 *coagulation*. *Biomarker research*, 2022. **10**(1): p. 4.
- 989 60. Rodencal, J. and S.J. Dixon, *A tale of two lipids: Lipid unsaturation commands ferroptosis*  
 990 *sensitivity*. *Proteomics*, 2023. **23**(6): p. 2100308.
- 991 61. Murata, M., et al., *Molecular substructure of the liquid-ordered phase formed by*  
 992 *sphingomyelin and cholesterol: Sphingomyelin clusters forming nano-subdomains are a*  
 993 *characteristic feature*. *Biophysical reviews*, 2022. **14**(3): p. 655-678.
- 994 62. Medfisch, S.M., et al., *Phosphatidylethanolamine-phosphatidylserine binding synergy of seven*  
 995 *coagulation factors revealed using Nanodisc arrays on silicon photonic sensors*. *Scientific*  
 996 *Reports*, 2020. **10**(1): p. 17407.
- 997 63. Scott, H.L., et al., *Model membrane systems used to study plasma membrane lipid asymmetry*.  
 998 *Symmetry*, 2021. **13**(8): p. 1356.



- 999 64. Engberg, O., et al., *Sphingomyelin acyl chains influence the formation of sphingomyelin-and*  
1000 *cholesterol-enriched domains*. Biophysical journal, 2020. **119**(5): p. 913-923.
- 1001 65. Dias, C. and J. Nylandsted, *Plasma membrane integrity in health and disease: significance and*  
1002 *therapeutic potential*. Cell discovery, 2021. **7**(1): p. 4.
- 1003 66. Bhattacharya, T., et al., *Advancement in biopolymer assisted cancer theranostics*. ACS Applied  
1004 Bio Materials, 2023. **6**(10): p. 3959-3983.
- 1005 67. von Schacky, C., *Importance of EPA and DHA blood levels in brain structure and function*.  
1006 Nutrients, 2021. **13**(4): p. 1074.
- 1007 68. Park, Y.-J., et al., *Phosphatidylserine synthase plays an essential role in glia and affects*  
1008 *development, as well as the maintenance of neuronal function*. Iscience, 2021. **24**(8).
- 1009 69. Sokolova, D., T. Childs, and S. Hong, *Insight into the role of phosphatidylserine in complement-*  
1010 *mediated synapse loss in Alzheimer's disease*. Faculty Reviews, 2021. **10**.
- 1011 70. Schumacher, D., et al., *Phosphatidylserine supplementation as a novel strategy for reducing*  
1012 *myocardial infarct size and preventing adverse left ventricular remodeling*. International  
1013 journal of molecular sciences, 2021. **22**(9): p. 4401.
- 1014 71. Preetam, S., et al., *Application of Nanobiosensor in Health Care Sector, in Bio-Nano Interface:*  
1015 *Applications in Food, Healthcare and Sustainability*, M. Arakha, A.K. Pradhan, and S. Jha,  
1016 Editors. 2022, Springer Singapore: Singapore. p. 251-270.
- 1017 72. Preetam, S., et al., *Revolutionizing Cancer Treatment: The Promising Horizon of Zein*  
1018 *Nanosystems*. ACS Biomaterials Science & Engineering, 2024.
- 1019 73. Zargarian, S., et al., *Phosphatidylserine externalization, "necroptotic bodies" release, and*  
1020 *phagocytosis during necroptosis*. PLoS biology, 2017. **15**(6): p. e2002711.
- 1021 74. Wang, F., et al., *Effects on tumor growth and immunosuppression of a modified Ta1 peptide*  
1022 *along with its circular dichroism spectroscopy data*. Data in brief, 2018. **20**: p. 126-131.
- 1023 75. Farhad, M., A.S. Rolig, and W.L. Redmond, *The role of Galectin-3 in modulating tumor growth*  
1024 *and immunosuppression within the tumor microenvironment*. Oncoimmunology, 2018. **7**(6):  
1025 p. e1434467.
- 1026 76. Rajan, R., et al., *Liposome-induced immunosuppression and tumor growth is mediated by*  
1027 *macrophages and mitigated by liposome-encapsulated alendronate*. Journal of Controlled  
1028 Release, 2018. **271**: p. 139-148.
- 1029 77. Beatty, G.L. and W.L. Gladney, *Immune escape mechanisms as a guide for cancer*  
1030 *immunotherapy*. Clinical cancer research, 2015. **21**(4): p. 687-692.
- 1031 78. Sharma, B. and S.S. Kanwar. *Phosphatidylserine: A cancer cell targeting biomarker*. in *Seminars*  
1032 *in cancer biology*. 2018. Elsevier.
- 1033 79. Orning, P. and E. Lien, *Multiple roles of caspase-8 in cell death, inflammation, and innate*  
1034 *immunity*. Journal of Leucocyte Biology, 2021. **109**(1): p. 121-141.
- 1035 80. Kur, I.-M. and A. Weigert, *Phosphatidylserine externalization as immune checkpoint in cancer*.  
1036 Pflügers Archiv-European Journal of Physiology, 2024: p. 1-14.
- 1037 81. Lo, C.-F., et al., *Targeting the phosphatidylserine-immune checkpoint with a small-molecule*  
1038 *maytansinoid conjugate*. Journal of Medicinal Chemistry, 2022. **65**(19): p. 12802-12824.
- 1039 82. Aehnlich, P., et al., *Tam Receptor inhibition—implications for cancer and the immune system*.  
1040 Cancers, 2021. **13**(6): p. 1195.
- 1041 83. Min, C., et al., *Tim-4 functions as a scavenger receptor for phagocytosis of exogenous particles*.  
1042 Cell Death & Disease, 2020. **11**(7): p. 561.
- 1043 84. Luque-Campos, N., et al., *The macrophage response is driven by mesenchymal stem cell-*  
1044 *mediated metabolic reprogramming*. Frontiers in Immunology, 2021. **12**: p. 624746.
- 1045 85. Belzile, O., et al., *Antibody targeting of phosphatidylserine for the detection and*  
1046 *immunotherapy of cancer*. ImmunoTargets and Therapy, 2018. **7**(null): p. 1-14.
- 1047 86. Byun, E.-B., et al., *Hesperidin structurally modified by gamma irradiation induces apoptosis in*  
1048 *murine melanoma B16BL6 cells and inhibits both subcutaneous tumor growth and metastasis*  
1049 *in C57BL/6 mice*. Food and Chemical Toxicology, 2019. **127**: p. 19-30.
- 1050 87. Oyler-Yaniv, J., et al., *Catch and release of cytokines mediated by tumor phosphatidylserine*  
1051 *converts transient exposure into long-lived inflammation*. Molecular cell, 2017. **66**(5): p. 635-  
1052 647. e7.



- 1053 88. Zhang, L., et al., *Phosphatidylserine-targeted bimodal liposomal nanoparticles for in vivo*  
 1054 *imaging of breast cancer in mice*. Journal of Controlled Release, 2014. **183**: p. 114-123.
- 1055 89. Kannadorai, R.K., S.K. Udumala, and Y.W.K. Sidney, *Noninvasive in vivo multispectral*  
 1056 *optoacoustic imaging of apoptosis in triple negative breast cancer using indocyanine green*  
 1057 *conjugated phosphatidylserine monoclonal antibody*. Journal of biomedical optics, 2016.  
 1058 **21**(12): p. 126002-126002.
- 1059 90. Skotland, T., et al., *Molecular lipid species in urinary exosomes as potential prostate cancer*  
 1060 *biomarkers*. European Journal of Cancer, 2017. **70**: p. 122-132.
- 1061 91. Zhang, J., et al., *Lipids in surgical aerosol as diagnosis biomarkers for discrimination of lung*  
 1062 *cancer*. Cancer Management and Research, 2019: p. 5537-5543.
- 1063 92. Zhao, D., et al., *Near-infrared optical imaging of exposed phosphatidylserine in a mouse glioma*  
 1064 *model*. Translational oncology, 2011. **4**(6): p. 355-364.
- 1065 93. Blanco, V.M., R. Curry, and X. Qi, *SapC-DOPS nanovesicles: a novel targeted agent for the*  
 1066 *imaging and treatment of glioblastoma*. Oncoscience, 2015. **2**(2): p. 102.
- 1067 94. Chu, Z., et al., *In vivo optical imaging of brain tumors and arthritis using fluorescent SapC-*  
 1068 *DOPS nanovesicles*. JoVE (Journal of Visualized Experiments), 2014(87): p. e51187.
- 1069 95. Winter, P.M., et al., *Imaging of brain tumors with paramagnetic vesicles targeted to*  
 1070 *phosphatidylserine*. Journal of Magnetic Resonance Imaging, 2015. **41**(4): p. 1079-1087.
- 1071 96. Ntziachristos, V., et al., *Visualization of antitumor treatment by means of fluorescence*  
 1072 *molecular tomography with an annexin V-Cy5. 5 conjugate*. Proceedings of the National  
 1073 Academy of Sciences, 2004. **101**(33): p. 12294-12299.
- 1074 97. Blankenberg, F.G., et al., *In vivo detection and imaging of phosphatidylserine expression during*  
 1075 *programmed cell death*. Proceedings of the National Academy of Sciences, 1998. **95**(11): p.  
 1076 6349-6354.
- 1077 98. Dumont, E., et al., *Real-time imaging of apoptotic cell-membrane changes at the single-cell*  
 1078 *level in the beating murine heart*. 2001, Nature Publishing Group US New York.
- 1079 99. Koopman, G., et al., *Annexin V for flow cytometric detection of phosphatidylserine expression*  
 1080 *on B cells undergoing apoptosis*. 1994.
- 1081 100. Vermes, I., et al., *A novel assay for apoptosis flow cytometric detection of phosphatidylserine*  
 1082 *expression on early apoptotic cells using fluorescein labelled annexin V*. Journal of  
 1083 immunological methods, 1995. **184**(1): p. 39-51.
- 1084 101. Reutelingsperger, C.P., G. Hornstra, and H.C. Hemker, *Isolation and partial purification of a*  
 1085 *novel anticoagulant from arteries of human umbilical cord*. European journal of biochemistry,  
 1086 1985. **151**(3): p. 625-629.
- 1087 102. Kietselaer, B.L., et al., *Noninvasive detection of plaque instability with use of radiolabeled*  
 1088 *annexin A5 in patients with carotid-artery atherosclerosis*. New England Journal of Medicine,  
 1089 2004. **350**(14): p. 1472-1473.
- 1090 103. Jennewein, M., et al., *Vascular imaging of solid tumors in rats with a radioactive arsenic-*  
 1091 *labeled antibody that binds exposed phosphatidylserine*. Clinical Cancer Research, 2008. **14**(5):  
 1092 p. 1377-1385.
- 1093 104. Wojton, J., et al., *Systemic delivery of SapC-DOPS has antiangiogenic and antitumor effects*  
 1094 *against glioblastoma*. Molecular Therapy, 2013. **21**(8): p. 1517-1525.
- 1095 105. Abu-Baker, S., et al., *Cytotoxicity and selectivity in skin cancer by SapC-DOPS nanovesicles*.  
 1096 Journal of cancer therapy, 2012. **3**(4): p. 321.
- 1097 106. Davis, H.W., et al., *Enhanced phosphatidylserine-selective cancer therapy with irradiation and*  
 1098 *SapC-DOPS nanovesicles*. Oncotarget, 2019. **10**(8): p. 856.
- 1099 107. De, M., et al., *A novel therapeutic strategy for cancer using phosphatidylserine targeting*  
 1100 *stearylamine-bearing cationic liposomes*. Molecular Therapy-Nucleic Acids, 2018. **10**: p. 9-27.
- 1101 108. Kenis, H., L. Hofstra, and C. Reutelingsperger, *Annexin A5: shifting from a diagnostic towards*  
 1102 *a therapeutic realm*. Cellular and Molecular Life Sciences, 2007. **64**: p. 2859-2862.
- 1103 109. Kenis, H., et al., *Cell surface-expressed phosphatidylserine and annexin A5 open a novel portal*  
 1104 *of cell entry*. Journal of Biological Chemistry, 2004. **279**(50): p. 52623-52629.
- 1105 110. Ran, S. and P.E. Thorpe, *Phosphatidylserine is a marker of tumor vasculature and a potential*  
 1106 *target for cancer imaging and therapy*. International Journal of Radiation Oncology\* Biology\*  
 1107 Physics, 2002. **54**(5): p. 1479-1484.





- 1108 111. Huang, X., M. Bennett, and P.E. Thorpe, *A monoclonal antibody that binds anionic*  
1109 *phospholipids on tumor blood vessels enhances the antitumor effect of docetaxel on human*  
1110 *breast tumors in mice*. *Cancer research*, 2005. **65**(10): p. 4408-4416. View Article Online  
DOI: 10.1039/D4MA00511B
- 1111 112. Luster, T.A., et al., *Plasma protein  $\beta$ -2-glycoprotein 1 mediates interaction between the anti-*  
1112 *tumor monoclonal antibody 3G4 and anionic phospholipids on endothelial cells*. *Journal of*  
1113 *Biological Chemistry*, 2006. **281**(40): p. 29863-29871.
- 1114 113. Willems, G.M., et al., *Competition of annexin V and anticardiolipin antibodies for binding to*  
1115 *phosphatidylserine containing membranes*. *Biochemistry*, 2000. **39**(8): p. 1982-1989.
- 1116 114. Li, R., et al., *Targeting Phosphatidylserine with Calcium-Dependent Protein-Drug Conjugates*  
1117 *for the Treatment of Cancer*. *Molecular cancer therapeutics*, 2018. **17**(1): p. 169-182.
- 1118 115. Guan, S., et al., *Phosphatidylserine targeting peptide-functionalized pH sensitive mixed*  
1119 *micelles for enhanced anti-tumor drug delivery*. *European Journal of Pharmaceutics and*  
1120 *Biopharmaceutics*, 2020. **147**: p. 87-101.
- 1121 116. Van Rite, B.D., et al., *Enzyme prodrug therapy designed to target L-methioninase to the tumor*  
1122 *vasculature*. *Cancer letters*, 2011. **301**(2): p. 177-184.
- 1123 117. Oldenborg, P.-A., *CD47: a cell surface glycoprotein which regulates multiple functions of*  
1124 *hematopoietic cells in health and disease*. *International Scholarly Research Notices*, 2013.  
1125 **2013**.
- 1126 118. Unanue, E.R., *Perspectives on anti-CD47 antibody treatment for experimental cancer*.  
1127 *Proceedings of the National Academy of Sciences*, 2013. **110**(27): p. 10886-10887.
- 1128 119. Haubelt, H., et al., *Variables influencing Platelet Function Analyzer-100TM closure times in*  
1129 *healthy individuals*. *British journal of haematology*, 2005. **130**(5): p. 759-767.
- 1130 120. Ennishi, D., et al., *TMEM30A loss-of-function mutations drive lymphomagenesis and confer*  
1131 *therapeutically exploitable vulnerability in B-cell lymphoma*. *Nature medicine*, 2020. **26**(4): p.  
1132 577-588.
- 1133 121. Advani, R., et al., *CD47 blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma*. *New*  
1134 *England Journal of Medicine*, 2018. **379**(18): p. 1711-1721.
- 1135 122. Budhu, S., et al., *Targeting phosphatidylserine enhances the anti-tumor response to tumor-*  
1136 *directed radiation therapy in a preclinical model of melanoma*. *Cell reports*, 2021. **34**(2).
- 1137 123. Kawakami, T., et al., *High titer of serum antiphospholipid antibody levels in adult*  
1138 *Henoch-Schönlein purpura and cutaneous leukocytoclastic angiitis*. *Arthritis Care & Research*,  
1139 2008. **59**(4): p. 561-567.
- 1140 124. Schuck-Paim, C., et al., *Effect of pneumococcal conjugate vaccine introduction on childhood*  
1141 *pneumonia mortality in Brazil: a retrospective observational study*. *The Lancet Global Health*,  
1142 2019. **7**(2): p. e249-e256.
- 1143 125. Nallanthighal, S., et al., *Inhibition of collagen XI alpha 1-induced fatty acid oxidation triggers*  
1144 *apoptotic cell death in cisplatin-resistant ovarian cancer*. *Cell death & disease*, 2020. **11**(4): p.  
1145 258.
- 1146 126. Qian, S., et al., *The role of BCL-2 family proteins in regulating apoptosis and cancer therapy*.  
1147 *Frontiers in Oncology*, 2022. **12**.
- 1148 127. Ahmadi-Kashani, M., H. Dehghani, and A. Zarrabi, *A biocompatible nanoplatfom formed by*  
1149 *MgAl-layered double hydroxide modified Mn3O4/N-graphene quantum dot conjugated-*  
1150 *polyaniline for pH-triggered release of doxorubicin*. *Materials Science and Engineering: C*,  
1151 2020. **114**: p. 111055.
- 1152 128. Bhattacharjee, R., et al., *Synergy of nanocarriers with CRISPR-Cas9 in an emerging technology*  
1153 *platform for biomedical appliances: Current insights and perspectives*. *Materials & Design*,  
1154 2022. **224**: p. 111415.
- 1155 129. Bhattacharjee, R., et al., *Theragnostic application of nanoparticle and CRISPR against food-*  
1156 *borne multi-drug resistant pathogens*. *Materials Today Bio*, 2022. **15**: p. 100291.
- 1157 130. Qin, X., et al., *Artificial Nucleotide-containing Aptamers Used in Tumor Therapy*. *Chemical*  
1158 *Research in Chinese Universities*, 2020. **36**(2): p. 164-170.
- 1159 131. Ahmed, E.M., *Hydrogel: Preparation, characterization, and applications: A review*. *Journal of*  
1160 *Advanced Research*, 2015. **6**(2): p. 105-121.
- 1161 132. Bansal, T., et al., *Novel formulation approaches for optimising delivery of anticancer drugs*  
1162 *based on P-glycoprotein modulation*. *Drug discovery today*, 2009. **14**(21-22): p. 1067-1074.



- 1163 133. Myers, K.V., S.R. Amend, and K.J. Pienta, *Targeting Tyro3, Axl and MerTK (TAM receptors):*  
 1164 *implications for macrophages in the tumor microenvironment.* Molecular cancer, 2019. **18(1):**  
 1165 p. 1-14. View Article Online  
DOI: 10.1039/D4MA00511B
- 1166 134. Aggarwal, M. and S. Kumar, *The Use of Nanorobotics in the Treatment Therapy of Cancer and*  
 1167 *Its Future Aspects: A Review.* Cureus, 2022. **14(9):** p. e29366.
- 1168 135. Tannock, I.F., et al., *Limited Penetration of Anticancer Drugs through Tumor Tissue.* Clinical  
 1169 Cancer Research, 2002. **8:** p. 878-884.
- 1170 136. Mittal, P., et al., *Unlocking the power of nanomedicine: the future of nutraceuticals in oncology*  
 1171 *treatment.* Frontiers in Nutrition, 2023. **10:** p. 1258516.
- 1172 137. Mukherjee, S., et al., *Reaction kinetics involved in esterification between the fatty acids in*  
 1173 *castor oil and furfuryl alcohol.* Industrial Crops and Products, 2024. **213:** p. 118393.
- 1174 138. Taniguchi, K. and M. Karin, *NF- $\kappa$ B, inflammation, immunity and cancer: coming of age.* Nature  
 1175 Reviews Immunology, 2018. **18(5):** p. 309-324.
- 1176 139. Theoharides, T.C., A. Twahir, and D. Kempuraj, *Mast cells in the autonomic nervous system*  
 1177 *and potential role in disorders with dysautonomia and neuroinflammation.* Annals of Allergy,  
 1178 Asthma & Immunology, 2023.
- 1179 140. Doo, D.W., et al., *Inhibition of the Wnt/ $\beta$ -catenin pathway enhances antitumor immunity in*  
 1180 *ovarian cancer.* Therapeutic advances in medical oncology, 2020. **12:** p. 1758835920913798.
- 1181 141. Mamuladze, T. and J. Kipnis, *Type 2 immunity in the brain and brain borders.* Cellular &  
 1182 Molecular Immunology, 2023. **20(11):** p. 1290-1299.
- 1183 142. Ledford, H., *US cancer institute to overhaul tumour cell lines.* Nature News, 2016. **530(7591):**  
 1184 p. 391.
- 1185 143. Minchinton, A.I. and I.F. Tannock, *Drug penetration in solid tumours.* Nature Reviews Cancer,  
 1186 2006. **6(8):** p. 583-592.
- 1187 144. Padera, T.P., et al., *Cancer cells compress intratumour vessels.* Nature, 2004. **427(6976):** p.  
 1188 695-695.
- 1189 145. Denekamp, J. and B. Hobson, *Endothelial-cell proliferation in experimental tumours.* British  
 1190 journal of cancer, 1982. **46(5):** p. 711-720.
- 1191 146. Felfoul, O., et al., *Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to*  
 1192 *tumour hypoxic regions.* Nature nanotechnology, 2016. **11(11):** p. 941-947.
- 1193 147. Sinclair, J., et al., *Profiling signatures of ovarian cancer tumour suppression using 2D-DIGE and*  
 1194 *2D-LC-MS/MS with tandem mass tagging.* Journal of proteomics, 2011. **74(4):** p. 451-465.
- 1195 148. Li, C., et al., *Effective control of DBPs formation and membrane fouling in catalytic ozonation*  
 1196 *membrane reactor for municipal wastewater reclamation.* Separation and Purification  
 1197 Technology, 2024. **330:** p. 125492.
- 1198 149. Jones, M.K., A. Nair, and M. Gupta, *Mast Cells in Neurodegenerative Disease.* Frontiers in  
 1199 Cellular Neuroscience, 2019. **13.**
- 1200 150. Niu, J., et al., *Construction of micro-nano robots: living cells and functionalized biological cell*  
 1201 *membranes.* Frontiers in Bioengineering and Biotechnology, 2023. **11.**
- 1202 151. Pedersen, L.E., et al., *Porcine major histocompatibility complex (MHC) class I molecules and*  
 1203 *analysis of their peptide-binding specificities.* Immunogenetics, 2011. **63:** p. 821-834.
- 1204 152. Schmidt, A., N. Oberle, and P.H. Krammer, *Molecular mechanisms of treg-mediated T cell*  
 1205 *suppression.* Frontiers in immunology, 2012. **3:** p. 51.
- 1206 153. Hoxhaj, G. and B.D. Manning, *The PI3K-AKT network at the interface of oncogenic signalling*  
 1207 *and cancer metabolism.* Nature Reviews Cancer, 2020. **20(2):** p. 74-88.
- 1208 154. Miricescu, D., et al., *PI3K/AKT/mTOR signalling pathway involvement in renal cell carcinoma*  
 1209 *pathogenesis.* Experimental and Therapeutic Medicine, 2021. **21(5):** p. 1-7.
- 1210 155. Hu, Q., et al., *JAK/STAT pathway: Extracellular signals, diseases, immunity, and therapeutic*  
 1211 *regimens.* Frontiers in Bioengineering and Biotechnology, 2023. **11:** p. 1110765.
- 1212 156. Owen, K.L., N.K. Brockwell, and B.S. Parker, *JAK-STAT signaling: a double-edged sword of*  
 1213 *immune regulation and cancer progression.* Cancers, 2019. **11(12):** p. 2002.
- 1214 157. Tannock, I.F. and S. Hayashi, *The proliferation of capillary endothelial cells.* Cancer research,  
 1215 1972. **32(1):** p. 77-82.
- 1216 158. Tesfay, L., et al., *Stearoyl-CoA desaturase 1 protects ovarian cancer cells from ferroptotic cell*  
 1217 *death.* Cancer research, 2019. **79(20):** p. 5355-5366.



- 1218 159. Hoop, M., et al., *A smart multifunctional drug delivery nanoplatform for targeting cancer cells*.  
1219 Nanoscale, 2016. **8**(25): p. 12723-12728.
- 1220 160. Aust, S., et al., *Absence of PD-L1 on tumor cells is associated with reduced MHC expression*  
1221 *and PD-L1 expression increases in recurrent serous ovarian cancer*. Scientific reports, 2017.  
1222 **7**(1): p. 42929.
- 1223 161. Fleury, H., et al., *Exploiting interconnected synthetic lethal interactions between PARP*  
1224 *inhibition and cancer cell reversible senescence*. Nature Communications, 2019. **10**(1): p. 2556.

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