

Review

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Review

# Strategies to Modulate Mast Cell Activation for Cancer Immunotherapy

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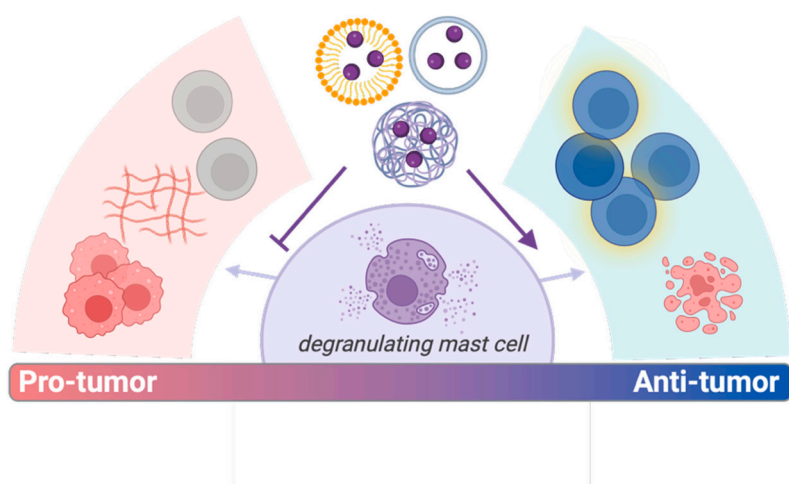
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## Abstract

Mast cells (MCs) are an emerging target in cancer immunotherapy due to their phenotypic plasticity, immunomodulatory capacity, and active recruitment into the tumor microenvironment (TME). While MCs can adopt both pro- and anti-tumor phenotypes, recent evidence suggests that tumors exploit this plasticity by recruiting immature MC progenitors and conditioning them toward tumor-supportive functions. Therapeutic strategies aimed at reprogramming MCs into anti-tumor effectors—rather than depleting them—are gaining traction. However, many of the most promising modulatory agents, including cationic peptides and small-molecule agonists, face significant delivery barriers due to poor systemic stability, off-target effects, and limited tumor penetration. Drug delivery systems (DDSs), particularly nanomedicine platforms, offer a powerful solution by enabling targeted, controlled, and localized delivery of these agents to MCs within the TME. In this review, we highlight the rationale for MC-targeted immunotherapy, evaluate current strategies for phenotypic modulation, and emphasize the critical role of DDSs in overcoming translational barriers. By integrating MC biology with advanced delivery technologies, we outline a path forward for harnessing this underexplored immune population in cancer treatment.

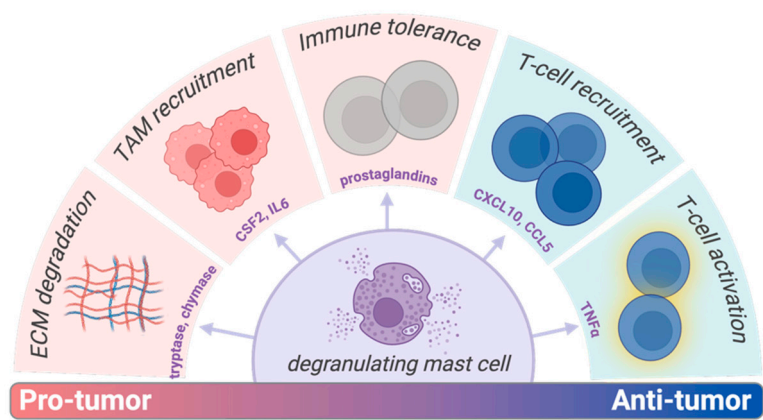
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## Graphical Abstract:



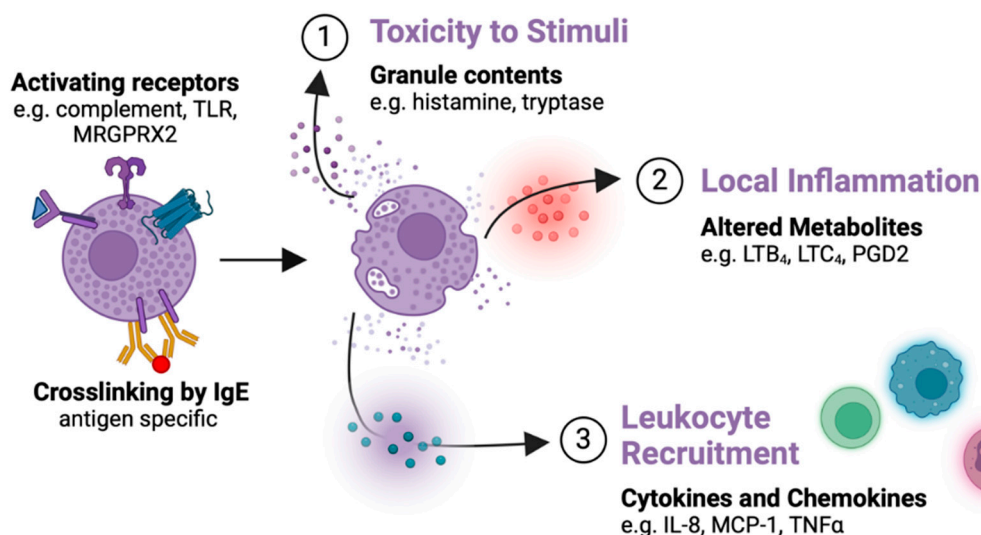
1. Introduction

Immunotherapy, including immune checkpoint blockade, chimeric antigen receptor T-cell therapy, and cancer vaccines, is increasingly becoming the frontline treatment for several cancers and has revolutionized cancer care. One emerging target of interest for tumor immunotherapy is the mast cell (MC). MCs are granulocytes that have been implicated in rapid, robust, and sustained inflammatory responses in the body (e.g., anaphylaxis), but primarily have roles in immunity against infectious pathogens [1,2]. The presence of MCs in various tumors has long been recognized and is closely associated with cancer prognosis. While increased MC density correlates with favorable outcomes in some cancers (e.g., gliomas, colorectal, ovarian), it is associated with poor prognosis in others (e.g., melanoma, thyroid, gastric) [3–16]. Additional conflicting results arise in breast and prostate cancer, where outcomes vary depending on MC location within the tumor [17–20]. Further, MC activation status has corresponded with both improved and worsened prognosis, sometimes within the same disease [7–10]. These mixed and sometimes even contradictory results highlight the dual role MCs play in cancer and hints at their immunotherapeutic potential (Figure 1).



**Figure 1.** Varied pro- and anti-tumor effects of mast cells within the tumor microenvironment (TME). The effector molecule is labeled in purple at the bottom of the wedge and the resultant immune effect in grey at the top. Chemokine ligand 5 (CCL5); colony-stimulating factor 2 (CSF2); C-X-C motif chemokine ligand 10 (CXCL10); extracellular matrix (ECM); tumor-associated macrophages (TAM); tumor necrosis factor (TNF); toll-like receptor (TLR).

MCs are often understudied in cancer research, partly because their activation is primarily associated with Type I hypersensitivity reactions, such as anaphylaxis. Anaphylaxis is typically triggered by antigen-specific IgE binding to the high-affinity IgE receptor (FcεRI) receptor on MCs. Upon exposure to an anaphylactic inducing antigen (e.g., peanut Ara h or shellfish tropomyosin), IgE crosslinking activates MCs, leading to rapid systemic inflammation and classic anaphylactic symptoms [21,22]. However, non-IgE activation mechanisms exist, mediated through receptors such as complement receptors (C3a/C5a), the Mas-related G protein receptor-X2 (MRGPRX2), or toll-like receptors (TLR) 2 and 4 [23–25]. Upon activation, MCs initiate a triphasic response designed to induce immediate toxicity, signal nearby immune cells, and recruit specialized immune populations. MCs secrete pre-synthesized granule contents, immunomodulatory lipid mediators, and chemokines and cytokines in each phase respectively [22] (Figure 2). The sum contribution of MC secreted factors within the tumor microenvironment (TME) defines whether its phenotype is pro- or anti-tumor.



**Figure 2.** Sequentially labeled mast cell activation events with their subsequent immune effects. Leukotriene C<sub>4</sub> and B<sub>4</sub> (LTC<sub>4</sub>, LTB<sub>4</sub>); monocyte chemoattractant protein-1 (MCP-1); mas-related G protein receptor-X2 (MRGPRX2); prostaglandin D<sub>2</sub> (PGD<sub>2</sub>); tumor necrosis factor (TNF); toll-like receptor (TLR).

The systemic dissemination of chemokines and cytokines is critical to MC homeostatic functioning and its immunotherapeutic potential as this ultimately initiates formation of an adaptive immune response [26]. For example, TNF- $\alpha$  released by MCs recruits adaptive immune populations to lymph nodes and enhances T-cell activation [27,28]. This functionality is facilitated by MCs localizing around blood vessels at host-environment interfaces [29]. Their perivascular location allows cytokines and chemokines to rapidly enter systemic circulation. An additional aspect that makes MCs uniquely intriguing for immunotherapy is that they leave the bone marrow as progenitors and mature within their final tissue of residence. MCs are recruited to the airways, intestinal linings, skin, and throughout regions of the brain where they undergo tissue-specific maturation shaped by local cytokines, extracellular matrix (ECM), and matrix metalloproteases (MMPs) [30]. This leads to a remarkably plastic and heterogeneous cellular population with distinct, tissue-dependent transcriptional programs [31] and resulting phenotypes [32,33]. Despite their heterogeneity, MCs are broadly classified in humans by protease content into MC<sub>T</sub> (tryptase-only) and MC<sub>CT</sub> (containing tryptase, chymase, carboxypeptidase, and cathepsin G) [34–36]. Although the full extent of their phenotypic flexibility is still being explored, MCs are increasingly recognized as dynamic players with therapeutic relevance in modulating the TME.

Strikingly, there is sufficient evidence to suggest that tumors are actively recruiting these immature MC progenitors to the TME. Stem cell factor (SCF) is a key growth factor for MC maturation and differentiation; however, it has been shown to recruit MCs in various cancers, including glioblastoma, hepatocellular carcinoma, and breast cancer [37–39]. Further, SCF and MCs have been implicated in promoting metastasis in breast cancer [40]. Several chemokines have been shown in tumor-mediated MC recruitment. CXCL12 colocalizes with CXCR4<sup>+</sup> MCs in human glioblastoma [39], and inhibition of CXCR4 prevents MC migration towards pancreatic adenocarcinoma [41]. Similarly, CCL5 colocalizes with MCs in uterine cancer, and lymphoma-secreted CCL5 causes *in vitro* MC chemotaxis [42,43]. There is significant evidence that MC progenitors are broadly recruited to the TME, where, upon arrival, they can be conditioned to adopt pro- and anti-tumor-supportive functions.

The role of MCs in the TME has been heavily reviewed [36,44–46]. Within the context of cancer, MCs are thought to play a dual role – being both pro- and anti-tumor depending on the environment. Their pro-tumor functions can be summarized as promoting angiogenesis, degrading the ECM, and engaging in immunosuppression [44]. These effects are mediated by vasoactive factors such as



vascular endothelial growth factor (VEGF) and platelet-derived growth factor- $\beta$  (PDGF- $\beta$ ), proteases including tryptase or chymase, and matrix metalloproteinases like matrix metalloprotein-9 (MMP-9), as well as inhibitory cytokines such as IL-10 and TGF- $\beta$  [47]. MC anti-tumor functions are in direct cytotoxicity, immune cell recruitment, and immune cell activation. These effects are mediated by factors like TNF- $\alpha$ , CCL5, and IL-6, respectively. The role of MCs in tumors is complex, and numerous MC modulating agents have been developed and tested for their anti-tumor potential. To improve delivery and efficacy, a few of these compounds have been formulated using drug delivery systems (DDSs). This review will examine MC modulators that have been evaluated for their activity in cancer.

**Table 1.** A series of polycationic peptides derived from, or derivatives of wasp venom. Mastoparan-L (M-L).

PEPTIDE	SEQUENCE	ORIGIN	REF
Mastoparan-L	INLKALAALAKKIL-NH <sub>2</sub>	<i>Vespa lewisii</i>	[48]
Mastoparan-X	INWKGIAAMAKKLL- NH <sub>2</sub>	<i>Vespa xanthoptera</i>	[49]
Mastoparan-C	LNLKALLAVAKKIL-NH <sub>2</sub>	<i>Vespa crabro</i>	[50]
Polybia-MPI	IDWKLLDAAKQIL-NH <sub>2</sub>	<i>Polybia paulista</i>	[51]
KM8	KLLKKNLKALAALAKKIL-NH <sub>2</sub>	M-L analog	[52]
[I <sup>5</sup> , R <sup>8</sup> ] Mastoparan	INLKILARLAKALL-NH <sub>2</sub>	M-L analog	[53]
Mastoparan-3	NLKALAALAKKIL-NH <sub>2</sub>	M-L analog	[54]
Mastoparan-7 (M7)	INLKALAALAKALL- NH <sub>2</sub>	M-L analog	[55]
MP12W	INLKALAALAWALL-NH <sub>2</sub>	M7 analog	[56]

2. Modulating MC Activation as Cancer Immunotherapy

Anti-tumor immunotherapy strategies involving MCs focus on either inhibiting or activating their function. Approaches aimed at inhibition, such as reducing MC numbers or stabilizing them, have been studied as well as MC activation via agonism of receptors like complement receptors or TLRs. Historically, MC activation has been the most studied. Activation of MCs is achieved historically through agents such as compound 48/80 (c48/80), mastoparan, and mastoparan 7 (M7). C48/80, a p-methoxy-N-methyl phenylethylamine crosslinked by formaldehyde, is believed to activate MCs by directly stimulating Gi/o-type G proteins or by activating phospholipase D [57–61]. Mastoparan is an oligopeptide derived from wasp venom and is the parent compound for several second-generation MC agonists such as M7 and third-generation agonists MP12W [55,56,62]. Polymyxins, a class of antibiotics, have also demonstrated MC agonist activity and are considered relatively safe. Aside from mastoparan, many of these compounds have been underexplored for their anti-cancer potential [63–67].

Given that mastoparan has been the most extensively studied, particularly in cancer research, Table 1 highlights a range of wasp venom-derived or -inspired peptides that have been investigated for their MC agonism and anti-cancer potential. These peptides share a characteristic amphipathic structure and are typically used in their amidated form (-NH<sub>2</sub>) rather than the carboxylated form (-COOH), as the former has been shown to exhibit greater biological potency [68]. Native mastoparan peptides originate from various wasp species, including *Vespa lewisii* (Mastoparan-L), *V. xanthoptera* (Mastoparan-X), *V. crabro* (Mastoparan-C), and *Polybia paulista* (Polybia-MPI) [48–51]. In addition to natural variants, several synthetic analogs have been engineered to enhance efficacy, stability, or selectivity. These include KM8 (a rationally designed analog of Mastoparan-L), [I<sup>5</sup>, R<sup>8</sup>] Mastoparan (a modified version with improved immunomodulatory effects), and Mastoparan-3 (a truncated derivative retaining core activity) [51–53]. Collectively, these cationic, membrane-penetrating peptides are of significant interest for MC activation and cancer immunotherapy research due to their potential to trigger immune responses or cytotoxic effects in the TME, with the Mas-related G protein-

coupled receptor (MRGPR) family identified as their putative target [69–71]. These wasp venom-derived peptides represent a promising class of immunomodulatory agents for targeted MC activation and anti-tumor therapy.

2.1. Direct Effects of MC Modulators on Cancer Cells

Inhibition and activation of MCs can be achieved by leveraging agents that selectively induce MCs activation and exhibit cytotoxicity toward tumor cells (Table 2). Table 2 provides a comparative overview of the cytotoxic effects of various MC modulators—both inhibitors and agonists—across a range of cancer and healthy cell lines. Modulator activity is reported at the 50% inhibitory concentration (IC<sub>50</sub>) as determined through an MTT assay.

Among the MC inhibitors, cromolyn sodium exhibited selective cytotoxicity, showing stronger inhibition of colon cancer cells (HT-29, IC<sub>50</sub> = 2.33 μM) compared to healthy breast epithelial cells (MCF-10, IC<sub>50</sub> = 7.33 μM), suggesting some degree of tumor selectivity. In contrast, MC agonists such as mastoparan derivatives displayed broad-spectrum cytotoxicity across multiple tumor types. The amidated form, mastoparan-L-NH<sub>2</sub>, demonstrated substantially greater potency than its carboxylated counterpart, evidenced by IC<sub>50</sub> values for the same breast cancer lines ranged from 20–24 μM for the amidated form compared to 250–432 μM for the carboxylated version. Synthetic analogs such as KM8 exhibited the most potent cytotoxicity, with IC<sub>50</sub> values as low as 5–7 μM in breast, lung, and esophageal cancers and significantly higher IC<sub>50</sub>s in healthy cells (~45 μM), indicating improved therapeutic indices. While natural mastoparans like mastoparan-C and Polybia-MPI show promise in lung, breast, and prostate models, their moderate cytotoxicity toward healthy cells warrants further optimization. The observed variability in IC<sub>50</sub> values across cell types and peptide formulations highlights the importance of structure-activity relationships in tuning both efficacy and safety. Future development of MC-targeted therapeutics may benefit from combining potent MC agonists like KM8 with delivery systems that enhance tumor specificity and minimize systemic toxicity. These findings underscore the potential of MC modulators as targeted anti-cancer agents, particularly when modified to enhance potency and selectivity.

**Table 2.** Cytotoxicity of various peptides and molecules against a range of tumor and healthy cells. Values were identified from MTT assay after incubation for the designated time with respective compound. Glioblastoma (GBM). Time at which in vitro model was evaluated is given in hours. The 50% inhibitory concentration is listed as IC<sub>50</sub> in micromolar. NA means not available.

MC Modulator	Cancer Type	In vitro model	Time (hrs)	IC <sub>50</sub> (μM)	Ref
MC Inhibitor					
Cromolyn sodium	Colon	HT-29	72	2.33	[72]
	Healthy	MCF-10		7.33	
MC Agonist					
Mastoparan-L-COOH	Lymphoma	Jurkat	24	77.9	[73]
	GBM	U87		311.7	
	Cervical	SiHA		172.1	
	Breast	MCF-7		432.5	
		MDA-MB-231		251.25	
		SK-BR3		320.3	
	Melanoma	A2058		140	
		B16F10-Nex2		165	

MC Modulator	Cancer Type	In vitro model	Time (hrs)	IC <sub>50</sub> (μM)	Ref	
Mastoparan-L-NH <sub>2</sub>	Healthy	Melan-a	48	411.5		
		HACaT		428		
	Lung	A549		~14	[74]	
				34.3	[75]	
	Colon	HCT-116 p53 double knockout		~30-35	[76]	
		HT-29		~40-45		
	Leukemia	Jurkat		8-9.2	[77]	
		THP-1				
	Myeloma	HOPC		11		
		Breast		MDA-MB-231		20–24
	T47D					
	MDA-MB-468					
	4T1					
	SKBR3					
	MCF7					
	MCF7-TX400					
	Prostate	PC3		<50		
	Ovarian	SCOV3		25		
	Cervical	HeLa		10		
	Healthy	PBMCs		48		
	Leukemia	HL60		NA	10	[54]
	Breast	MCF-7		48	26.6	[52]
		MCF-7/Dox			27.6	
	Lung	A549			28.3	
		NCI-446			28.4	
	Esophageal	Eca109			31.9	
Healthy	LO2	53.2				
	HEK-293	51.5				
KM8	Breast	MCF-7	5.3			
		MCF-7/Dox	5.5			
	Lung	A549	6.2			
		NCI-446	6.3			
	Esophageal	Eca109	7.4			
	Healthy	LO2	45.9			
		HEK-293	45.0			
Mastoparan-C	Lung	H157	24	13.57	[78]	
	Breast	MCF-7		25.27		
	Prostate	PC-3		6.29		

MC Modulator	Cancer Type	In vitro model	Time (hrs)	IC <sub>50</sub> (μM)	Ref
	GBM	U251-MG		36.65	
	Healthy	HMEC-1		57.15	
Polybia-MPI	Prostate	PC-3		64.68	[79]
	Bladder	Biu87		52.16	
		EJ		75.51	
	Healthy	HUVEC		55.6	
	GBM	T298G	2	32.7	[80]
Mastoparan-X				18.05	
Mastoparan-3	Leukemia	HL60	NA	10	[54]
				200	
Mastoparan [I <sup>5</sup> , R <sup>8</sup> ]	Leukemia	THP-1	72	24.5	[53]
	Healthy	HEK-293		>200	

2.2. Inhibition of MC Function as Cancer Immunotherapy

In cancer immunotherapy, inhibiting MC function is commonly achieved by reducing their numbers or stabilizing their degranulation. Stabilizing degranulation can result in a reduction in the expression of MC-derived factors such as VEGF and histamine. VEGF plays a central role in angiogenesis, making it a promising target for limiting tumor vascularization and growth [81,82]. Histamine signals through four distinct receptors (H1–H4) that can be expressed on both tumor cells and various immune cells [83–85]. However, the complexity of histamine signaling poses a challenge—particularly since individual receptors can have opposing effects. For example, the H2 receptor has been shown to both promote and inhibit tumor progression, depending on the context [86,87].

Historically, reducing MC numbers has been accomplished by targeting c-KIT (CD117) which is the receptor for stem cell factor, an essential growth factor for MC survival and development [88]. One such agent is imatinib, an FDA-approved tyrosine kinase inhibitor used to treat cancers with aberrant c-KIT signaling [89,90]. However, because c-KIT is also expressed on other cell types, such as epithelial cells, imatinib can cause off-target effects. In a prostate cancer model, imatinib reduced adenocarcinoma development but unexpectedly promoted an aggressive neuroendocrine prostate cancer phenotype [91,92]. Similarly, in a triple-negative breast cancer model, imatinib increased tumor growth and induced peripheral tumor-associated clotting [93]. These findings suggest that MC depletion strategies may limiting. Given the variable outcomes of MC depletion, alternative strategies such as stabilizing MC degranulation have also been explored.

With regards to inhibiting MCs through stabilizing degranulation, inhibitory receptors like, Siglec-8 and PD-L1, can be targeted using humanized antibodies, like antolimab (anti-Siglec-8) or infliximab (anti-TNF-α) [28,94]. Blockade of MC-associated PD-L1 was shown to increase T-cell infiltration and activation products in a gastric cancer model [95]. However, these strategies are rarely specific to MCs, as the targeted receptors—such as Siglec-8, which is also expressed on eosinophils and basophils, and PD-L1, which is found on antigen-presenting cells, T cells, B cells, and various other cell types—are broadly expressed. In place of inhibitory antibodies, primarily two small molecules have been used in the context of cancer to stabilize MCs: ketotifen and cromolyn sodium (CS).

Ketotifen is a non-competitive antagonist of H1 receptors that prevents MC degranulation [96,97]. Interestingly, it was able to prolong survival and suppress metastasis formation of



neuroendocrine prostate cancer [98]. This is an aggressive prostate cancer subtype promoted by c-KIT targeting, suggesting MCs in prostate cancer can be protective and deleterious depending on the subtype. These findings are supported by contradictory roles of MCs on tumor growth in prostate cancer depending on peri- vs intratumoral localization [17,92]. Cromolyn sodium, an FDA-approved MC stabilizer used for asthma, allergic rhinitis, and mastocytosis, has shown promising preclinical anti-tumor effects in several models, including thyroid and pancreatic cancers (Table 2) [12,99–101]. In a thyroid cancer xenograft model, Melillo et al. demonstrated that co-delivery of exogenous MCs with cromolyn significantly reduced tumor growth, accompanied by a decrease in proliferating (Ki67+) cells, compared to delivery of MCs alone [12]. Similarly, in a Myc-driven  $\beta$ -cell carcinoma model, Soucek et al. found that cromolyn halted tumor expansion and induced widespread cell death in established islet tumors, indicating a critical role for MCs in pancreatic tumor maintenance [101]. In contrast, Aliabadi et al. reported only a non-significant reduction in subcutaneous colon tumor mass using a higher cromolyn dose (50 mg/kg) from the previous study, suggesting tumor-type or model specific differences in MC involvement [72]. Together, these findings underscore the nuanced therapeutic role of MCs in tumor treatment and highlight the potential and limitations of both depletion and stabilization strategies.

**Table 3.** Relevant preclinical model details and outcomes of soluble delivery of MC-modulating agents. Tumor size was determined via a web-based data digitizer. Daily (qd), every two days (q2d), intraperitoneal (IP), intravenous (IV), not significant (n.s.), and subcutaneous (subQ).

MC AGONIST	CANCE R	IN VIVO MODEL	DOSE	DOSIN G	CONCLUSIONS	REF
Cromolyn sodium	Thyroid	8505-C subQ in BALB/c-nu	IP 10 mg/kg	qd	Co-injection of MCs with tumor resulted in accelerated tumor growth, treatment with cromolyn reversed this	[12]
	Pancreati c	<i>pIns- mycER<sup>TAM</sup>;RI P7-bcl- xl</i> transgenic	IP 10 mg/kg	qd	Induced apoptosis of pre-existing $\beta$ - cell tumors; MCs critical for tumor expansion	[101 ]
	Colon	CT-26 subQ in BALB/c	IP 50 mg/kg	q2d	Non-significant reduction in tumor weight and after survival change	[72]
Mastoparan	Melano ma	B16F10-Nex2 subQ in C57BL/6	Peritumor al 5 mg/kg	qd 5x	70.29% growth inhibition rate 28.26% prolonged survival ratio	[73]
	Breast	4T1 orthotopic in BALB/c	IP 6 mg/kg	q2d	Non-significant reduction in tumor growth.	[68]

					significant reduction when combined with gemcitabine		
		MCF-7/Dox in BALB/c-nu	IV 10 μmol/kg	q2d 7x	33.6% growth inhibition rate	No signs of damage by histology or blood chemistry	[52]
KM8					81.6% growth inhibition rate		

2.3. Activation of MCs as Cancer Immunotherapy

While a limited number of cancer therapies focus on inhibiting MCs, a growing number of studies instead aim to activate them. Once activated, MCs have been shown to have direct cytotoxic effects on tumor cells via TNF-α and indirect cytostatic effects through stimulating DC histamine release[87,102]. MCs can also modulate macrophages, eosinophils, dendritic cells, NK cells, and T cells within the TME and recruit additional immune populations [36,81]. For example, activated MCs can release CCL2 and CCL3 that have been shown to recruit plasmacytoid DCs, CD8 T-cells, and NK cells [103,104]. MC-secreted TNF-α then facilitates better CD8 T-cell priming by DCs and can directly activate T cells [28,105]. These agonists predominantly target MRGPR due to non-specificity and anaphylaxis being associated with other MC activating receptors. While activation by these agonists sometimes results in robust degranulation, evidence suggests they can facilitate protective adaptive immune functions [106–108].

There is a limited number of *in vivo* studies regarding cancer and MC agonists, with studies primarily focusing on Mastoparan-L. The greatest reduction in tumor growth was reported by De Azevedo et al., who observed a 70% inhibition in a low-immunogenicity melanoma model following peri-tumoral treatment [73]. Except for this study, all studies used systemic, rather than local, delivery routes (I.V. or I.P.) and evaluated mastoparan and its analogues in breast cancer models. Hilchie et al. saw a non-significant reduction in tumor growth with systemic mastoparan-L treatment, while Zhang et al. observed a 33.6% growth inhibition rate [52,68]. Notably, Hilchie et al. used a murine tumor model whereas Zhang et al. used a xenograft model. Using the same model and route of administration, Zhang et al. showed that mastoparan analog KM8 had an 81.6% inhibition rate. These findings highlight the potential of MC agonists—particularly mastoparan analogs—as anti-tumor agents, though further *in vivo* studies are needed to optimize delivery methods and clarify their therapeutic efficacy across cancer types and within species.

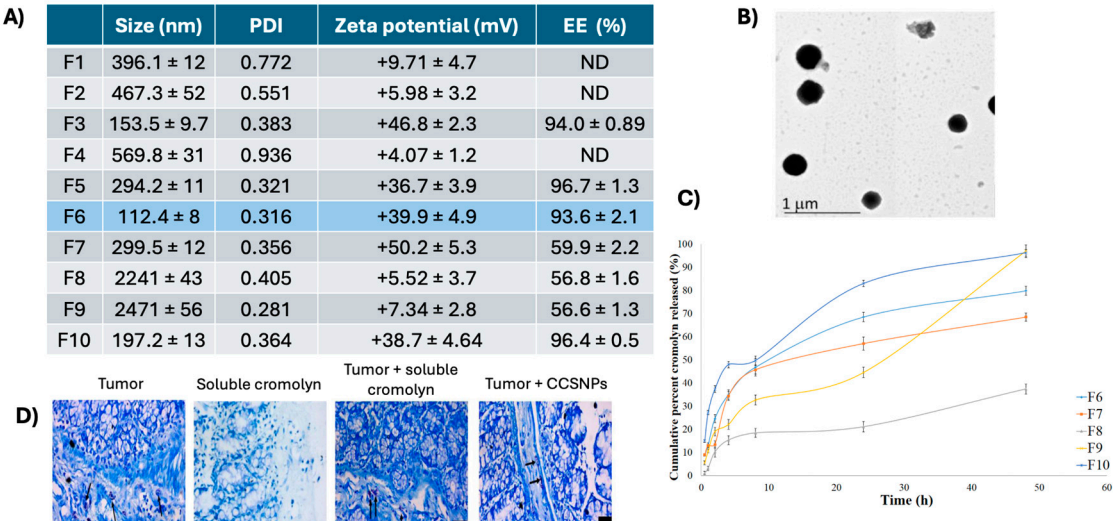
3. Formulation of MC Modulators in Drug Delivery Systems

To improve stability, bioavailability, and targeted delivery of MC modulators, various advanced DDSs have been applied to modulate MC responses (Table 4). These systems help address key challenges to delivery of MC modulators such as poor solubility, enzymatic degradation, and off-target cytotoxicity [109]. Among the many carriers applied, PEGylated liposomes are noted to enhance circulation time and reduce immune clearance, offering controlled release of the encapsulate. Chitosan nanoparticles are well studied because of their known mucoadhesive properties which can improve mucosal delivery. Similarly, alendronate sodium (e.g. Fosamax) nanoconjugates have been formed with MC modulating compounds for lung delivery. The fluvastatin-mastoparan

nanocomplex represents a dual-functional system combining the anti-inflammatory statin properties of fluvastatin with the MC-activating capacity of mastoparan. Soya phosphatidylcholine-based phytosomes enhance the stability and bioavailability of MC modulators by encapsulating them in the phospholipid carriers. Other innovative platforms include outer membrane vesicles (OMVs) from gram-negative bacteria, which serve as natural carriers for immune modulators, and fluorinated polyethylenimine (PEI) nanoparticles, which improve endosomal escape and transfection efficiency. Also, micelles offer a targeted approach for delivering of MC modulators. Collectively, these DDS technologies offer promising strategies for harnessing or inhibiting MC functions in cancer, allergy, and infection, while minimizing systemic toxicity and enhancing therapeutic precision [110–114].

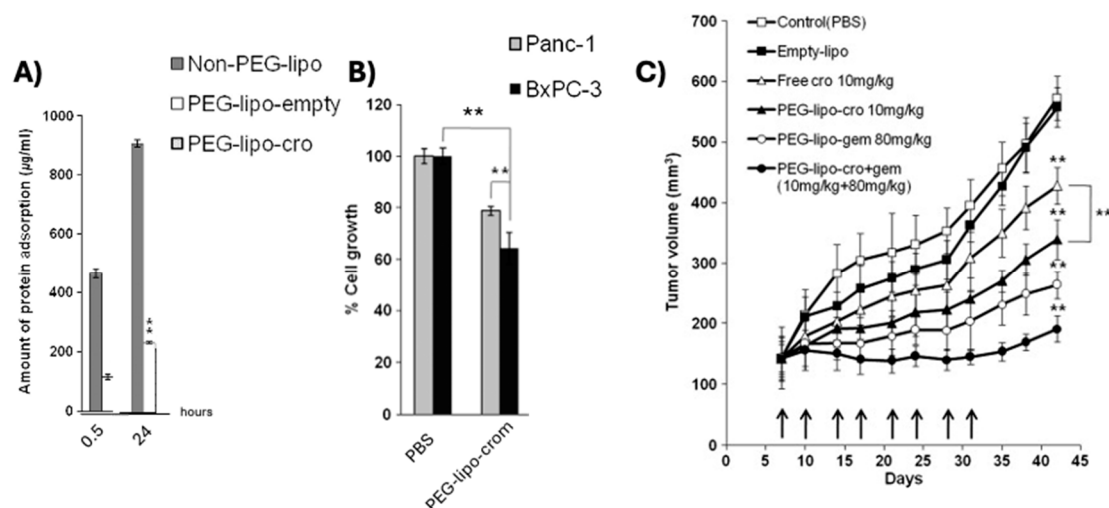
3.1. Cromolyn Formulations for MC Inhibition

Cromolyn has been encapsulated into chitosan nanoparticles and liposomes to facilitate better delivery [115–117]. Using ionic gelation techniques, Motwai et al. fabricated 10 formulations of cromolyn-containing chitosan nanoparticles (CCSNPs) [116]. These formulations displayed a wide range of physiochemical characteristics: 100 nm to 2 μm diameter, 0.3-0.9 polydispersity index (PDI), and +5 to +50 mV surface charge (Figure 3A). The formulation “F6” was selected for *in vivo* interrogation considering output measures of encapsulation efficiency, morphology, and release kinetics (Figure 3A,B). These roughly spherical, positively charged particles displayed a burst release of ~20% in two hours before plateauing at 80% release in 48 hours (Figure 3C). In a chemically induced colorectal cancer model, Motawi et al. showed 33% of mice treated with cromolyn-containing chitosan NPs had tumor lesions compared to 67% for mice receiving soluble cromolyn. Colorectal cancer leads to loss of colon architecture, a phenotype that was rescued only with treatment with cromolyn-containing NPs but not the vehicle or soluble controls. When evaluating colon MCs by toluidine blue staining, they observed that without tumor induction, there were limited MCs present, even with cromolyn treatment. In disease-bearing mice, treatment with cromolyn NPs decreased MC numbers while treatment with soluble cromolyn had no apparent effect on the number of MCs (Figure 3D). Survival was not assessed in these studies. Overall, the study demonstrates that encapsulating the MC inhibitor cromolyn in polymeric chitosan NPs significantly enhances its therapeutic efficacy in a colorectal cancer model.



**Figure 3.** Characterization and *in vivo* evaluation of cromolyn chitosan nanoparticles (CCSNP) by Motwai et. al. (A) The physicochemical characteristics of all formulations made with the selected optimized CCSNP “F6” highlighted in blue. (B) TEM images of spherical F6 particles. (C) Release kinetics of five of the ten formulations, F6-F10. (D) MC staining by toluidine blue of the colon of mice with chemically induced tumors including control, soluble cromolyn (without and with tumor) or CCSNP groups. Not determined (ND) [116].

Kim et al. produced PEGylated liposomes containing cromolyn using a reverse-phase evaporation vesicle method [115]. Liposomes were produced with a fixed dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), and distearoylphosphatidylcholine (DSPC) molar ratio (4:1:1), while the concentration of a fourth excipient, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000] (DSPE-mPEG<sub>2000</sub>), was optimized by varying its molar ratio from 0.1 to 1.0. A ten-fold increase in DSPE-mPEG<sub>2000</sub> resulted in a 3.5x reduction in encapsulation efficiency of cromolyn. The formulation with the lowest ratio of DSPE-mPEG<sub>2000</sub> (4:1:1:0.1) was selected for stability evaluations and found to not increase surface-adsorbed serum proteins after 48 hours (Figure 4A). Further the liposomal formulation was able to reduce cell growth of two pancreatic lines *in vitro* (Figure 4B). In an immunodeficient model of pancreatic cancer, they observed improved tumor growth with their PEGylated liposomal formulation over a soluble cromolyn control, both dosed at 10 mg/kg (Figure 4C). While treatment with a gemcitabine PEGylated liposome outperformed the cromolyn formulation, it was dosed at 80 mg/kg. However, the greatest tumor reduction was observed in mice receiving separate cromolyn and gemcitabine liposomal formulations at 10 and 80 mg/kg respectively. Overall, formulated cromolyn demonstrated potent effects *in vivo* whether delivered through chitosan particles or liposomes.



**Figure 4.** Stability and efficacy studies of a cromolyn liposomal formulation. (A) Surface adsorbed protein levels after liposome incubation with serum at 30 minutes and 24 hours. Liposomes were either non-PEG (Non-PEG-lipo), PEGylated but blank (PEG-lipo-empty), or PEGylated with cromolyn (PEG-lipo-cro). (B) Cell growth inhibition of the optimized liposomal formulation against two pancreatic cancer lines, Panc-1 and BxPC-3. (C) Tumor growth curves, as measured by calipers, of treatments including PBS, soluble cromolyn, and liposomal formulations of cromolyn, gemcitabine, and a combination of both; \*\*  $p < 0.01$ , with significance compared to controls where not clarified. [115].

### 3.2. Mastoparan Nanoconjugates

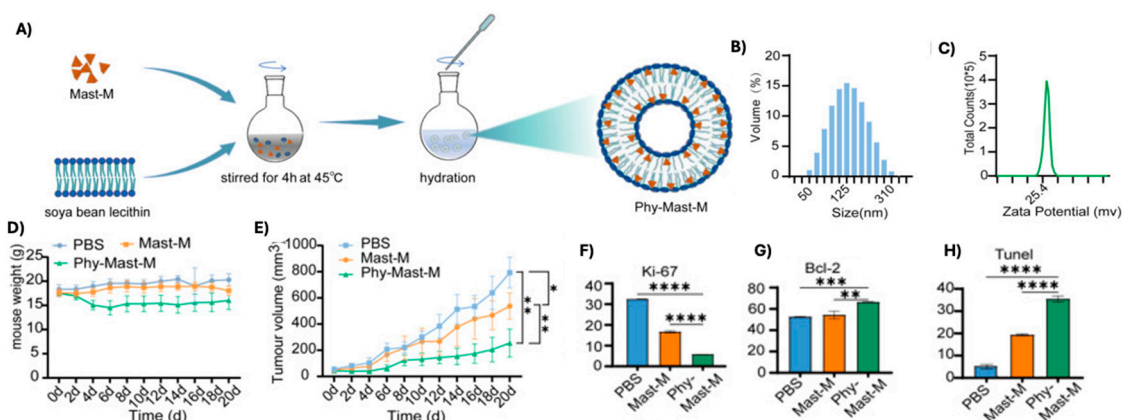
Alhakamy et al. (2021, 2022) developed two distinct systems by conjugating mastoparan to small-molecule drugs, enhancing their therapeutic activity and characterizing their performance *in vitro*. In 2021 they produced a mastoparan and fluvastatin (a statin) conjugate optimized for a 77.6 nm size [75]. Complexation with mastoparan nearly halved its 24-hour IC<sub>50</sub> value against the A549 lung cancer cell line, reducing it from 34.3 to 18.6 µg/mL. They subsequently developed a nanoconjugate composed of mastoparan and alendronate sodium (Fosamax), resulting in a larger particle size of 134.9 nm [118]. When the IC<sub>50</sub> assay was conducted over two hours in the same A549 lung cancer cell line, the nanoconjugate demonstrated a markedly enhanced potency, reducing the IC<sub>50</sub> from ~14 µM for mastoparan alone to just 1.3 µM. Notably fluvastatin and alendronate sodium have their own anti-tumor effects, and while significance against soluble controls was assessed,



synergy was not. Interestingly, mastoparan appears to be more potent at shorter timepoints, which is supported by Hilchie et al.'s finding that cytotoxicity peaked at 8 hours [68]. Together, these studies by Alhakamy et al. demonstrate that conjugating mastoparan to anti-tumor small molecules like fluvastatin and alendronate sodium can significantly enhance cytotoxic potency *in vitro*, though the potential for synergistic effects remains to be fully explored.

### 3.3. Phytosome Encapsulation of Mastoparan

Phytosomes are liposome-like vesicular systems formed by complexing bioactive compounds with plant-derived phospholipids, such as soya bean lecithin. Like liposomes, these carriers enhance the stability, bioavailability, and cellular uptake of encapsulated agents. In one study, mastoparan-M was encapsulated into phytosomes to generate positively charged nanoparticles optimized for *in vivo* delivery (Figure 5A–D) [119]. When applied in a breast cancer xenograft model, Zhao et al. reported that phytosome-encapsulated mastoparan-M led to a significant reduction in tumor volume by day 20, compared to administration of soluble mastoparan-M. Histological analysis using hematoxylin and eosin (HE) staining further demonstrated a marked decrease in proliferative activity and a corresponding increase in apoptosis within tumor tissues treated with the phytosomal formulation (Figure 5E–H). These results suggest that phytosomal delivery enhances the therapeutic efficacy and tumor-targeting potential of mastoparan-based therapies.



**Figure 5.** Characterization of mastoparan-M phytosome formulation. (A) Self-complexation of mastoparan-M with soybean lecithin to create spherical phytosomes. (B) DLS and (C) zeta potential of the final formulation with results being 125 nm and +32 mV respectively. (D) Mouse weight and (E) tumor size shown after the first treatment. Quantitation of HE staining of (F) Ki67+, (G) Bcl-2, and (H) TUNEL on excised tumors. [119].

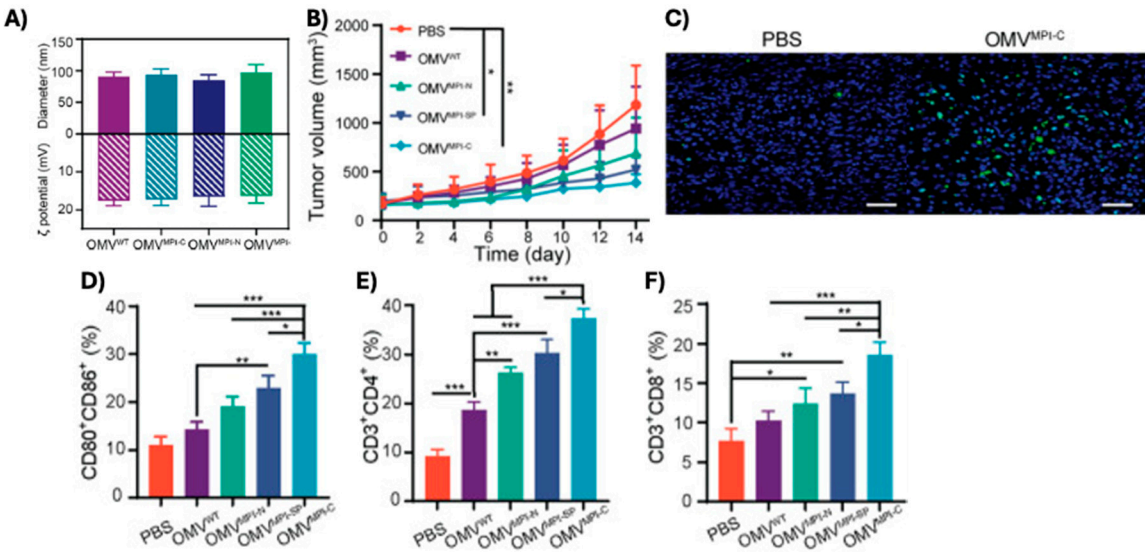
### 3.4. Polybia-MP1 Nanoparticle Formulations

MC agonist Polybia-MP1 (MP1) was formulated into natural and synthetic carrier systems by Ren et al. and Li et al., respectively, to treat bladder cancer [120,121].

Ren et al. genetically engineered gram-negative bacteria to express modified MP1 peptides for improved expression, which were subsequently passively loaded into the bacteria's naturally secreted outer membrane vesicles (OMVs) [121]. Four OMV formulations were made with all found to be ~90 nm with a -17 mV charge (Figure 6A). When taken *in vivo* in a subcutaneous immunocompetent model of bladder cancer, they identified a lead formulation that both significantly reduced tumor growth and displayed greater apoptosis by TUNEL immunofluorescence staining (Figure 6B,C). They went on to show this formulation improved intratumoral antigen presentation by dendritic cells as well as increasing infiltration by both CD4 and CD8 T-cells (Figure 6D–F). These elevated levels of effector populations correlated with increased IL-6, TNF- $\alpha$ , and IFN- $\gamma$  within the tumor. Critically, many of these results were shown to be significant compared to the OMV vehicle control, suggesting the heightened inflammation was due to MP1. These findings highlight that MP1-

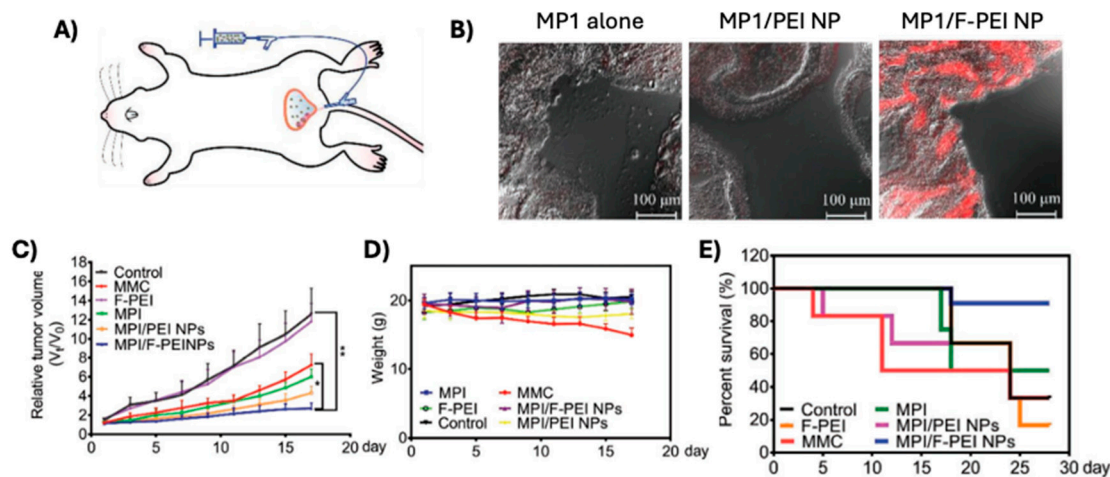


loaded OMVs can enhance anti-tumor immunity through targeted MC activation and modulation of the TME.



**Figure 6.** Results showing successful TME immune modulation after treatment with OMV particles containing MP1. A) size and charge assessed by DLS for four OMV formulations. B) Tumor growth control by caliper measurements and C) apoptosis by immunofluorescent TUNEL assay. Changes in intratumoral immune populations showing increased D) CD80+ CD86+ dendritic cells, E) CD4+ T-cells, and F) CD8+ T-cells. Dendritic cells were defined as CD11c+. [121].

Li et al. encapsulated MPI into polyethylenimine (PEI), with or without fluorination (F-PEI), the latter aimed at enhancing transport across the bladder mucosa following intravesical therapy [120]. After successfully producing 260-296 nm sized NPs for F-PEI and PEI, respectively, Li et al. assessed the NP's mucosal transport *in vivo* in an orthotopic xenograft bladder cancer model (Figure 7A). Using a fluorescently labeled MP1, they saw an increased penetration of the fluorescent signal into the bladder 60 minutes after infusion (Figure 7B). Initial results in a subcutaneous tumor showed their MP1-containing F-PEI NPs improved tumor control, even when compared to a chemotherapeutic agent, as well as minimal reduction in mouse weights (Figure 7C,D). Finally, they supported their results with an orthotopic bladder cancer model and showed that their F-PEI NPs containing MP1 improved survival at four weeks to 83%, compared to 50% for soluble MPI (Figure 7E). These findings highlight the potential of fluorinated PEI NPs as an effective delivery system for MPI, offering enhanced mucosal penetration, superior tumor control, and improved survival outcomes in bladder cancer models. MCs are thought to promote bladder cancer invasiveness through ECM remodeling [122].



**Figure 7.** Successful transmembrane delivery of MP1 for the treatment of bladder cancer. A) schematic of intravesicular therapy. B) Confocal images of resected bladders 60 minutes after treatment with soluble MP1, MP1 encapsulated in PEI NPs, and MP1 encapsulated in F-PEI NPs; MP1 has been fluorescently labeled with Cy5.5 for visualization. C) Tumor growth control in a subQ bladder cancer model and D) mouse weights during time of treatment. E) Survival of mice bearing orthotopic bladder tumors after treatment with MP1 in F-PEI NPs along with several controls. Fluorinated polyethylenimine (F-PEI), mitomycin-c (MMC); polybia-MP1 (MP1); polyethylenimine (PEI) [120].

3.5. Micellar Polymyxin E Co-Delivered with Doxorubicin

Lan et al. investigated the use of polymyxin E (PE) co-loaded with doxorubicin into polymeric micelles as a therapeutic strategy for cervical cancer [123]. In a subcutaneous U14 tumor model in Kunming mice, treatment with micelles containing polymyxin E alone showed no significant impact on tumor growth. However, when combined with doxorubicin and administered intravenously at 2.5 mg/kg every other day for a total of seven doses, the co-loaded micelles resulted in a significantly greater reduction in tumor volume compared to either agent alone. These results suggest that while PE may lack direct anti-tumor activity, its combination with chemotherapy can enhance therapeutic efficacy, likely through synergistic mechanisms or improved drug delivery.

**Table 4.** Relevant preclinical model details and outcomes of MC-modulating agents in drug delivery system. Tumor size was determined via a web-based data digitizer. Doxorubicin (DOX), dimethylhydrazine (DMH), intraperitoneal (IP), intravenous (IV), mitomycin C (MMC), not significant (n.s.), (fluorinated) polyethylenimine [(F-)PEI], polymyxin E (PE), subcutaneous (subQ), every two days (q2d), every 3 days (q3d), and every 4 days (q4d).

MC AGONIST	CAN CER	MODEL	VEHICLE	DOSE	SCHE DULE	CONCL USIONS	R EF
Cromolyn sodium	Pancr eatic	Bx-PC-3 subQ in BALB/c mice	PEGylated liposomes	IV 10mg/kg	Twice weekl y for 4 weeks	Encapsul ated cromolyn outperfor med soluble in tumor growth inhibition	[1 15 ]

						, more apoptosis 24 hr after treatment	
	Color ectal	DMH induced in Wister albino rats	Chitosan nanoparticles	IP 5mg/kg	Twice weekly for 16 weeks	Survival not assessed; maintenance of colon architecture, decreased MC frequency	[16]
Mastoparan	Lung	<i>In vitro</i>	Alendronate sodium nanoconjugate	A549 24 hr MTT	NA	IC50 = 1.3 µM; vehicle alone 37.6 µM, alone ~13	[74]
			Fluvastatin mastoparan nanoconjugate	A549 4hr MTT		More apoptotic proteins present in MAS-FLV-NC; IC50 = 18.6 µM; vehicle alone = 58.4 µM, mas = 34.3 µM	[75]
Mastoparan-M	Breast	4T1 subQ in BALB/c mice	Soya phosphatidylcholines phytosomes	IV 2.7mg/kg	q4d 4x	Encapsulated mastoparan outperformed	[124]

						soluble in tumor growth inhibition with more apoptosis (Bcl-2 and TUNEL) and decreased proliferation (Ki-67)	
Polybia-mastoparan I (MPI)	Bladder	MB49 subQ in C57/BL6 mice	Outer membrane vesicles of gram-negative bacteria	IT 100 µg vesicle protein	q3d 4x	DC maturation, macrophage recruitment, CD4+ and CD8+ infiltration	[121]
		T24 subQ in BALB/c nude mice	Fluorinated PEI nanoparticles	0.5 mg/kg intravesical	60min long treatment 1x	NPs resulted in superior tumor control a SOC chemo (MMC), no weight loss	[120]
Polymyxin E	Cervical	U14 subQ in Kunming mice	PE-Doxorubicin loaded	2.5mg/kg DOX	q2d 7x	PE not efficacious alone,	[123]

			micelles	I.V.		but when combined with DOX, better than either alone	
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4. Conclusions

MCs represent a unique and underexplored target for cancer immunotherapy. Significant evidence suggests MCs play a role in tumor development, with the environmental context dictating whether that role is pro- or anti-tumor. Targeting the process of functional polarization within the TME offers a unique opportunity to potentially reprogram progenitor MCs before they are co-opted by the tumor’s immunosuppressive environment. While more work needs to be done to understand the underlying biology, advances are already being made in potential therapeutics. Approaches to reduce MC numbers and to target their secreted factors are often non-specific and have mixed results across tumor models. MC agonists and stabilizing agents have been shown to decrease tumor growth *in vivo* in cancer models; however, many of these agents display unfavorable physiochemical properties for systemic administration. Drug delivery systems—particularly those leveraging nanomedicine platforms—offer a promising solution by enabling cell-specific targeting, controlled release kinetics, and co-delivery of synergistic agents. Indeed, some *in vivo* studies only observed significant reductions in tumor burden upon codelivery of MCs agonists with chemotherapeutic agents [68]. Likely, the success of MC-targeted therapies will be contingent on combination with other immunotherapies[125,126]. As such, the integration of advanced delivery technologies is not merely advantageous but essential for the achieving the precision necessary for effective immunological re-education of MCs in cancer therapy.

Future work should exploit DDS for delivery of MC modulators that affect MC development within the TME. MCs are a distinctive immunotherapeutic target, as many tumors actively recruit progenitor cells and condition them within the TME. Targeting the mechanisms that drive this functional polarization presents a promising strategy to disrupt tumor-mediated immune education—offering the potential to reprogram or eliminate MCs before they are co-opted into supporting tumor growth and immune suppression. In summary, leveraging drug delivery systems to precisely modulate mast cell behavior within the TME may unlock new avenues in cancer immunotherapy, transforming these often-overlooked cells from tumor accomplices into allies in anti-cancer defense.

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