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Neurokinin-1 receptor (NK-1R) antagonists: potential targets in the treatment of glioblastoma multiforme

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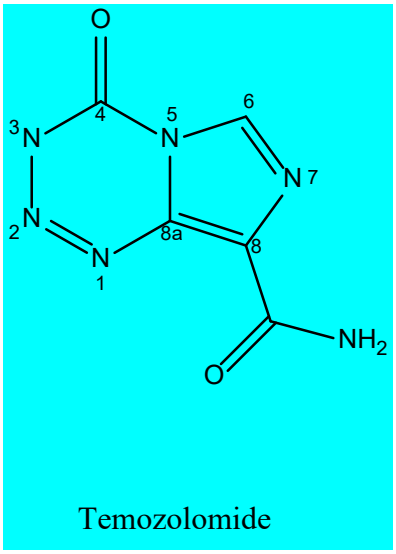
Abstract

The current standard of care in glioblastoma multiforme (GBM), as the most morbid brain tumor, is not adequate, despite substantial progress in cancer therapy. Among patients receiving current standard treatments, including surgery, irradiation, and chemotherapy, the overall survival (OS) period with GBM is less than one year. The high mortality frequency of GBM is due to its aggressive nature, including accelerated growth, deregulated apoptosis, and invasion into surrounding tissues. The understanding of the molecular pathogenesis of GBM is, therefore, crucial for identifying, designing, and repurposing potential agents in future therapeutic approaches. In recent decades, it has been apparent that several neurotransmitters, specifically substance P (SP), an undecapeptide in the family of neuropeptides tachykinins, are found in astrocytes. After binding to the neurokinin-1 receptor (NK-1R), the SP controls cancer cell growth, exerts antiapoptotic impacts, stimulates cell invasion/metastasis, and activates vascularization. Since SP/NK-1R signaling pathway is a growth driver in many cancers, this potential mechanism is proposed as an additional target for treating GBM. Following an evaluation of the function of both SP and its NK-1R inhibitors in neoplastic cells, we recommend a unique and promising approach for the treatment of patients with GBM.

Keywords: Glioblastoma multiforme; Apoptosis, Substance P; Neurokinin-1 receptor

1. Introduction

Glioblastoma multiforme (GBM), as the most frequent and malignant brain tumor, has an accelerated growth trajectory with a median survival rate of 12-14 months (Afshari et al., 2019, Afshari et al., 2020, Batash et al. , 2017). The pathogenesis of GBM consists of numerous genetic alterations, which deregulates multiple molecular pathways, including angiogenesis, cell motility, apoptosis, micrometastasis, and invasion (Afshari et al., 2020, Jalili-Nik et al. , 2020, Yabroff et al. , 2012). The current standard of care of GBM includes maximal safe surgical resection, radiotherapy, and chemotherapy with temozolomide (TMZ, Fig. 1) and bevacizumab (Avastin®, a vascular endothelial growth factor [VEGF] inhibitor, which increases an average of two-months survival of affected patients (Alifieris and Trafalis, 2015, Soukhtanloo et al. , 2020). Besides that, adjuvant treatments have potentially significant clinical resistance since the central nervous system (CNS) is a highly shielded region and the cancerous cells have a wide intra-tumoral genetic and epigenetic heterogeneity. Therefore, the development of effective treatment strategies to regulate tumor progression and improve the median survival of GBM patients are urgently required (Kadiyala et al. , 2019).



Temozolomide

Figure 1. Chemical structure of temozolomide (TMZ, 194.151 g/mol)

Redesigning existing medications promotes and opens up avenues for the discovery of novel cancer therapies. These medications have key benefits such as approved use for therapeutic purposes, generally inexpensive, well-characterized adverse effects and safety profile (El Demerdash et al. , 2020, Stylli, 2020). Nowadays, numerous studies have shown that the Substance P (SP)/neurokinin-1 receptor (NK-1R), as a new repurposing system, may play a key role in various cancers such as hepatoblastoma and esophageal squamous cell carcinoma (Muñoz and Coveñas, 2019a). Several studies have shown that SP promotes a series of NK-1R signaling pathways that intervene with cell sensitivity in such tumor cells (Javid et al. , 2020a). Various studies demonstrate that tumor cells have more NK-1R on their surface and bind more firmly to SP than healthy human cells (Davoodian et al. , 2019, Gharaee et al. , 2018). In this regard, it has been indicated that GBM malignant cells (e.g., GAMG, SNB-19, U-87 MG, U-373 MG, and UC-11) express significantly more NK-1Rs (Akazawa et al. , 2009a, Mou et al. , 2013). Moreover, it has been confirmed that SP advances the proliferation of GBM cells, which could be inhibited by NK-1R antagonists (e.g., aprepitant, L-732,138, and L-733,060) (Javid et al. , 2020b, Muñoz and Rosso, 2010). Herein, in this review, we will focus on the role of chemical products affecting SP/NK-1R system as potential antagonists for treating GBM.

2. Properties of substance P (SP)/neurokinin-1 receptor (NK-1R) system

The family of mammalian tachykinin has three classical members: SP, NK-A (both encoded with the TAC1 gene), and NK-B (encoded with the TAC3 gene), other members such as hemokinins and endokinins are encoded with the TAC4 gene (Palma, 2006, Patacchini et al. , 2004, Zhang et al. , 2000). Tachykinin receptors, according to their affinity ligands are divided into three distinct sorts: TACR1 (NK-1R), TACR2 (NK-2R), and TACR3 (NK-3R) (Table 1), which have different preferred affinities for SP, NK-A, and NK-B (Page, 2005, Pennefather et al. , 2004, Werge, 2007).

Table 1. The genes and the affinity of human tachykinin receptors (Garcia-Recio and Gascón, 2015).

Receptor	Gene	Affinity of receptor
NK-1	TACR1	SP>NK-A>NK-B
NK-2	TACR2	NK-A>NK-B>SP
NK-3	TACR3	NK-B>NK-A>SP

SP is a small undecapeptide, highly conserved member of the tachykinin peptides made of 11-aminoacids, binding to the NK-R family (NK-1R, NK-2R, and NK-3R). SP regulates different biological functions, mainly in the peripheral nervous system and CNS (Majkowska-Pilip et al. , 2018, Muñoz et al. , 2015). SP is commonly distributed in different organs (e.g., genitourinary, nervous, immune, and cardiovascular system) and related to various activities, such as microvascular permeability, wound repair, leukocyte transport, hematopoiesis, cell endurance, metastasis, and inflammation of the nervous system (Elhousiny, 2019, Garcia-Recio and Gascón, 2015, Hong et al. , 2019, Liew and Yong, 2019, Park et al. , 2016). It has been reported that SP performs physiological activities at low concentrations. When the peptide is released at higher levels, it modulates pathophysiological events, such as inflammation, nausea, vomiting, fatigue, and depression. SP also enhances angiogenesis by increasing the proliferation of endothelial cells and promotes the proliferation and migration of tumor cells (Hökfelt et al. , 2000, Seegers et al. , 2003).

Generally, SP has been detected in retinoblastoma, GBM, neuroblastoma, and breast cancer cells (Muñoz et al. , 2005, Ruan et al. , 2018). SP has also been identified in the peritumor and tumor mass, especially in the tumor periphery, although in comparison with typical mammalian epithelial cells, preprotachykinine A expression has been increased (Cuesta et al. , 2002). Besides, oral squamous cell carcinoma and larynx carcinoma tissues have been documented to produce SP in the cytoplasm and the tumor cell nucleus (Muñoz et al., 2015, Muñoz et al. , 2012). For instance, our previous research has shown that the SP could induce the human

esophageal squamous cell carcinoma progression through overexpression of metastasis and angiogenesis-related factors (Mohammadi et al. , 2020).

As mentioned earlier, the NK-R is the principal receptor for the tachykinin group of peptides, one of the most significant preserved peptide families. NK-R engages with inflammatory processes and neurotransmission through NK-1, NK-2, and NK-3Rs (Garcia-Recio and Gascón, 2015). NK-1R is commonly distributed in both central and peripheral nervous systems. They are engaged in cell reactions, such as endocrine and paracrine discharge, vasodilatation, pain, regulation of cell proliferation, and modulator of the nervous system (Grewal, 2016). Interestingly, the number of NK-1R found in healthy human cells has been reported to be lower than those shown in human tumor cells (Muñoz and Coveñas, 2019b).

The involvement of NK-1R was found to play an essential role in the viability of tumor cells in multiple human cell/tissue samples. The degree of tumor malignancy contributes to the number of NK-1Rs: a more significant amount, higher fatality, and a worse prognosis correlate with more NK-1R overexpression (Feyer and Jordan, 2011, Garcia-Recio and Gascón, 2015). Notably, it has been reported that NK-1R mRNA expression has been upregulated in malignant cells compared to healthy tissues. On the other hand, it has been shown that NK-1R antagonists could interact with the attachment of neuropeptide SP to NK-1R. Indeed, NK-1R antagonists cause suppression of tumor cell growth and apoptosis. The primary anti-tumor activity of these antagonists (e.g., suppression of the migration of tumor cells and neoangiogenesis) happens via the NK-1R. One of the NK-1R antagonists is aprepitant/fosaprepitant, which has antiemetic effects following chemotherapy or surgical procedures (Muñoz et al. , 2019). Our previous studies have shown that aprepitant could induce anti-cancer effects against squamous cell carcinoma (Javid et al. , 2020c). Altogether the NK-1R is a therapeutic target for cancer, and its antagonists may be conceived as an expansive range of anti-tumor medications for cancer therapy.

Another critical fact to remember is that following the NK-1R attachment, the SP regulates cancer cell proliferation, induces an antiapoptotic effect, initiates invasion/metastasis/migration, and activates neo-angiogenesis of endothelial cells (Esteban et al. , 2006, Munoz and Covenas, 2013). Therefore, in the field of GBM treatment, the potential roles of the SP/NK-1R system seem to be fundamental, and NK-1R antagonists could potentially be a unique class of redesigned anti-GBM medications.

3. Role of NK-1R antagonists in GBM

As discussed in the introduction section, GBM is the most malignant type of primary brain tumor with a poor prognosis (Sharifzad et al. , 2020). The standard treatment is a multi-modality approach that requires complete/adequate surgical resection, concurrent irradiation, and chemotherapy (Mollazadeh et al. , 2020). Regrettably, the mean survival time following the diagnosis of GBM is 15 months, despite this multi-modal intensive therapy; therefore, new therapeutic agents involving specific mechanisms are urgently needed (Jayabalan et al. , 2020). In brain tumors, neuropeptide SP is overexpressed alongside the primary receptor NK-1R and is a major player in inflammation, progression, and metastasis. The SP and the NK-1R genes in human stem cell lines and primary stem cells are known to boost proliferative and migratory activities (Javid et al. , 2019). However, the reliable SP-mediated signaling that promotes progression of the brain tumors remains undefined.

Mechanistically, the NK-1R, as a G protein-coupled membrane receptor (GPCR), is regularly activated via GBM cells and may, therefore, be considered as a promising element in peptide-specific receptor therapy (Cordier et al. , 2016). In this regard, various hypotheses evaluate the involvement of NK-1R in the biology of GBM. It has been confirmed that NK-1R is present on several GBM cell lines, including U-251 MG, U-87 MG, DBTRG-05 MG, U373 MG, and SNB-19 (Akazawa et al. , 2009b, Muñoz and Coveñas, 2019c).

NK-1R agonists have been shown to facilitate GBM cell proliferation, migration, and cytokine secretions, whereas GBM cell development was effectively inhibited *in vitro* and *in vivo* by NK-1R antagonists. Park *et al.* have shown that in U373 MG cells, the molecular activation of NK-1R enhances mitogenesis, cell growth, and secretion of interleukin-6 (IL-6) (Park et al. , 2008). A pathological production of original signal transduction pathways throughout reactive astrocytes may be due to SP's role in GBM. SP actively triggers phospholipase C, which induces the release of IL-6 and prostaglandin E2 from human fetal astrocytes in culture, and there is evidence that reactive proliferating astrocytes upregulate this receptor (Melani et al. , 2019).

Consistently, it has been shown that SP, as a neuro-immune mediator, upregulates the activation of the transcription factor nuclear factor-kappa B (NF-κB) and κB-dependent gene expression (Lieb et al. , 1997). In a phase I clinical trial study involving recurring GBM, it has been shown that local intratumor injection of radiolabeled DOTAGA–SP inhibits significant additional growth, leading to a radionecrotic tumor transformation, with low toxicity (Cordier et al. , 2010).

Initially identified for the negative control of GPCR, β-Arrestins (ARRBs, like ARRB1 and ARRB2) were used to serve as scaffold proteins and as adapters to regulate intracellular signal transduction (Song et al. , 2018). However, recent studies have shown that ARRBs can mediate different cell signaling mechanisms independent of the G protein (Ghali and Ghali, 2020). Their function is binding to specific kinases and proteins, including mitogen-activated protein kinase (MAPK), mouse double minute 2 homolog (MDM2), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt), and NF-κB (McDonald et al. , 2000, Miller and Lefkowitz, 2001). Numerous studies have shown that ARRB1 deficiency could increase the sensitivity of GBM cells to the treatment of NK-1R antagonists (Lan et al. , 2017). It has been demonstrated that the ARRB1-mediated signaling pathway is vital for NK-1-mediated GBM cell

proliferation (Sobolesky and Moussa, 2013). In line with this, the knockdown of ARRB1 significantly inhibited the proliferation of cancer cells. It caused the arrest of cycle phase G2/M, inducing apoptosis, as revealed by Zhang *et al*. Besides, the transcription of both NF-κB and AP-1, which are involved in cyclin B1, has been controlled by ARRB1-mediated extracellular signal-regulated kinase (ERK)1/2 and Akt phosphorylation (Zhang et al. , 2017). These findings indicate that ARRB1 plays a crucial role in NK-1R-intervened cell proliferation and cell cycle in GBM cells.

Recently, Akazawa *et al.* have reported that in human GBM cells, NK-1R activation increases the phosphorylation and activity of Akt, a serine-threonine protein kinase triggered by PI3K and consequently inhibits apoptosis (Cherry and Stella, 2014). NK-1R has been involved in these regulatory pathways to boost the synthesis of DNA and cytokine secretion and to mediate the activity against apoptosis, suggesting that NK-1R is a potential regulator of human GBM cell apoptosis (Alifieris and Trafalis, 2015). A sequence of sequential phases is involved in the GBM invasion process (Vollmann-Zwerenz et al. , 2020). Due to their function in degrading local extracellular matrices, matrix metalloproteinases (MMPs) play a crucial role in this process, enabling tumor cells to infiltrate into surrounding tissues (Kwiatkowska and Symons, 2020). Specific MMPs like MMP-2 and MMP-9 have been directly linked to the progression and malignancy of GBM (Goranci-Buzhala et al. , 2020). These enzymes are tightly controlled at various levels, such as gene expression, by their unique inhibitors of active enzymes (metalloproteinase tissue [MT] inhibitors). Mou *et al.* have found that NK-1R directly mediates GBM cell migration by up-regulation of MMP-2 and MT1-MMP (Mou et al., 2013). Nowadays, it has been discovered that NK-1R has an essential role in the pathogenesis of GBM. Hence, agents that act as antagonists of these receptors will be beneficial for the treatment of GBM (Muñoz and Coveñas, 2019a). In the next sections, we have reviewed some

promising chemical products that were used to block NK-1R, both preclinical and clinical studies.

3.1. *Preclinical studies*

3.1.1. L-733,060 and L-732,138

L-733,060 is a unique, intense, and long-acting non-peptide tachykinin NK-1R antagonist, demonstrating a high affinity for the human NK-1R *in vitro* (Baker, 1994). The administration of L-733,060 results in pain relief and has antidepressant effects. Also, L-733,060 has been used to treat anxiety, depression, and hepatotoxicity in animal studies (Bang et al. , 2003, Munoz et al. , 2005b).

In vitro models, L-733,060 inhibits the migration activity of SP in tumor cells, exerting anti-tumor effects against human GBM, neuroblastoma, retinoblastoma, and melanoma (Lang and Drell, 2004, Muñoz et al. , 2006, Munoz et al. , 2005a). Consistently, Muñoz *et al.* performed an *in vitro* study to explore the capability of SP as a trigger for tumor cell growth and inhibitory effect of L-733,060 in the GAMG GBM (IC_{50} 21 μM) and SKN-BE2 neuroblastoma cells. This study indicated that SP has a mitogen activity, and L-733,060 has an antitumoral action on these cancer cells by triggering the SP/NK-1R system (Muñoz et al., 2005). Akazawa *et al.* have also demonstrated that blockage of NK-1R in human GBM cells with L-733,060 activates caspase-3 cleavage and proteolysis of poly (ADP-ribose) polymerase, proposing that NK-1R plays a significant role in GBM cells apoptosis (Akazawa et al., 2009a).

L-732,138 is a specific and competitive NK-1R antagonist. It is more potent for cloned human NK-1R (almost 1,000-fold) than cloned human NK-2R and NK-3R, and also more potent (200-fold) for human in comparison with rat NK-1Rs (Munoz et al. , 2007). The administration of L-732,138 partially reverses the cell proliferation caused by exogenous SP. In this regard, L-732,138 suppressed the attachment of SP to the cloned human NK-1R expressed in Chinese hamster ovarian cells at 2.3 nM concentration (Cascieri et al. , 1994). It has been shown that

the administration of L-732,138 suppresses hyperalgesia (Cahill and Coderre, 2002). Also, Muñoz *et al.* have been demonstrated that the tryptophan-based antagonist L-732,138 has an antagonizing effect against the GAMG GBM cell line, with an IC_{50} of 48 μM (Muñoz et al. , 2010a).

3.1.2. FK-888 and MEN 11467

FK-888 is a selective and potent antagonist for NK-1R (Hirayama et al. , 1993), which is active both *in vitro* and *in vivo*. FK-888 could inhibit the SP-induced contraction of the isolated guinea-pig trachea (IC_{50} 32 nM) and mitigate SP-induced airway constriction *in vivo* (Aramori et al. , 1994). MEN 11467 is a potent selective and orally- effective peptidomimetic tachykinin NK-1R antagonist. It has been recently shown that MEN 11467 potently inhibits the binding of SP to NK-1R in the IM9 lymphoblastoid cells (Cirillo et al. , 2001).

Recently, MEN 11467 and FK-888 have been studied in U373 MG human astrocytoma cells. These antagonists exert a strong suppression of SP-induced physiological reactions, such as aggregation of inositol monophosphate and IL-6 discharge. Nevertheless, because of their strong sensitivity to human tachykinin NK-1R, MEN 11467 demonstrated a higher capacity to suppress functional responses than FK-888. These results suggest that the slight discrepancies in the chemical compound of MEN 11467 and FK-888 establish the specific binding properties of the NK-1R and are answerable for the more noteworthy intensity of MEN 11467 to inhibit functional reactions in GBM (Palma et al. , 1999a).

Palma *et al.* have indicated the immunoreactivity features of SP in the U373 MG GBM cell line. Long term use of subcutaneous NK-1R antagonists, MEN 11149 and MEN 11467, which have a strong affinity to humans but not precisely for murine receptors, suppressed the growth of U373 MG cell lines xenograft for at least six weeks. Interestingly, the administration of NK-1R antagonists (both subcutaneously and intravenously) has been successful in preventing tumor development for around ten days after the last administration (Palma, 2006).

3.1.3. Aprepitant

The small molecule aprepitant (L-754,030, MK-869, Emend®) has various pharmacological effects depending on its concentration, antagonizing the human SP/NK-1R. In clinical pharmacology, aprepitant decreases nausea and chemotherapy-induced vomiting at low doses (Mohammadi et al., 2020). Aprepitant, a lipid-soluble agent, readily crosses the blood-brain barrier (BBB), reaching a high concentration in the CNS. In addition to aprepitant, its intravenous pro-drug fosaprepitant (L-758,298, MK- 0517, Ivemend®) and, lately, rolapitant (Varubi®) have been licensed as a therapy for nausea and vomiting in humans (Muñoz and Coveñas, 2013).

Aprepitant has been used as an anxiolytic, antidepressant, and antiemetic agent at medium doses. Interestingly, higher doses of NK-1R-antagonist aprepitant contribute to robust tumor growth inhibition and apoptosis in experimentally *in vitro* and *in vivo*, including hepatoblastoma, melanoma, and colon cancer (Berger et al. , 2014, Munoz et al. , 2010). Recently, Harford-Wright *et al.* utilized an *in vivo* model to describe the function of SP in the pathogenesis of brain tumors. Also, it has been shown in an *in vivo* preclinical study with an experimental brain tumor model enhances the peritumor edema of brain cancer by using aprepitant (3 mg/kg/day). The intravenous administration of aprepitant has also been shown to decrease tumor volume and cellular growth, displaying that SP can play a role in brain pathophysiology (Harford-Wright et al. , 2014). Aprepitant (5–70 μM) has been shown to induce a concentration-dependent GBM cell growth inhibition (GAMG, IC_{50} 33 μM). Muñoz *et al.,* in their *in vitro* study, have demonstrated that aprepitant is a novel and potentially effective anti-tumor medication in the treatment of GBM with an IC_{50} of 33.1 μM and an IC_{100} of 66.2 μM (Muñoz and Rosso, 2010). It has been shown that combined therapy with aprepitant and maraviroc is expected to exert synergistic inhibition of growth-enhancing signaling in GBM (Kast and therapeutics, 2010). In the study by Kast *et al.*, four antiviral drugs, cidofovir,

293 acyclovir, maraviroc, ritonavir, and aprepitant, have been investigated for the treatment of
294 GBM. In this study, cytotoxic effects of aprepitant (7%) in GAMG GBM cells were lower than
295 a combination of TMZ + aprepitant (19%), suggesting that this combination has beneficial anti-
296 cancer effects in the treatment of GBM (Kast et al. , 2016).

297 ***3.1.4. Cyclosporin A***

298 Cyclosporin A (CsA), an immunosuppressive agent, is a tachykinin receptor antagonist,
299 demonstrating significant sensitivity for both NK-1R and NK-2R (Lavagno et al. , 2001). It has
300 been shown that CsA inhibits the growth of C6 GBM cells and initiates apoptosis via activation
301 of caspase-3 and DNA fragmentation. CsA treatment results in increased levels of p53 proteins
302 and the transcriptional activation of p53-dependent genes in C6 GBM cells containing a wild-
303 type p53 tumor-suppressor (Han et al. , 2010, Mosieniak et al. , 1997). Interestingly, in a study
304 done by Zupanska *et al.*, CsA induced a growth arrest or programmed cell death of T98G cells
305 independently on their p53 status. The U-87 MG cells did not experience apoptosis following
306 the administration of CsA, except an arrest in G1 of the cell cycle, indicating the β-
307 galactosidase-associated activity of the senescent cells (Zupanska et al. , 2005).

308 Also, Muñoz *et al.* performed an *in vitro* growth-inhibiting analysis of CsA against all of seven
309 human tumor cell lines, including GAMG, WERI-Rb-1 retinoblastoma, SKN-BE2
310 neuroblastoma, CAPAN pancreas carcinoma, HEp-2 larynx carcinoma, 23132/87 gastric
311 carcinomas, and SW-403 colon carcinoma. They demonstrated that CsA has a broad spectrum
312 of anti-tumor functions. CsA blocked these cancer cells' proliferation at μM concentrations; the
313 suppression appeared on a dose-dependent basis. Besides, CsA inhibits SP-induced mitogen
314 activation of cancer cells, indicating that NK-1R is active in this process. Upon administration
315 of CsA, apoptosis of these seven cancer cell lines, including GBM, was detected. Such results
316 indicate that the anti-cancer activity of CsA is at least attributed to the NK-1R antagonist

317 pharmacological mechanism. Simultaneously, the presence of NK-2R in this action must not
318 be ignored; altogether, CsA seems to have wide-spectrum anti-cancer effects (Muñoz et al. ,
319 2010b).

320 ***3.1.5. Ketamine***

321 Astrocytes have now been reported to be used as essential modulators for neuronal synaptic
322 transmission. Astrocytic activities have been inhibited by general anesthetics, particularly
323 ketamine (an intravenous anesthetic). Zhang *et al.* have observed that ketamine blocks
324 glutamate transmission from astrocytes to neurons within the therapeutic concentrations and is
325 presumably regulated by the extrasynaptic activation of the GluN1/GluN2B receptors (Zhang
326 et al. , 2019). Recently, it has been proved that ketamine acts as a competitive antagonist of the
327 N-methyl-D-aspartate (NMDA) excitatory neurotransmission receptor and also antagonizes the
328 NK-1R by interacting with the SP attachment (Okamoto et al. , 2003).

329 Numerous studies have indicated that ketamine exerts potential pharmacological effects,
330 including apoptosis and autophagy induction, attenuation of PI3K/Akt/mammalian target of
331 rapamycin (mTOR), increase in eNOS gene expression, and blockage of NMDA receptor
332 against various tumoral cell lines (Duan et al. , 2019, Wang et al. , 2017, Yuhas et al. , 2017,
333 Zhao et al. , 2019, Zhou et al. , 2018). As mentioned earlier, SP was shown to cause the release
334 of pro-inflammatory cytokines mediated by the NK-1R in the pathogenesis of many human
335 brain disorders (Martinez et al. , 2016). Recently, Yamaguchi *et al.* have examined the anti-
336 inflammatory impact of ketamine on the SP-induced NK-1R activation in the human U373 MG
337 GBM cells. The findings of their studies revealed that ketamine inhibited the synthesis and
338 secretion of IL-6 and IL-8 in the U373 MG cells and SP-induced ERK1/2, p38 MAPK, and
339 NF-κB activation, exerting anti-inflammatory effects (Yamaguchi et al. , 2017).

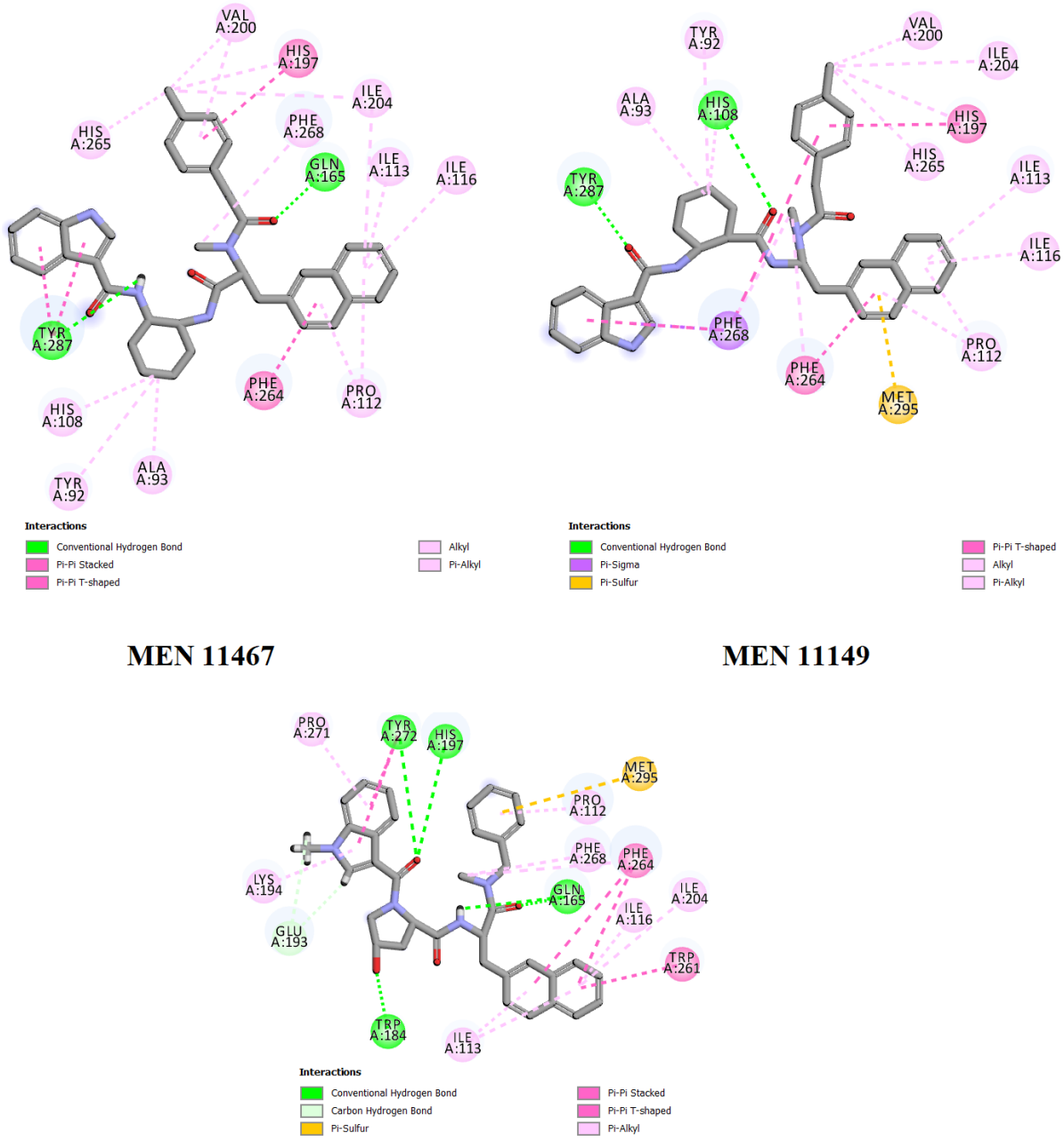
340 ## 4. Molecular docking study

Notably, in this review, a molecular docking study was performed using the Genetic Optimization for Ligand Docking (GOLD) Suite version 5.2.2 software. The outcomes revealed binding free energies (Chemscore.dG) for MEN 11467 (−58.21 kJ/mol), MEN 11149 (−53.38 kJ/mol), and FK-888 (−52.98 kJ/mol) into the active site of NK-1R, indicating that the inhibition of NK-1R activity by these compounds could be more promising for future directions. For the molecular docking study, the co-crystal structure of the target protein NK-1R (Protein Data Bank [PDB] ID: 6HLP) in complex with its co-crystallized inhibitor was obtained from the PDB (https://www.rcsb.org) (Yau et al. , 2019).

As shown in Table 2 and Figs. 2 and 3, we have summarized the binding free energies (Chemscore.dG) of the mentioned chemical compounds.

Table 2. The ChemScore.dG function of the NK-1R antagonists.

Compounds	ChemScore.DG
MEN 11467	-58.21
MEN 11149	-53.38
FK-888	-52.98
Aprepitant	-32.28
L-732,138	-30.56

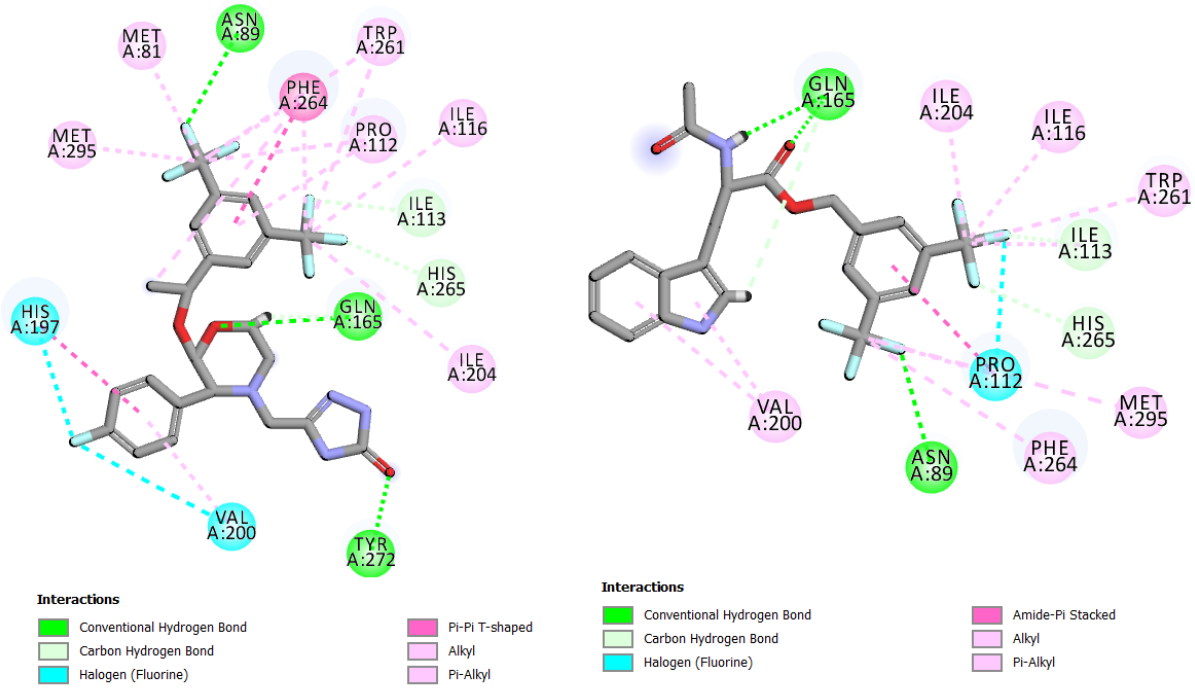


353

354 **Figure 2.** Molecular docking of MEN 11467, MEN 11149, and FK-888 in the binding pocket of NK-1R

355 (PDB:6HLP).

356



Aprepitant **L-732,138**

357

358 **Figure 3.** Molecular docking of aprepitant and L-732,138 in the binding pocket of NK-1R (PDB:6HLP).

359

5. Concluding remarks and future directions

According to the molecular heterogeneity of GBM, current approaches targeting oncogenic mechanisms have faced limited effectiveness, showing a dismal OS in affected patients. The existing chemotherapy could also be the source of drug resistance in GBM therapy, as the cell metabolism and signaling process are significantly destabilized. In this review, we have investigated several studies on chemical compounds targeting NK-1R, showing substantial improvements in the treatment of GBM both *in vitro* and *in vivo*.

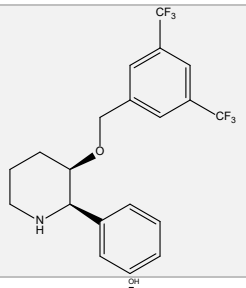
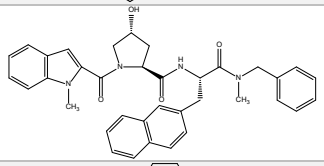
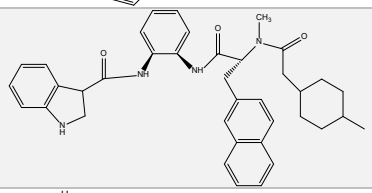
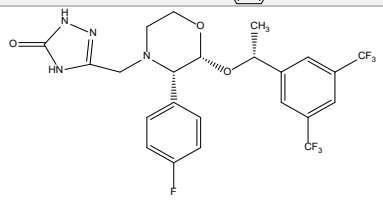
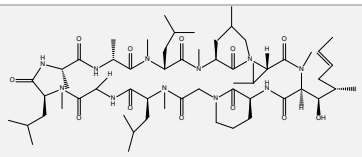
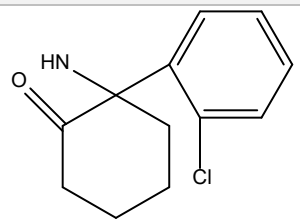
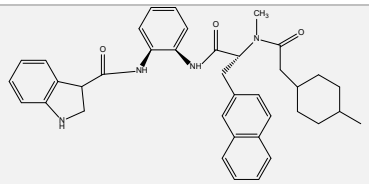
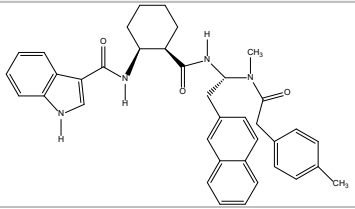
The potential clinical benefit of NK1R antagonists for a wide variety of pathological disorders, including pain, anxiety, arthritis, asthma, emesis, migraine, and schizophrenia, have previously been described. In this regard, the use of NK-R antagonists against cancer has to date been a focus of only a few clinical studies. Recently, a CUSP9 strategy repurposed drugs, including aprepitant, auranofin, captopril, celecoxib, disulfiram, itraconazole, minocycline, quetiapine, and sertraline combined with TMZ, have preclinical data demonstrating potential anti-GBM effects, *in vitro* (patient-derived glioblastoma stem cell [GSC]). CUSP9 was shown to have a mixed impact compared with individual pharmaceuticals, and 50% of GSC cultures were particularly susceptible to the therapeutic combination. Interestingly, the benefit of CUSP9 with TMZ was higher than TMZ monotherapy in clinical plasma concentrations (Halatsch et al. , 2018, Skaga et al. , 2019).

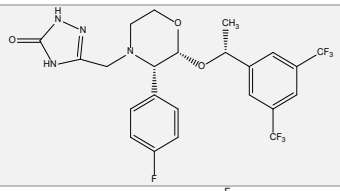
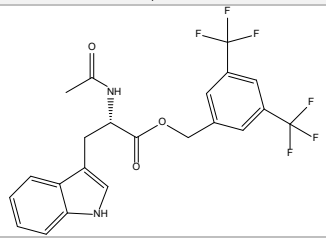
Interestingly, some promising traditional therapies, which modulate SP/NK-1R system, might be more productive. In this regard, the Japanese herbal medicine, *Yokukansan*, has an anti-inflammatory effect, suppressing the production of IL-6 and IL-9 induced by SP and reducing COX-2 expression in U373 MG GBM cells, apparently by inhibiting signals, including p38 MAPK, ERK1/2 and NF-κB activation (Yamaguchi et al. , 2020).

Collectively, according to the data reported in the previous sections, Table 3 shows the key-points of potential chemical compounds in future GBM studies. The present findings indicate that NK-1R is a novel therapeutic target in treating GBM because NK-1R antagonists provide anti-tumor activities, such as antiproliferative, apoptosis-inducing, and tumor volume reduction against GBM cells. Besides, NK-1R antagonists like ketamine modify the activity of some molecular signaling pathways, including NF-κB, p38 MAPK, and ILs. This suggests that NK-1R antagonists could exert a dual anti-tumor action in GBM, including antiproliferative and anti-inflammation, thereby SP/NK-1R system plays a vital role in the tumor progression.

More interestingly, based on our molecular docking study, MEN 11467, MEN 11149, and FK-888 have shown potential affinity in the binding pocket of NK-1R; therefore, these chemical compounds could be more beneficial as NK-1R inhibitors. Based on these data, a clinical trial should be conducted to analyze the anti-tumor actions of these potential compounds given in GBM patients pre- and post-surgery. In summary, NK-1R is a new marker and a critical target for the treatment of GBM, showing that NK-1R antagonists could exert anti-tumor actions against GBM. The studies, as mentioned above, suggest the anti-tumor potential of the chemical compounds as well as their possibilities for an alternative approach to GBM treatment (Fig. 4).

Table 1. Potential effects of NK-1R antagonists in GBM, *in vitro,* and *in vivo*.

Drugs	Chemical structure	Type of study	Main mechanism (s)	Reference (s)
L-733,060		*In vitro*	- Induction of apoptosis - A decrease in Tumor volume/size - Inhibits the migration activity of SP in tumor cells	(Akazawa et al., 2009a, Muñoz et al., 2015, Muñoz et al., 2005)
FK888		*In vitro*	- Full and robust suppression of SP induced physiological reactions - Inhibition of functional responses of GBM cells	(Hirayama et al., 1993, Palma et al., 1999a)
MEN 11467		*In vitro*	- Blocking all reinforcing activity of SP/NK-A on NK-1R+glioma cell lines	(Palma et al. , 1999b)
Aprepitant		*In vitro*	- Inhibition of tumor cell growth - Suppressed the migration and proliferation of tumor cell - Induction of apoptosis	(Dikmen, 2016, Kast et al., 2016, Muñoz and Coveñas, 2013, Muñoz and Rosso, 2010)
Cyclosporin A		*In vitro*	- Suppression of cell growth in human tumor cells - Inhibition of SP-induced mitogen activation of cancer cells - Induction of apoptosis	(Muñoz et al., 2010b)
Ketamine		*In vitro*	- Preventing the attachment of SP to NK-1R - Inhibiting the synthesis of IL-6 and IL-8 by U373 MG cells - Modifying SP-induced inflammatory responses in U373 MG cells by suppressing the signaling molecules (NF-κB, p38 MAPK, and ERK1/2)	(Yamaguchi et al., 2017)
MEN 11467		*In vivo*	- Preventing SP binding to tachykinin NK-1 locales - Strongly antagonizing tachykinin and antigen-intervened inflammatory reactions of the respiratory system	(Cirillo et al. , 1998, Cutrufo et al. , 1999, Palma et al. , 2000)
MEN 11149		*In vivo*	- Suppressing the development of U373 MG cell lines xenograft for at least six weeks - Preventing tumor growth for around ten days since the last usage	(Palma, 2006)

Aprepitant		*In vivo*	- A decrease in tumor volume/size	(Harford-Wright et al., 2014)
L-732,138		*In vivo*	- Suppressing the attachment of SP	(Muñoz et al., 2010a)

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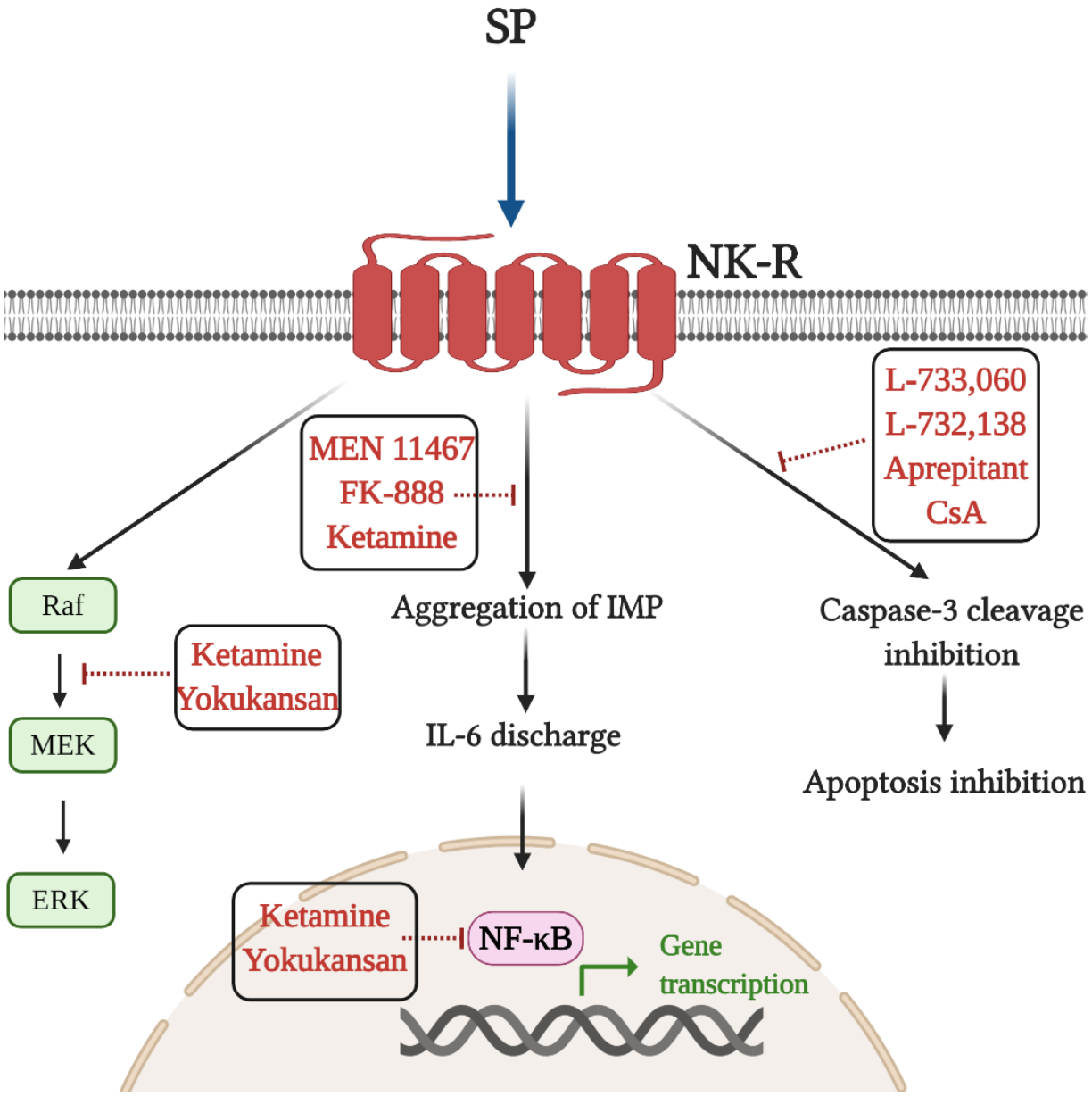

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404 **Figure 4.** The schematic presentation of the SP/NK-1R system regulating multiple signaling pathways involved

405 in GBM progression.

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