

# Nanocarrier-Enabled Melittin Therapy: Mechanistic Advances, Therapeutic Applications, and Translational Challenges

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**Abstract:** Melittin, a 26-amino-acid amphipathic peptide derived from bee venom, has attracted sustained interest in nanomedicine because of its broad anticancer, antiviral, and anti-inflammatory activities. These effects arise not only from direct membrane disruption, but also from secondary intracellular stress signaling and context-dependent immunomodulatory responses. The same mechanism, however, also underlies melittin's major limitation: potent biological activity is closely coupled to hemolysis, nonspecific cytotoxicity, and poor systemic tolerability. For this reason, the central question in melittin nanomedicine is no longer whether the peptide is bioactive, but how that activity can be spatially, temporally, and pharmacologically constrained in vivo. Nanocarrier engineering has therefore become integral to melittin development, with lipidic, polymeric, inorganic, and hybrid systems being explored to reduce premature toxicity, improve lesion-site accumulation, regulate peptide release, and enable active or stimulus-responsive targeting. In this review, we synthesize current progress from three linked perspectives: the mechanistic basis of melittin action, the disease-specific logic of its reported applications, and the translational criteria that distinguish proof-of-concept platforms from development-relevant ones. This review was prepared by searching peer-reviewed literature published primarily between 2020 and early 2026 in PubMed, Web of Science, and Scopus, with priority given to studies addressing melittin biological activity, nanocarrier formulation design, toxicity modulation, and translational considerations. Across the current literature, the most decisive issues are not efficacy alone, but the extent to which toxicity attenuation, exposure control, PK/PD interpretability, and formulation tractability can be achieved within the same platform. The available evidence suggests that melittin is most plausibly advanced in delivery-controlled settings, particularly localized, route-constrained, or selected combination-oriented applications, whereas broader systemic use still requires stronger evidence in long-term safety, pharmacological standardization, and product-level reproducibility.

**Keywords:** nanocarriers, melittin, multi-target therapy, clinical translation, toxicity modulation

## Introduction

Melittin is the major bioactive peptide component of bee venom and consists of 26 amino acids arranged in an amphipathic sequence that readily interacts with lipid membranes.<sup>1,2</sup> This structural feature underlies a wide range of reported biological effects, including anticancer, antiviral, antimicrobial, and anti-inflammatory activities.<sup>3</sup> At the same time, it also explains why melittin remains difficult to translate: the same membrane activity that enables therapeutic action can also produce hemolysis, nonspecific cytotoxicity, vascular irritation, and a narrow tolerability window after systemic exposure.<sup>4,5</sup> In melittin research, potency and liability are therefore mechanistically inseparable rather than sequentially distinct problems.



Compared with other well-characterized antimicrobial peptides such as defensins and magainins, melittin is distinguished by a short linear amphipathic  $\alpha$ -helical architecture and pronounced cationic membrane affinity, which support rapid membrane insertion and pore-forming or membrane-destabilizing activity.<sup>2,6</sup> These structural and biophysical properties help explain why melittin has been investigated beyond antimicrobial use, including anticancer, antiviral, and anti-inflammatory contexts, but they also intensify the need for delivery systems that restrict nonspecific membrane injury.<sup>3,7</sup>

The therapeutic interest in melittin persists because it can act across disease settings that differ markedly in biology yet share one common feature: membrane vulnerability or membrane-dependent signaling remains relevant to pathology. In cancer, melittin can directly injure tumor cells, induce secondary stress responses, and in some settings promote immunogenic or microenvironmental changes that reshape antitumor immunity.<sup>8,9</sup> In antiviral applications, its most consistent activity arises at structurally accessible stages of infection, particularly where virion integrity or early entry processes remain susceptible to peptide-mediated disruption.<sup>10,11</sup> In inflammatory disease, melittin has been shown to modulate macrophage- and cytokine-associated pathways that contribute to tissue injury.<sup>7</sup> These observations explain why melittin has continued to attract attention despite its formulation challenges, but they do not by themselves resolve the central translational problem: therapeutic plausibility depends on whether these effects can be expressed selectively in pathological tissue rather than indiscriminately across normal blood and organs.<sup>12</sup>

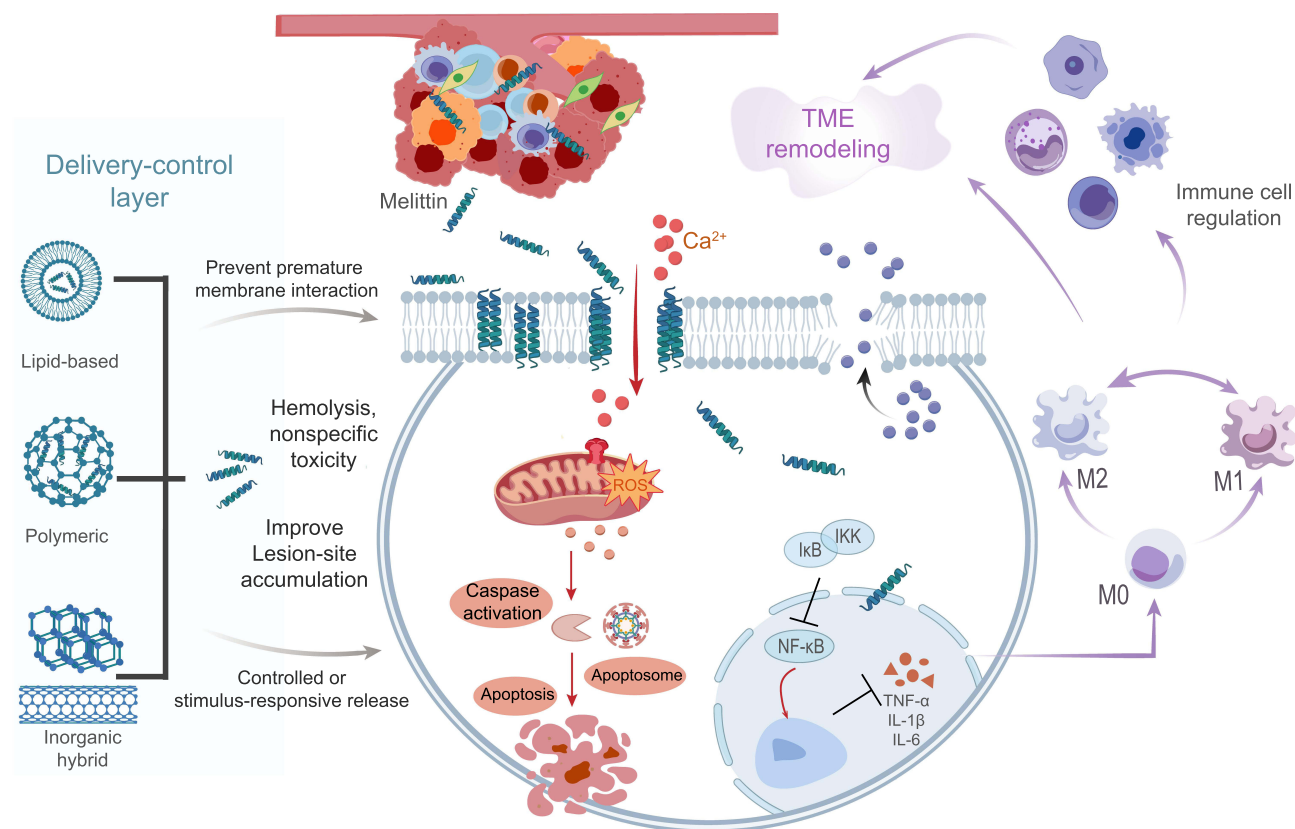
Nanocarrier engineering has therefore become central to the field rather than adjunct to it. Liposomes, micelles, lipid disks, lipid-coated polymeric particles, inorganic or magnetic carriers, hydrogels, peptide-based assemblies, and multi-functional bioinspired systems have all been used to alter how melittin is loaded, shielded, distributed, activated, or released.<sup>13,14</sup> In this context, delivery design does more than improve convenience or stability; it determines whether membrane-active exposure occurs in circulation, at lesion sites, or after intracellular entry, and thus whether melittin behaves as a broadly lytic toxin or as a controllable therapeutic agent.<sup>2,5</sup> The significance of nanocarriers in melittin research should therefore be understood at the mechanistic level as well as at the formulation level.

Much of the literature has emphasized individual formulation successes, often by showing reduced hemolysis, increased uptake, improved retention, or stronger biological efficacy in selected models.<sup>15–17</sup> However, the field now requires a more integrated framework for comparison. The key questions are not simply which platforms produce biological effects, but which ones do so in a manner that is pharmacologically interpretable and developmentally credible. In practical terms, four issues are especially important across the literature: whether a platform attenuates toxicity without abolishing activity, whether it restricts exposure to relevant tissues or cell populations, whether it provides a controllable and interpretable pharmacological profile, and whether its composition and behavior are sufficiently defined to support product-oriented development. These questions connect mechanistic understanding to disease application and ultimately to translational prioritization.<sup>18–20</sup>

To define the scope of this review, peer-reviewed studies published primarily between 2020 and early 2026 were searched in PubMed, Web of Science, and Scopus, with emphasis on melittin biological activity, nanocarrier-enabled delivery, toxicity modulation, PK/PD considerations, CMC issues, and translational readiness. Accordingly, this review examines melittin nanomedicine through three linked levels of analysis. First, we revisit the mechanistic basis of melittin action, beginning with membrane disruption and extending to intracellular stress and microenvironmental regulation. Second, we evaluate the current literature across anticancer, antiviral, and anti-inflammatory applications by asking how route, selectivity, and delivery control shape disease-specific plausibility. Third, we compare major nanocarrier classes from a translational perspective, focusing on formulation clarity, PK/PD interpretability, long-term safety maturity, and manufacturing burden. With this structure, this review aims to clarify how the mechanistic activity of melittin can be controllably regulated through delivery design and which carrier classes currently hold the most promising prospects for clinical translation.

## Mechanistic Basis of Melittin and the Role of Nanocarriers

Melittin should not be understood as a single-function cytolytic peptide. Its biological effects are better viewed as a cascade that begins with membrane interaction and extends into secondary intracellular responses and broader changes in inflammatory or immune signaling.<sup>7,13</sup> This sequence is important because it explains both the therapeutic versatility



**Figure 1** Mechanistic basis of melittin activity and nanocarrier-enabled delivery control. Melittin activity involves membrane disruption, intracellular stress responses, inflammatory signaling regulation, macrophage polarization, and tumor microenvironment remodeling. Nanocarriers can reduce premature membrane interaction, attenuate nonspecific toxicity, improve lesion-site accumulation, and enable controlled or stimulus-responsive release.

of melittin and the difficulty of controlling it *in vivo*. Primary membrane disruption provides the initiating event, but the downstream consequence depends strongly on cell type, tissue context, exposure level, and whether delivery systems restrict when and where active peptide is released.<sup>6,21</sup> The mechanistic basis of melittin activity and the delivery-control functions of major nanocarrier classes are summarized in Figure 1.

## Primary Membrane-Disruptive Activity

The first and most fundamental event in melittin action is its direct interaction with lipid bilayers.<sup>2</sup> Owing to its amphipathic and cationic structure, melittin can associate with cell membranes, insert into the phospholipid bilayer, and assemble into pore-forming or membrane-destabilizing structures.<sup>22</sup> At sufficiently high local concentrations, this process leads to loss of membrane integrity, ion imbalance, leakage of intracellular components, and cell death.<sup>8</sup> In cancer and antiviral studies, this membrane-active behavior is often the most immediate source of efficacy; in toxicity studies, it is also the clearest explanation for hemolysis and nonspecific injury.<sup>5,10</sup> The key implication is that therapeutic use depends not on suppressing membrane activity altogether, but on restricting where and when that activity is expressed.

This point is especially important because membrane disruption is only partly disease-selective in its native form.<sup>23</sup> Tumor cells, infected cells, activated immune cells, and erythrocytes can all be affected if exposure is not spatially controlled.<sup>24</sup> As a result, free melittin often shows strong bioactivity *in vitro* but a limited safety margin *in vivo*.<sup>4</sup> Many nanocarrier strategies can be understood as attempts to solve precisely this problem by shielding the peptide during circulation, delaying membrane-active exposure until lesion sites are reached, or confining interaction to cells defined by route, ligand recognition, or microenvironmental triggers.<sup>25,26</sup> In mechanistic terms, nanocarriers

are therefore not merely passive containers for melittin; they are regulators of the initiating event that determines whether downstream pharmacology becomes therapeutically useful or systemically harmful.

## Secondary Intracellular Responses

Although membrane disruption initiates melittin activity, many reported effects cannot be explained by lysis alone.<sup>13</sup> Once membrane permeability is altered, cells may undergo a broader range of intracellular responses that include calcium dysregulation,<sup>27</sup> mitochondrial dysfunction, oxidative stress, caspase activation, and changes in survival-related signaling pathways.<sup>28–30</sup> Depending on exposure conditions and cell context, these events may culminate in apoptosis, necrosis, immunogenic stress, or mixed death phenotypes.<sup>31,32</sup> In tumor cells, such secondary responses help explain why melittin can remain pharmacologically effective even when membrane damage is only partial or transient.

These downstream effects are also important because they create the bridge between direct cytotoxicity and disease-specific therapeutic logic. In oncology, intracellular stress and apoptosis-related cascades support the use of melittin not only as a lytic peptide but also as a trigger of immunogenic or combination-sensitive tumor killing.<sup>33,34</sup> In antiviral studies, membrane perturbation may be followed by inhibition of entry-related processes or other early infection events.<sup>35</sup> In inflammatory settings, intracellular signaling changes in activated macrophages or related effector cells can reduce the production of proinflammatory mediators without requiring unrestricted tissue damage.<sup>36</sup> Seen in this way, melittin's therapeutic profile is not determined by membrane activity alone, but by how that activity is converted into interpretable intracellular consequences. This is one reason why delivery systems that control local concentration, uptake, or intracellular release can materially reshape melittin pharmacology even when the peptide sequence itself remains unchanged.

## Immunomodulatory and Microenvironmental Regulation

Beyond direct membrane and intracellular effects, melittin can also act as a regulator of the surrounding biological environment.<sup>37</sup> Reported studies have shown that melittin or melittin-based formulations can influence inflammatory cytokines, dendritic cell maturation, macrophage polarization, and the immune composition of tumor or inflamed tissue.<sup>38,39</sup> In some cancer platforms, these effects are linked to features consistent with immunogenic cell death, antigen presentation, or relief of local immunosuppression.<sup>32,40</sup> In inflammatory disease, the same peptide has been associated with downregulation of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, NO, or related mediators in activated macrophages and injured tissue.<sup>41,42</sup> These observations indicate that melittin should be understood not only as a membrane-lytic peptide, but also as a context-dependent modulator of microenvironmental and immune processes that shape therapeutic outcome.

This microenvironmental regulatory activity is critical for understanding melittin's therapeutic value. Anticancer efficacy is not explained solely by tumor-cell lysis if treatment also changes dendritic-cell activation or suppressive immune populations. Antiviral plausibility is not simply a matter of *in vitro* virucidal activity if tolerability depends on restricting exposure to infection-relevant compartments. Anti-inflammatory benefit is not reducible to general cytotoxicity if the dominant effect is reduction of activated macrophage signaling in a route-defined tissue context. In each case, the same mechanistic breadth that makes melittin attractive also makes indiscriminate exposure harder to justify. Thus, the more melittin is recognized as a modulator of microenvironmental biology, the more necessary delivery design becomes for separating useful immunopharmacology from unwanted tissue injury.

## Mechanistic Significance of Nanocarrier-Enabled Delivery

For melittin, delivery design is inseparable from mechanism. Encapsulation, conjugation, surface engineering, and route-specific administration do more than improve formulation stability; they reshape when membrane-active exposure occurs, how much peptide reaches pathological tissue, whether intracellular delivery is favored, and whether secondary immunological effects emerge in a restricted rather than systemic manner.<sup>12,43,44</sup> Lipidic, polymeric, inorganic, and hybrid carriers may achieve this through passive retention, receptor-mediated targeting, trigger-responsive release, or local retention after topical, intratumoral, lymphatic, or transdermal administration.<sup>14,17,31</sup> The resulting biological profile of melittin *in vivo* is therefore determined as much by carrier behavior as by peptide potency itself.

Once delivery is recognized as a mechanistic regulator rather than a formulation afterthought, differences among disease settings become easier to understand. Some applications benefit from route-defined exposure and therefore require less structural complexity. Others depend on highly selective or responsive delivery to make melittin usable at all. Likewise, translational comparison among carrier classes can no longer be based only on whether they produce efficacy, but on whether they provide a credible degree of control over the mechanisms outlined above. Melittin's clinical feasibility lies not in avoiding its membrane activity, but in controlling it via delivery design.

## Applications of Melittin

### Anticancer Activity

Oncology remains the setting in which melittin has been explored most extensively, largely because membrane vulnerability, apoptotic susceptibility, and microenvironmental dysregulation create several potential points of intervention at once.<sup>9,13,14,21</sup> The central challenge is not whether melittin can damage tumor cells, but whether that activity can be directed toward malignant tissue without reproducing the same membrane injury in blood and healthy organs.<sup>4,5,12</sup> As summarized in Table 1, currently reported anticancer studies span free melittin, targeted micelles, lymphatic delivery systems, pH-responsive mesoporous silica platforms, bioinspired multicomponent nanovehicles, and other formulation classes, with evidence distributed across orthotopic, xenograft, metastatic, and in vitro-resistant tumor models.<sup>32,37,43,45–51</sup>

Table 1 supports a consistent pattern across these systems: antitumor activity is relatively easy to demonstrate, whereas selectivity, exposure control, and formulation tractability are harder to achieve simultaneously. Targeted platforms such as LC09-MLT@F127 and MLT-HA-HPPS show how lesion-oriented delivery can preserve melittin activity while improving hemocompatibility or regional tolerability, particularly in osteosarcoma and sentinel lymph node-associated disease settings.<sup>32,43</sup> Multicomponent systems, including the bioinspired BTN platform and the pH-labile artificial natural killer cell construct, further illustrate that melittin can be integrated into activatable or resistance-reversal strategies with strong biological effect, but at the cost of greater formulation complexity.<sup>37,47</sup> By contrast, free-melittin studies in colorectal, ovarian, and bladder tumor models remain useful as benchmarks for intrinsic peptide activity and mechanistic plausibility, yet they also underscore that efficacy without delivery engineering does not by itself resolve the exposure problem.<sup>46,50,51</sup> Taken together, the evidence in Table 1 suggests that the most credible oncologic role of melittin lies in tumor-directed, delivery-controlled, or combination-oriented strategies rather than unrestricted systemic use.<sup>12,32,37,43,45–51</sup>

### Antiviral Activity

Antiviral applications of melittin are conceptually attractive for a different reason from oncology.<sup>10,35,52</sup> Rather than relying on a single intracellular oncogenic pathway, antiviral activity can arise from structurally vulnerable stages of infection, particularly where virion integrity, envelope stability, or early attachment processes remain accessible to peptide intervention.<sup>10,35,52</sup> As summarized in Table 2, the current antiviral literature spans herpes simplex virus, SARS-CoV-2, HIV-1, vesicular stomatitis virus, and enteroviruses, and is dominated by free-peptide studies, with only limited evidence from formulation-based systems such as the SIT-MEL nanoformula.<sup>35,52–56</sup> Across these reports, the most consistent signal is not broad post-entry suppression, but direct virucidal or early-stage interference under controlled in vitro conditions.<sup>35,52,53,55,56</sup>

Table 2 supports a clear hierarchy within the available antiviral evidence.<sup>35,52–56</sup> The strongest and most reproducible findings concern direct inactivation of viral particles or interference with viral attachment, as illustrated by the anti-HSV studies and the broad virucidal effects reported against VSV and EV-71.<sup>35,52</sup> For SARS-CoV-2, both free melittin and the SIT-MEL nanoformula show measurable in vitro activity, but these data still primarily establish proof of concept rather than development-ready antiviral positioning.<sup>53,54</sup> The literature on HIV-1 further suggests that melittin-related antiviral effects may extend beyond simple membrane disruption to suppression of viral gene expression, yet these observations also remain preclinical.<sup>55,56</sup> Taken together, the antiviral evidence indicates that melittin is best understood at present as

**Table 1** Nanocarrier-Enabled Melittin Strategies for Cancer Therapy

| Platform/Agent  | Tumor Setting                                     | Delivery Format/Route   | Key Antitumor Outcome   | Safety/ Selectivity Signal  | Evidence-Based Note  | Translational Limitation  | Reference |
|---|---|---|---|---|--|---|-----------|
| LC09-MLT@F127   | Osteosarcoma                                      | Aptamer-micelle; IV, 2.0 mg/kg weekly   | 49% tumor-volume reduction; 65.8% TUNEL-positive cells  | Hemolysis 13.9% at 70 $\mu\text{g}/\text{mL}$ ; lower than free melittin                  | OS-targeting micelle with ICD induction and reduced hemolysis  | Targeting benefit requires stronger validation beyond uptake and short-term efficacy; ligand-density and micelle reproducibility remain important   | [32]      |
| BTN carrying tumor-activated melittin pro-peptide, HPPH, and ROS-responsive gemcitabine prodrug | 4T1 breast tumor                                  | Bioinspired lipoprotein-based activatable co-delivery nanovehicle; IV   | 82.8% tumor-growth inhibition; increased CRT exposure   | Legumain-responsive activation reduced nonspecific hemolysis in physiological media       | Tumor-permeating, activatable multicomponent platform with ICD induction and immunosuppression-relieving effects | High formulation complexity; contribution of each component, coordinated release, and scalable quality control require clarification                | [37]      |
| MLT-HA-HPPS   | 4T1 breast cancer/ sentinel lymph node metastasis | Dual-targeted HDL-mimic nanoparticle; paracancerous/ peritumoral, 20 nmol/ mouse every 2 days $\times$ 3                            | 81.3% primary-tumor inhibition; approximately 78.0% decrease in metastatic sentinel lymph node weight | No obvious systemic toxicity reported   | Regional lymphatic delivery strategy for primary tumor and sentinel lymph node control                           | Strong route-defined translational logic, but indication scope depends on clinically accessible regional administration                             | [43]      |
| M-M NPs   | Melanoma/ lung metastasis                         | TME-responsive MnO <sub>2</sub> -melittin nanoparticle; intratumoral (subcutaneous tumors) or IV (lung metastasis model), 5.0 mg/kg | Contralateral tumor suppression; $\sim$ 50% fewer lung metastatic nodules                             | Reduced hemolysis and no obvious in vivo toxicity reported                                | Tumor killing combined with APC activation and systemic antitumor immunity                                       | Promising immune-remodeling effect, but manganese-containing and TME-responsive systems require stronger long-term fate and safety characterization | [45]      |
| Free melittin   | SW480 colorectal xenograft                        | IP, 1.0 mg/kg for 18 days   | Apoptosis increased from 8.6% to 23.3%  | No obvious body-weight loss or major hematologic/ biochemical toxicity reported           | Free-peptide proof-of-concept showing ER stress-mediated apoptosis   | Demonstrates intrinsic activity but does not solve systemic exposure, hemolysis, or tumor-selectivity problems                                      | [46]      |
| pH-labile artificial natural killer cell based on melittin-gated Dox-loaded MSNs                | MCF-7/Adr drug-resistant breast cancer            | pH-responsive dendritic mesoporous silica platform; IV, 5.0 mg/kg Dox every 4 days  | Dox IC <sub>50</sub> reduced from 35.79 to 0.77 $\mu\text{g}/\text{mL}$ ; xenograft growth inhibited  | Selective inhibition under mildly acidic conditions; no obvious systemic toxicity in vivo | Drug-resistance reversal through melittin-assisted intracellular Dox accumulation                                | Trigger reliability, silica-material fate, and Dox/melittin dose attribution need stronger PK/PD characterization                                   | [47]      |

|   |   |   |   |  |   |  |      |
|---|---|---|---|--|---|--|------|
| FA-PENs carrying miR-34a, LTX-315, and melittin | MDA-MB-231 triple-negative breast cancer cells                      | Folate-targeted polymeric system; in vitro      | 98.0% uptake; melittin/miR-34a co-delivery increased death induction by 54% | Carrier alone showed low in vitro cytotoxicity; no in vivo validation                            | Folate-targeted multi-cargo synergy at proof-of-concept stage             | Early in vitro evidence only; multi-cargo dose ratio, release coordination, and in vivo safety remain unresolved                 | [48] |
| PEG-GO-Fe <sub>3</sub> O <sub>4</sub> /MEL      | HeLa cervical cancer cells  | Magnetic graphene oxide nanocomposite; in vitro | 87.6% inhibition at 72 h; apoptosis 51.6%                                   | PEG-GO-Fe <sub>3</sub> O <sub>4</sub> vehicle was biocompatible in vitro; no in vivo safety data | Magnetic, sustained-release proof-of-concept in HeLa cells                | Development limited by lack of in vivo validation, chronic material safety, degradation profile, and manufacturing comparability | [49] |
| Free melittin                                   | ID8 ovarian xenograft   | IP, 0.05–0.1 mg/kg every 3 days × 8             | Tumor weight reduced to 37% of control                                      | No significant liver/kidney biochemical or histologic toxicity reported                          | Confirms free-peptide in vivo efficacy without delivery engineering       | Useful benchmark, but narrow therapeutic margin and lack of exposure control limit systemic translational credibility            | [50] |
| Free melittin                                   | T24/EJ melittin-sensitive bladder tumor; BIU87 resistant comparator | Intratumoral, 1.0 mg/kg every 2 days × 2 weeks  | Marked suppression of T24/EJ xenografts, but limited effect in BIU87 model  | Minimal effect on SV-HUC-1 viability at 4 µg/mL; intratumoral delivery used in vivo              | Local free-peptide proof-of-concept with epigenetic selectivity mechanism | Local administration reduces systemic exposure but limits indication breadth; resistance heterogeneity remains a key concern     | [51] |

**Abbreviations:** APC, antigen-presenting cell; BTN, bioinspired therapeutic nanovehicle; CRT, calreticulin; Dox, doxorubicin; ER, endoplasmic reticulum; FA-PENs, folate-targeted polyelectrolyte nanoparticles; HDL, high-density lipoprotein; ICD, immunogenic cell death; IP, intraperitoneal; IV, intravenous; MSN, mesoporous silica nanoparticle; ROS, reactive oxygen species; SLN, sentinel lymph node; TME, tumor microenvironment.

**Table 2** Antiviral Activities of Melittin and Melittin-Based Formulations

| Agent/ Platform                        | Virus             | Delivery Format                        | Antiviral Mechanism   | Potency Indicator  | Safety/Selectivity Note  | Translational Limitation  | Reference |
|--|-------------------|--|---|--|--|---|-----------|
| Melittin variants<br>MEL-AM<br>/MEL-AF | HSV-1/HSV-2       | Free peptide                           | Virucidal inactivation and inhibition of viral attachment                               | MEL-AF virucidal EC <sub>50</sub> : 0.29 µg/mL for HSV-1 and 0.46 µg/mL for HSV-2; >50% plaque inhibition at 5 µg/mL during attachment | Vero-cell CC <sub>50</sub> : 12.05 µg/mL for MEL-AM and 10.50 µg/mL for MEL-AF       | Strong in vitro activity, but lacks delivery optimization, mucosal retention strategy, and in vivo PK/PD validation               | [35]      |
| Melittin                               | SARS-CoV-2        | Free peptide                           | Likely membrane destabilization, possible RBD interaction, and host-response modulation | EC <sub>50</sub> 0.656 µg; approximately 95% viral reduction at 12 h and 99% at 24 h   | No or very low cytolytic activity at the tested EC <sub>50</sub> range in Vero cells | Potent in vitro evidence, but systemic safety, targeted delivery, and in vivo antiviral efficacy remain unresolved                | [53]      |
| SIT-MEL optimized nanoformula          | SARS-CoV-2        | Nanoformula/ nano-conjugate            | 3CLpro inhibition with antiviral activity in infected Vero E6 cells                     | Antiviral activity in infected-cell assays; 3CLpro inhibition observed at low micromolar range   | Reported as non-cytotoxic in Vero E6 cells under tested conditions                   | Formulation-based proof-of-concept only; in vivo tolerability, release behavior, and exposure control remain undefined            | [54]      |
| Melittin/ melittin derivatives         | HIV-1             | Free peptide/ peptide-based approaches | Suppression of viral gene expression and reduction of infectivity                       | Complete inhibition reported at 2.5 µg/mL in selected in vitro systems   | Narrow therapeutic range reported for free melittin                                  | Mechanistically broader than simple lysis, but lacks mature nanocarrier design, in vivo pathway, and therapeutic-index resolution | [55,56]   |
| Melittin                               | GFP-fused VSV     | Free peptide                           | Rapid virucidal inactivation before cell attachment                                     | EC <sub>50</sub> 1.18 ± 0.09 µg/mL; SI 5.27  | Moderate selectivity in vitro  | Supports broad virucidal activity, but lacks formulation, tissue targeting, and systemic safety evidence                          | [52]      |
| Melittin                               | Influenza A (PR8) | Free peptide                           | Inhibition of infection and reporter expression   | EC50 1.15 ± 0.090 µg/mL; SI 6.66   | Better selectivity than some tested viral models                                     | Cell-based evidence only; respiratory delivery, mucosal tolerability, and local retention remain undeveloped                      | [52]      |
| Melittin                               | EV-71             | Free peptide                           | Virucidal activity with reduced cytopathic effect and infectivity                       | EC50 0.760 ± 0.030 µg/mL; SI 5.73  | Moderate selectivity in HeLa cells   | Extends activity to a non-enveloped virus, but no delivery system and limited selectivity constrain translation                   | [52]      |

**Abbreviations:** CC<sub>50</sub>, 50% cytotoxic concentration; EC<sub>50</sub>, 50% effective concentration; EV-71, enterovirus 71; HSV, herpes simplex virus; IC<sub>50</sub>, 50% inhibitory concentration; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SI, selectivity index; VSV, vesicular stomatitis virus.

a potent but exposure-sensitive antiviral candidate whose most plausible applications are likely to be localized, exposure-limited, or adjunctive rather than broadly systemic.<sup>10,35,52–56</sup>

## Anti-Inflammatory Activity

In inflammatory disease, melittin is most relevant where pathology is spatially concentrated and immune dysregulation can be modulated without requiring broad systemic exposure.<sup>7,36,57–60</sup> This is why the anti-inflammatory literature tends to favor topical, regional, organ-directed, or otherwise route-defined interventions rather than unrestricted delivery.<sup>36,39,57–60</sup> As summarized in Table 3, currently reported evidence spans gastrointestinal inflammation, acute liver injury, wound-associated inflammation, allergic skin disease, vascular inflammatory remodeling, arthritis, lumbar spinal stenosis, and cerebral ischemia-associated neuroinflammation.<sup>28,29,36,38,41,42,57–60</sup> Across these settings, both free melittin and formulation-based systems have shown the ability to reduce inflammatory mediators, limit tissue injury, and improve functional or histologic outcomes, but the most interpretable studies are generally those in which route of administration itself helps define tissue selectivity.<sup>28,29,36,39,41,42,57–60</sup>

Table 3 supports a consistent distinction within the anti-inflammatory literature.<sup>7,28,29,36,38,39,41,42,57–60</sup> In localized or organ-confined settings, such as ulcerative colitis, wound repair, allergic dermatitis, and microneedle-assisted arthritis therapy, melittin-based treatment can be linked more directly to reduced cytokine burden, improved histology, and better local tolerability.<sup>36,42,57,58,60</sup> By contrast, studies using systemic administration in acute liver failure, lumbar spinal stenosis, cerebral ischemia, or vascular inflammatory remodeling remain valuable, but they depend more heavily on mechanistic interpretation and controlled dosing to separate therapeutic benefit from the intrinsic exposure liability of free melittin.<sup>28,29,38,41</sup> A second pattern is that formulation engineering does not simply enhance efficacy; in several models, it also improves interpretability by lowering hemolysis, reducing local irritation, or concentrating activity in inflammatory macrophages or lymphoid compartments.<sup>36,39,41,60</sup> Taken together, the evidence in Table 3 indicates that melittin is most plausibly developed for anti-inflammatory use in settings where route, lesion localization, and formulation control can be aligned within the same therapeutic strategy.<sup>7,36,39,42,57–60</sup>

## Translational Considerations for Melittin Nanocarriers

At this stage, the central issue is no longer whether nanocarrier incorporation can improve the apparent in vivo performance of melittin in isolated preclinical models. That has been shown repeatedly across lipidic, polymeric, inorganic, targeted, responsive, and multifunctional systems.<sup>16,61–63</sup> The more difficult question is which formulation classes can carry that improvement into a development setting where product definition, reproducibility, long-term safety, pharmacological interpretability, and manufacturing control matter as much as biological activity.<sup>18,64</sup> In this sense, translational evaluation of melittin nanomedicine depends less on the number of available platforms than on whether the evidence supporting each platform can be read as product-relevant rather than proof-of-concept alone.<sup>20,65</sup> A stepwise translational evaluation framework for development-relevant melittin nanocarriers is presented in Figure 2.

## Nonclinical and Preclinical Evidence

Preclinical evidence already supports a clear conclusion: nanocarrier incorporation can materially alter how melittin is distributed, tolerated, and administered in vivo.<sup>5,16,39</sup> Across current studies, formulation design has repeatedly been associated with reduced hemolysis, improved lesion-site accumulation, prolonged retention, route-specific delivery, or more favorable short-term tolerability compared with free melittin.<sup>12,44,63</sup> At the same time, the field is characterized by marked heterogeneity in model selection, administration route, formulation architecture, and readout depth, which weakens direct comparison across studies and limits how confidently one can rank platforms on the basis of efficacy alone.<sup>18,65</sup>

This issue is especially relevant for melittin because the same membrane activity that underlies therapeutic benefit also defines the main source of risk.<sup>4,23</sup> A formulation that shows tumor suppression, antiviral activity, or attenuation of inflammatory injury is not necessarily the one that offers the clearest development path.<sup>5,39,66</sup> The relevant distinction is whether efficacy is accompanied by interpretable exposure control, evidence of tolerability, and a formulation structure that can plausibly support reproducible nonclinical evaluation.<sup>63,67</sup> For this reason, the most informative next studies are

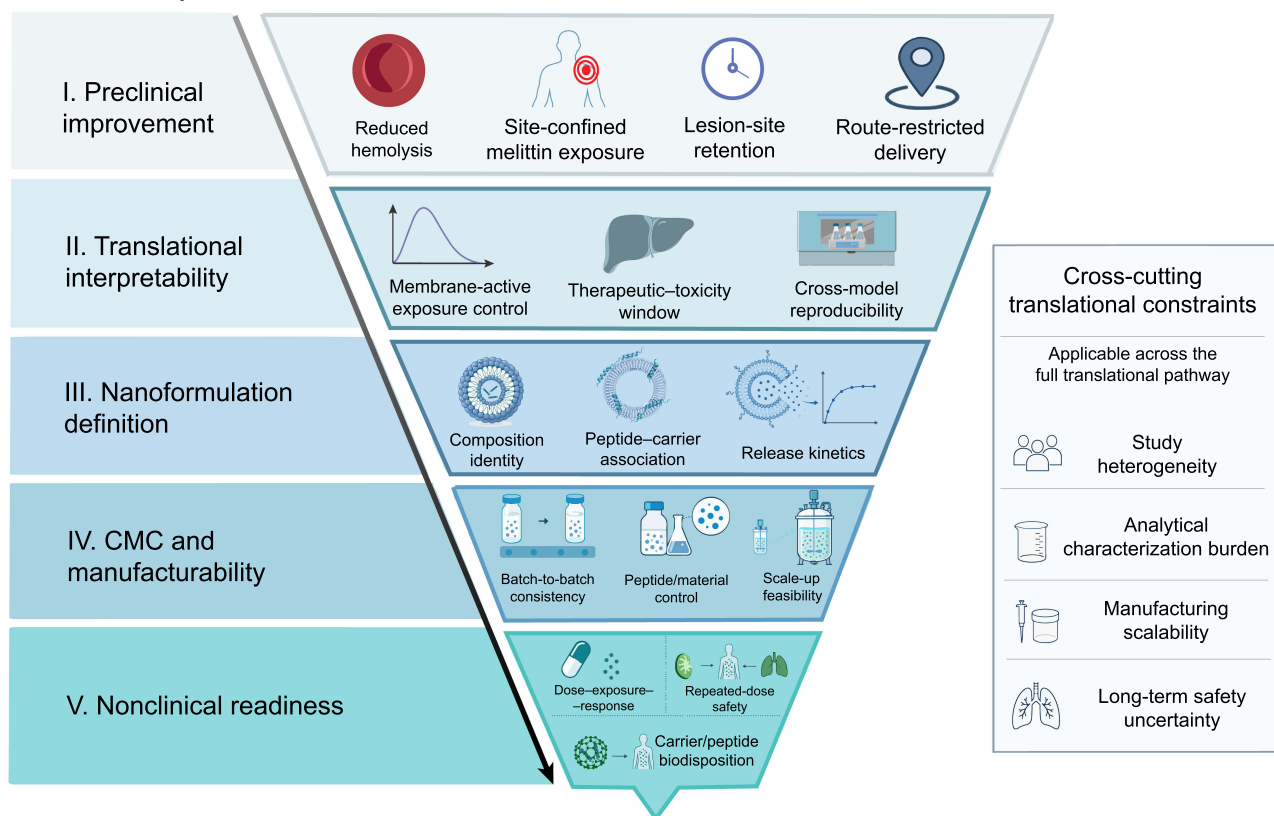
**Table 3** Melittin-Based Strategies for Inflammatory Diseases

| Platform/<br>Agent | Inflammatory<br>Setting                                | Delivery<br>Format/Route   | Key Anti-Inflammatory<br>Outcome   | Immune/Signaling<br>Effect  | Safety/<br>Selectivity   | Exposure<br>Suitability                              | Translational note   | Reference |
|--------------------|--|--|--|---|--|--|--|-----------|
| Melittin           | Skin inflammation/<br>acne-like inflammatory<br>models | Free peptide or<br>topical formulation                                     | Reduced inflammatory<br>swelling and granuloma-like<br>tissue responses in skin<br>inflammation-related<br>models          | Inhibition of IKK/I $\kappa$ B/NF- $\kappa$ B and p38 signaling<br>pathways   | Topical or local<br>use may<br>restrict<br>systemic<br>exposure  | Strong local<br>suitability                          | Topical or local use may<br>be clinically practical, but<br>irritation, sensitization,<br>and formulation stability<br>require assessment            | [7]       |
| Melittin           | Acute liver failure,<br>D-GalN/LPS-induced             | Free peptide; IP,<br>2–8 mg/kg, optimal<br>4 mg/kg                         | Improved survival; reduced<br>ALT/AST and liver injury;<br>decreased TNF- $\alpha$ and IL-1 $\beta$                        | Inhibited PKM2-mediated<br>Warburg effect in<br>activated macrophages   | No significant<br>in vivo toxicity<br>reported within<br>the effective-<br>dose range                  | Systemic acute-<br>use scenario                      | Supports intestinal anti-<br>inflammatory potential  | [28]      |
| Melittin           | Cerebral ischemia-<br>associated<br>inflammation       | Free peptide; IP,<br>0.1–0.4 $\mu$ g/g                                     | Improved motor function<br>and blood-flow recovery;<br>reduced infarct volume,<br>edema, and inflammatory<br>cytokines     | Increased MCP1<br>expression and<br>suppressed NF- $\kappa$ B<br>activation   | Protected BV-2<br>cells from<br>inflammation-<br>induced death<br>in vitro                             | Systemic<br>experimental<br>use                      | Biologically promising, but<br>brain-targeted or<br>exposure-controlled<br>delivery is needed to<br>reduce systemic liability                        | [29]      |
| MSC@NP-<br>FA-MN   | Adjuvant-induced<br>inflammatory arthritis             | Folate-targeted<br>melittin<br>nanoparticle<br>microneedle;<br>transdermal | Improved joint<br>inflammation and bone-<br>related changes; reduced<br>inflammatory cytokine<br>expression in foot joints | Folate receptor-mediated<br>targeting of inflammatory<br>M1 macrophages   | Lower<br>hemolysis than<br>free melittin;<br>no systemic<br>toxicity or skin<br>irritation<br>reported | Strong local/<br>regional<br>suitability             | Disease-directed delivery<br>with translational promise;<br>absorption variability and<br>device–formulation<br>reproducibility remain key<br>issues | [36]      |
| Melittin           | Lumbar spinal stenosis                                 | Free peptide; SC,<br>100 or 250 $\mu$ g/kg                                 | Improved locomotor<br>recovery and reduced<br>inflammatory injury  | Increased M2<br>macrophage proportion;<br>decreased M1-related<br>changes; regulated iron<br>homeostasis and<br>macrophage polarization | Reduced<br>apoptotic cell<br>death; lowered<br>IL-6 and<br>increased IL-10                             | Regional / local-<br>leaning<br>systemic<br>exposure | Suggests<br>neuroinflammatory<br>modulation potential, but<br>delivery optimization and<br>chronic safety are needed                                 | [38]      |
| Fa-MpG<br>@LNP     | Acute lung injury /<br>cytokine storm                  | Macrophage-<br>targeted lipid<br>nanoparticle; IV                          | Reduced alveolar thickening<br>and inflammatory<br>infiltration  | Suppressed NO, ROS,<br>and inflammatory-cell<br>influx  | Targeting<br>strategy may<br>reduce<br>nonspecific<br>exposure<br>relative to free<br>peptide          | Systemic use<br>with<br>macrophage<br>targeting      | Promising acute<br>inflammatory platform,<br>but macrophage targeting,<br>repeat-dose safety, and<br>pulmonary biodistribution<br>need consolidation | [39]      |

|                       |   |  |  |  |  |   |  |      |
|-----------------------|---|--|--|--|--|---|--|------|
| MeLioN                | Intracranial arterial inflammation / dolichoectasia model | Melittin-loaded iron oxide nanoparticle; IV, 2.5 mg/kg, five doses   | Prevented arterial-wall enlargement and ECM degeneration; reduced MCP-1, CD68 <sup>+</sup> macrophage recruitment, TNF- $\alpha$ , NF- $\kappa$ B, and MMP-9 | Macrophage-associated nanoparticle accumulation with suppression of vascular inflammatory remodeling | Favorable biosafety profile and brain clearance reported in healthy mice             | Organ-directed systemic delivery        | Interesting vascular/ neuroinflammatory niche; iron oxide fate, repeat dosing, and long-term neurovascular safety need stronger evidence | [41] |
| Melittin              | Ulcerative colitis, acetic acid-induced                   | Free peptide; oral, 40 $\mu$ g/kg for 5 days                         | Improved body-weight gain; reduced colon mass index; decreased TNF- $\alpha$ , IL-6, PGE <sub>2</sub> , COX-2, and sPLA <sub>2</sub> ; improved histology    | Suppressed TLR4/ TRAF6-mediated NF- $\kappa$ B and p38 MAPK signaling                                | Reduced oxidative stress, with decreased MDA and increased SOD/ GSH                  | Organ-focused gastrointestinal exposure | Useful oral proof-of-concept, but luminal stability, absorption, and exposure control require clarification                              | [42] |
| Melittin              | Ulcerative colitis, DSS-induced                           | Free peptide; oral, 2.4 mg/kg/day                                    | Reduced disease activity index; restored colon length and colon weight/ length ratio; improved histological damage   | Antioxidant and anti-inflammatory effects, including decreased MDA and increased SH/ SOD/CAT         | Improved colitis severity as monotherapy and with sulfasalazine                      | Organ-focused gastrointestinal exposure | Supports intestinal anti-inflammatory potential, but formulation-based retention could improve interpretability                          | [57] |
| MEL-DCL nano-complex  | Full-thickness wound healing                              | Nano-complex hydrogel; topical                                       | Near-complete wound closure by day 10; improved re-epithelialization, granulation tissue formation, and collagen-related markers                             | Reduced IL-6, IL-1 $\beta$ , and TNF- $\alpha$ ; increased HIF-1 $\alpha$ and TGF- $\beta$ 1         | Enhanced antioxidant activity; effect attenuated by TGF- $\beta$ receptor inhibition | Strong local suitability                | Clinically approachable topical scenario; requires hydrogel reproducibility, local release control, and irritation testing               | [58] |
| Melittin              | Rheumatoid/ inflammatory arthritis                        | Free peptide or formulation-based approaches; SC, IP, or transdermal | Reduced swelling, pain, stiffness, and inflammatory injury   | Modulated inflammatory signaling, including JNK- and cytokine-associated pathways                    | Local delivery preferable because systemic exposure may amplify toxicity             | Local or regional delivery preferred    | Broad efficacy signal, but models, schedules, and delivery formats remain heterogeneous  | [59] |
| $\alpha$ -melittin-NP | Allergic contact dermatitis/AD-like skin inflammation     | Melittin-carrying lipid nanoparticle; intradermal low dose           | Inhibited ear swelling; reduced serum IFN- $\gamma$ ; decreased leukocyte infiltration and tissue injury   | Restricted DC maturation, RelB activity, and effector T-cell activation                              | Reduced local skin damage and mast-cell degranulation relative to melittin           | Strong local suitability                | Strong local immune-modulation evidence; long-term local immune effects should be assessed   | [60] |

**Abbreviations:** AD, atopic dermatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; COX-2, cyclooxygenase-2; DC, dendritic cell; DSS, dextran sulfate sodium; ECM, extracellular matrix; GSH, glutathione; HIF-1 $\alpha$ , hypoxia-inducible factor-1 $\alpha$ ; IFN- $\gamma$ , interferon- $\gamma$ ; IP, intraperitoneal; IV, intravenous; LNP, lipid nanoparticle; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MMP-9, matrix metalloproteinase-9; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NO, nitric oxide; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; ROS, reactive oxygen species; SC, subcutaneous; SOD, superoxide dismutase; sPLA<sub>2</sub>, secretory phospholipase A<sub>2</sub>; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

## Stepwise translational evaluation framework for melittin nanocarriers



### Integrated criteria for development-relevant melittin nanocarriers

**Figure 2** Stepwise translational evaluation framework for melittin nanocarriers. The framework summarizes key development criteria for melittin nanocarriers, including preclinical improvement, translational interpretability, nanoformulation definition, chemistry, manufacturing, and controls (CMC) feasibility, and nonclinical readiness.

not simply additional proof-of-concept reports, but better-standardized studies that connect formulation attributes, tissue exposure, and biological effect within the same framework.<sup>18,65</sup>

## Regulatory Grounding for Peptide Nanotherapeutics

Melittin enters development first as a peptide drug substance and only then as part of a nanocarrier product.<sup>68,69</sup> Retained biological activity is therefore necessary but insufficient; the formulation must also be definable, reproducible, and interpretable as a product.<sup>70,71</sup> This principle becomes especially important in melittin systems because relatively small changes in loading stability, release behavior, or surface structure may alter both efficacy and membrane-associated toxicity.<sup>4,12,44</sup> In practical terms, regulatory readiness in this field is inseparable from scientific maturity.<sup>18</sup>

The current literature already reflects a gradual shift from exploratory carrier design toward more product-oriented thinking.<sup>18,19</sup> Simpler lipidic or composition-defined systems often make this transition more readily because their material identity and quality attributes are easier to define.<sup>72–74</sup> By contrast, highly layered targeted, responsive, or multifunctional systems may show stronger biological breadth but usually demand more extensive analytical characterization before their preclinical results can be interpreted as development-relevant.<sup>64,75,76</sup> Regulatory considerations in melittin nanomedicine should therefore not be viewed as external constraints imposed after discovery, but as part of the criteria by which platform credibility is recognized from the outset.<sup>18,71,72</sup>

## CMC and Manufacturability Considerations

Once melittin nanocarriers are considered as candidate products rather than experimental constructs, CMC becomes part of the core scientific question.<sup>70,71</sup> A platform is valuable not only for what it does biologically, but for whether its

composition, critical attributes, and manufacturing behavior can be defined with sufficient consistency to support development.<sup>18,64</sup> In melittin systems, this matters acutely because changes in formulation behavior may alter both the degree of target exposure and the extent to which hemolytic or nonspecific cytolytic activity remains restrained.<sup>4,12</sup>

### Source and Material Control

The reliability of any melittin nanocarrier begins with the definition of the peptide input itself.<sup>68–78</sup> Purity, impurity profile, degradation tendency, and source-related reproducibility all influence downstream loading, release, and batch comparability.<sup>79–81</sup> This issue is often understated when the formulation focus dominates the discussion, but for melittin it is particularly relevant because peptide integrity directly affects membrane activity and therefore the biological behavior of the final product.<sup>2,4</sup> Source definition should thus be treated as part of the formulation quality framework rather than as a separate upstream consideration.<sup>71,82</sup>

### Particle Reproducibility and Formulation Robustness

Particle reproducibility is the next defining layer of formulation control.<sup>72,77</sup> Early-stage reports often describe successful nanoparticle formation through particle size, zeta potential, morphology, or loading efficiency measured in a single prepared batch.<sup>12,16,17</sup> These readouts remain important, but translationally the relevant issue is whether they behave as controlled quality attributes rather than descriptive outputs.<sup>70,71</sup> This distinction is especially important in melittin systems because small shifts in particle structure or loading stability may change release behavior and safety margins.<sup>12,44</sup> The problem is therefore not successful encapsulation per se, but whether those particle features remain stable across batches, media, and preparation conditions.<sup>64,77</sup>

This burden increases as platform architecture becomes more sophisticated.<sup>20,64</sup> Ligand-modified systems require additional control of surface density and binding performance.<sup>16,32,75</sup> Responsive systems depend on trigger reliability under biologically realistic conditions.<sup>31,47,75</sup> Co-delivery platforms must preserve coordinated loading and release of more than one active component.<sup>33,83</sup> These demands do not diminish the scientific value of such systems, but they do mean that formulation robustness, comparability, and process windows become integral to how their biological results should be interpreted.<sup>64,70,71</sup>

### Endotoxin, Sterility, and Injectability Suitability

For parenteral melittin nanocarriers, endotoxin control, sterility assurance, and injectability suitability are not ancillary technical details.<sup>72,84,85</sup> They are part of mechanistic credibility. Where cytokine modulation, inflammatory injury, immune activation, or macrophage behavior are central readouts, uncertainty in formulation cleanliness can blur the distinction between peptide effect, carrier effect, and product-related artifact.<sup>39,84,86</sup> This is particularly relevant in melittin research, where many of the most interesting biological outcomes overlap with inflammatory and immunological pathways.<sup>2,8</sup>

### Scale-up Feasibility

Scale-up feasibility is where formulation elegance is tested against product reality.<sup>64,87</sup> Favorable loading, toxicity attenuation, or lesion-targeting under optimized laboratory conditions does not automatically translate into scalable product behavior.<sup>19,20</sup> The relevant question is whether those same features remain intact when manufacturing conditions broaden, batch numbers increase, and control over composition and release must be maintained prospectively rather than retrospectively.<sup>70,72</sup> Simpler lipidic, liposomal, or composition-defined systems generally offer a clearer path in this regard, whereas highly layered bioinspired, multifunctional, or hybrid systems impose a heavier development burden as process complexity rises.<sup>73,88,89</sup> In melittin formulations, that increase matters because structural inconsistency may directly affect the very toxicity profile that nanocarrier design is meant to improve.<sup>4,77</sup>

## Nonclinical Evaluation: Long-Term Safety and PK/PD Characterization

Nanocarrier engineering has most consistently improved the short-term tolerability profile of melittin *in vivo*.<sup>5,12,60</sup> Reduced hemolysis, preserved body weight, limited acute organ injury, lower local irritation, and improved lesion-site restriction are all repeatedly reported across several formulation classes.<sup>12,39,44</sup> These findings are important, but short-

term tolerability is not equivalent to nonclinical safety maturity.<sup>90,91</sup> For melittin, that distinction is central because the mechanism of benefit and the mechanism of harm remain closely linked.<sup>2,4</sup>

A development-relevant safety package requires more than acute tolerability signals.<sup>91,92</sup> Repeated-dose toxicity, delayed organ injury, recovery-phase reversibility, complement-related effects, vascular compatibility, hypersensitivity risk, and the fate of carrier materials become especially important once melittin is evaluated as a formulation-dependent therapeutic rather than as an isolated proof-of-concept peptide.<sup>86,93,94</sup> This issue is less pressing in localized or route-defined applications, where exposure is partly constrained by administration strategy itself, but becomes much more important when the intended use involves intravenous delivery, complex multifunctional carriers, or prolonged circulation.<sup>67,73,95</sup>

PK/PD characterization remains less mature than formulation innovation.<sup>63,67,96</sup> Many studies still infer in vivo behavior from imaging-based biodistribution, lesion accumulation, or stimulus-responsive release-related observations.<sup>16,41,43</sup> These approaches are useful for showing that a system reaches the intended site, but they do not by themselves establish a comparable dose–exposure–response framework across platforms.<sup>63,69,97</sup> What is often still missing is a direct connection between administered dose, tissue-level exposure, released peptide, and pharmacological consequence.<sup>67,98</sup> This gap becomes particularly important when platform classes differ substantially in structural complexity, trigger dependence, or local versus systemic administration, because the same efficacy signal can arise from very different exposure conditions.<sup>64,76,83</sup>

At this point, additional proof-of-concept studies are less informative than better-standardized ones.<sup>18,65</sup> The field stands to gain more from comparable nonclinical evaluation than from continued expansion in platform diversity alone.<sup>19,64</sup> The formulations most worth prioritizing are those that can link long-term safety, PK/PD behavior, and therapeutic effect within one coherent framework.<sup>63,90,91</sup> That requirement does not exclude targeted, responsive, or multifunctional systems, but it does raise the evidentiary threshold they must meet before they can be regarded as product-relevant rather than conceptually promising.<sup>75,76,83</sup>

## Translational Prioritization and Future Outlook

Not all melittin nanocarriers occupy the same developmental position, even when several produce clear biological effects in preclinical models.<sup>5,39,99</sup> What separates them is no longer efficacy alone, but the balance they achieve among formulation simplicity, exposure control, pharmacological interpretability, and manufacturing burden.<sup>18,64</sup> As summarized in Table 4, the most development-relevant systems at present are generally those with clearer material architecture and more tractable quality attributes, particularly simple lipid or composition-defined platforms and route-constrained local or regional delivery systems.<sup>73,88,95</sup> These classes are not intrinsically superior in all biological settings, but they are more readily interpreted as candidate products rather than as single-study constructs.<sup>19,20</sup>

Table 4 also clarifies why several more sophisticated platform classes occupy a more conditional position.<sup>64,75,76</sup> Targeted systems can improve lesion-site binding or cell-class selectivity, as shown by aptamer-, folate-, estrone-, or integrin-directed melittin carriers, but they also introduce additional demands in ligand control, surface reproducibility, and cross-study comparability.<sup>5,32,101</sup> Stimulus-responsive systems, including pH- or microenvironment-activated formulations, are mechanistically attractive because they seek to confine melittin activity to tumor-relevant conditions.<sup>31,47,76</sup> In both cases, the central question is not whether the concept is compelling, but whether the added functional sophistication yields a sufficiently clear advantage to justify the extra analytical and manufacturing burden.<sup>64,70</sup>

A similar distinction applies to co-delivery, inorganic, and bioinspired multifunctional platforms.<sup>33,89,100</sup> These systems have generated some of the most conceptually ambitious results in the current literature, including improved intracellular uptake, immune remodeling, photodynamic or photothermal synergy, and resistance-reversal effects.<sup>33,47,100</sup> Yet their interpretive burden is correspondingly heavier: multiple active components, layered structures, or hybrid materials make it harder to isolate the contribution of each element, define critical quality attributes, and project scalable product behavior.<sup>64,83,104</sup> For that reason, these platforms remain highly informative as innovation engines for the field, but they are not automatically the most immediate translational candidates simply because they are biologically broader.<sup>18,19</sup> In melittin nanomedicine, greater functionality often improves mechanistic reach while simultaneously raising the threshold for reproducibility, PK/PD interpretation, and CMC maturity.<sup>64,70</sup>

**Table 4** Translational Outlook of Melittin Nanocarrier Platforms

| Platform                                    | Therapeutic Rationale  | Translational Advantage   | IND-enabling Gap   | CMC Concern   | PK/PD & Safety Maturity  | Translational Outlook  | Reference              |
|---|--|---|--|---|--|--|------------------------|
| Simple lipid or composition-defined systems | Reduce free-melittin membrane toxicity while enabling more controllable delivery | Clearer composition, relatively mature formulation logic, stronger scale-up potential, and more straightforward quality-attribute control | Missing integrated evidence linking product identity, reproducibility, tissue exposure, released melittin, efficacy, and safety        | Batch consistency, peptide loading stability, release control, storage stability, sterility, and endotoxin control          | PK/PD: limited to moderate; long-term safety: limited, mainly short-term | Higher priority for early development, especially when paired with route-defined or regional exposure strategies | [12,15,25,26,44,73,74] |
| Localized or regional delivery systems      | Confine melittin exposure to accessible lesions and reduce systemic burden       | Better risk–benefit profile and clearer route-specific exposure–response interpretation   | Insufficient evidence for route consistency, human absorption behavior, local retention, and device/ formulation integration           | Dose uniformity, fabrication consistency, local release control, sterility, and tissue compatibility                        | PK/PD: context-dependent; long-term safety: still limited                | Higher priority when disease localization is clinically realistic  | [16,36,43,60,95,100]   |
| Targeted systems                            | Improve lesion selectivity and local accumulation through active targeting       | Potentially higher specificity and lower off-target exposure than passive delivery alone  | Need to show that targeting adds development-relevant value beyond improved uptake or short-term efficacy                              | Ligand-density control, conjugation reproducibility, surface stability, binding-performance assays, and batch comparability | PK/PD: limited; long-term safety: limited                                | Moderate priority; conditionally favorable if targeting provides a clear functional advantage                    | [5,32,39,43,75,101]    |
| Stimulus-responsive systems                 | Trigger melittin release or activation under disease-associated conditions       | Conditional release may improve lesion-confined activity and reduce premature membrane disruption   | Insufficient proof of reproducible trigger sensitivity, activation selectivity, and release behavior in heterogeneous in vivo settings | Trigger standardization, release-test robustness, process complexity, and stability of responsive linkages                  | PK/PD: limited; long-term safety: limited                                | Moderate priority; dependent on trigger robustness and in vivo activation clarity                                | [31,47,76]             |

(Continued)

**Table 4** (Continued).

| Platform  | Therapeutic Rationale  | Translational Advantage  | IND-enabling Gap  | CMC Concern  | PK/PD & Safety Maturity                             | Translational Outlook  | Reference          |
|---|--|--|---|--|---|--|--------------------|
| Co-delivery or combination-oriented systems     | Use melittin as a sensitizer, membrane-disruptive enhancer, or cooperative payload rather than a standalone agent  | Better aligned with current evidence showing synergy with chemotherapy, phototherapy, nucleic-acid delivery, or immunomodulation | Need coordinated exposure, justified dose ratio, and clear mechanistic contribution of melittin relative to other payloads    | Payload-ratio control, co-loading reproducibility, co-release consistency, multi-component specifications, and comparability       | PK/PD: limited; long-term safety: limited           | Moderate to higher scientific value, but development-intensive when multiple active components are integrated    | [33,47,48,83,102]  |
| Inorganic, magnetic, or MOF-based systems       | Exploit tunable structure, high loading, imaging compatibility, external guidance, or stimulus-responsive behavior | Strong mechanistic novelty and flexible materials design   | Chronic biocompatibility, degradation fate, tissue retention, and material-specific liabilities remain underdefined           | Material reproducibility, impurity control, degradation profile, residual metals, colloidal stability, and scale-up comparability  | PK/PD: very limited; long-term safety: very limited | Lower to conditional priority until biodegradation, tissue clearance, and repeat-dose safety are consolidated    | [31,45,49,103,104] |
| Bioinspired, hybrid, or multifunctional systems | Combine targeting, immune modulation, responsiveness, penetration enhancement, and co-delivery within one platform | Strong proof-of-concept efficacy and high mechanistic sophistication   | Development burden is high because multiple structural and functional variables must be defined and controlled simultaneously | Process complexity, analytical verification, component comparability, release control, product specifications, and reproducibility | PK/PD: very limited; long-term safety: very limited | Lower immediate development priority; primarily innovation-oriented unless product-level control is strengthened | [31,37,47,48,89]   |

**Notes:** Translational outlook does not represent a simple efficacy ranking. It reflects the relative likelihood that each platform class can support IND-enabling advancement under current evidence conditions. In the “PK/PD and safety maturity” column, PK/PD status is listed first, followed by long-term safety status.

**Abbreviations:** CMC, chemistry, manufacturing, and controls; IND, investigational new drug; MOF, metal–organicframework; PK/PD, pharmacokinetics/pharmacodynamics.

Taken together, the comparison in Table 4 suggests a practical near-term principle for the field.<sup>18,64</sup> Melittin becomes more clinically credible when its potency is made governable through delivery design rather than merely amplified through structural complexity. In that sense, the most plausible early-entry applications are those in which route, formulation clarity, and exposure restriction can be aligned within the same therapeutic strategy.<sup>67,73,95</sup> More elaborate targeted, responsive, and multifunctional systems remain important, but their progression depends on stronger evidence that biological sophistication is matched by product-level control.<sup>75,76,83</sup> The near-term future of melittin nanomedicine is therefore likely to favor platforms that simplify interpretation while still restraining peptide toxicity, rather than those that maximize functional layering without equivalent gains in development readiness.<sup>18,64</sup>

## Conclusion

Melittin remains a compelling but intrinsically difficult therapeutic molecule. Its appeal lies in the unusual breadth of its biological activity: the peptide can act directly on membranes, trigger downstream intracellular stress responses, and reshape inflammatory or immune signaling in ways that are relevant across cancer, infection, and inflammatory disease. Yet this same breadth also defines its central limitation. Melittin is not a conventional drug whose efficacy and toxicity can be cleanly separated at the molecular level; rather, its therapeutic effect and its liability arise from the same membrane-active foundation. For that reason, the central problem in melittin nanomedicine is not simply how to enhance activity, but how to govern it.

The literature reviewed here suggests that nanocarrier design should be understood in exactly that sense: not as a secondary formulation refinement, but as the primary means by which melittin becomes pharmacologically usable *in vivo*. Across diverse platforms, the most meaningful advances have come from restricting premature membrane exposure, improving tissue or cellular selectivity, and creating conditions under which melittin can act within a more interpretable therapeutic window. This is why the field has gradually moved beyond asking whether melittin is active and toward asking what kind of delivery architecture is required to make that activity spatially, temporally, and biologically controllable.

Viewed across application settings, melittin does not occupy a single therapeutic role. In oncology, it is most persuasive when incorporated into delivery-controlled strategies that balance tumoricidal potency with selectivity and tolerability. In antiviral research, the evidence is strongest where structurally accessible stages of infection remain vulnerable to direct peptide action, but broader systemic development remains less mature. In inflammatory disease, the therapeutic logic is often clearest when route of administration, lesion localization, and immune modulation can be aligned within the same intervention. These differences matter because they show that the value of melittin is not fixed; it depends on how disease context interacts with exposure pattern and formulation behavior.

This review also makes clear that preclinical promise is not evenly translatable across all nanocarrier classes. Platforms that appear highly sophisticated biologically are not always those best positioned for development, whereas simpler or more route-defined systems may offer a clearer path because their behavior is easier to reproduce, interpret, and control. The decisive distinctions at this stage therefore lie less in whether a platform can generate efficacy in a favorable model, and more in whether it can support a coherent product logic that includes formulation robustness, PK/PD interpretability, long-term safety relevance, and manageable manufacturing burden. In melittin nanomedicine, translational credibility begins where biological performance and product-level discipline start to converge.

Taken together, the field no longer lacks proof that melittin can be therapeutically useful. What it still lacks, in many cases, is a sufficiently mature framework for deciding when that usefulness is developmentally credible. The most plausible near-term future for melittin nanomedicine is therefore not unlimited expansion into ever more complex systems, but the disciplined advancement of platforms that make melittin's potency governable, interpretable, and clinically defensible. Under those conditions, melittin is best viewed not as a broadly deployable peptide drug in its native form, but as a high-potential therapeutic component whose real clinical value emerges only when delivery design successfully converts intrinsic activity into controlled pharmacology.

## Disclosure

The authors report no conflicts of interest in this work.

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