The Future of Nanoparticle-Directed Venous Therapy

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Abstract

Keywords

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Nanoparticles, structures of less than 200 nm capable of delivering pharmacotherapeutics to sites of disease, have shown great promise for the treatment of many disease states. While no nanoparticle therapies for deep vein thrombosis are currently approved by the Food and Drug Administration, many of the unique features of these therapies have the potential to treat both deep vein thrombosis and its most significant sequela, postthrombotic syndrome, while limiting the hemorrhagic complications of current antithrombotic therapies. Nanoparticles are complex structures with several important variables that must be considered to engineer effective therapies. This article will review the structure and engineering of nanoparticles, as well as promising molecular targets for future investigation.

Objectives: Upon completion of this article, the reader will be able to (1) describe those targets that demonstrate the greatest potential for nanoparticle therapy for deep vein thrombosis; (2) identify the basics of nanoparticle engineering, as it relates to nanoparticle therapy for deep vein thrombosis; and (3) describe the current and future developments in nanoparticle therapy for deep vein thrombosis.

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Deep vein thrombosis (DVT) is a common disease that can be life threatening, and has sequelae that can profoundly impair a patient's quality of life. Recent estimates of annual

venous thromboembolism (VTE) incidence ranges from 100 to 200 events per 100,000 person-years.¹ Postthrombotic syndrome is a chronic complication of DVT, characterized by swelling, pain, and ulceration of the lower extremity,² with the 1-year incidence of PTS after an initial episode of DVT reported to be between 25 and 50%.^{3–5}

Current therapies for DVT rely on interruption of the normal hemostatic and thrombotic process. The American College of Chest Physicians (ACCP) guidelines provide clinicians with evidence-based recommendations for treatment of DVT.⁶ Recommended anticoagulants—including intravenous unfractionated heparin, subcutaneous low-molecular-weight heparin, vitamin-K antagonists (VKAs), and the non-VKA oral anticoagulants (NOACs)—prevent propagation of thrombus and rely on innate fibrinolytic processes to break down clot. However, this therapeutic modality often does not decrease clot burden nor does it eliminate the risk of long-term recurrence or PTS. ACCP guidelines recommend 3 months of anticoagulation in most cases, but in patients with certain risk factors or with recurrent DVT, the recommendation is for indefinite anticoagulation—exposing the patient to many years of risk for complications.⁶ Because these therapies alter the coagulation mechanism

systemically, all antithrombotic therapies have undesirable systemic effects, and severe hemorrhagic complications represent an unavoidable risk of current treatment paradigms.

VKA treatment is plagued by difficulty in maintaining patients within the therapeutic range, and decreased time-in-therapeutic-range (TTR) is associated with a greater number of hemorrhagic events, and difficulty of maintaining a high TTR in patients is a result of both patient- and agent-specific factors.⁷ Though incidence of major hemorrhagic events seems to be decreased in patients treated with NOACs,⁸ perhaps more concerning is that these new anticoagulants have limited options for reversal.⁹ No clear consensus guidelines for reversal and management of bleeding or perioperative patients have yet been promulgated by any of the surgical or medical societies. Importantly, oral anticoagulants seem to have limited effect on the development of PTS.⁵ LMWH is an attractive alternative to oral anticoagulants, as it is associated with improvement in quality of life⁵—because monitoring is not required. However, well-designed trials have shown no difference in hemorrhagic complications between LMWH and VKAs.^{10,11}

Early thrombus removal strategies have been shown to reduce the incidence of postthrombotic syndrome, as duration of clot contact with vein wall is a key factor in reducing development of PTS.^{12,13} However, systemic effects are greater with the use of intrathrombus administration of thrombolytic agents. Catheter-directed thrombolysis carries a relative risk for hemorrhagic complications of 2.23 (95% CI: 1.41–3.52, $p = 0.0006$), and estimates of bleeding complications from these procedures range between 4 and 12%.¹⁴

Nanoparticle therapies hold promise for the treatment of DVT. Nanoparticles (NPs) represent a new paradigm in drug delivery, with the potential to maximize drug delivery to the site of desired action with minimization of systemic and off-target effects. A wide array of investigational NPs has therapeutic potential, with a host of available materials, structures, shapes, sizes, and targets. NPs can be engineered with any combination of characteristics—each with potential advantages and disadvantages—which affect pharmacodynamics and kinetics, delivery, intracellular trafficking, and site and mechanism of action.

Peripheral intravenous administration of therapeutics exposes them to inhibition or elimination while in the circulation of the patient. Encapsulation or incorporation of therapeutics—such as proteins, DNA, or drugs—within a nanoparticle can alter the pharmacokinetics of the drug by protecting it from inhibition or elimination.

Utilizing targeting ligands to localize delivery, nanoparticle therapies for DVT would direct NPs to the affected vessel—targeting inflammatory and/or thrombotic pathways. These therapies can be targeted directly to the thrombus, reducing hemorrhagic risk of anticoagulant or thrombolytic therapies or to the diseased vessel wall itself, potentially reducing venous remodeling that leads to postthrombotic syndrome. Furthermore, because they can deliver high doses while minimizing hemorrhagic risk, directed nanoparticle therapies may reduce duration of clot contact with vein wall, and subsequent development of PTS.

Here, we will review the recent advances in nanoparticle therapy for DVT. Currently, there are only few FDA-approved nanoparticle therapies, and none approved for DVT. Liposomal doxorubicin formulations (Doxil) are currently FDA approved for the treatment of recurrent or progressive ovarian cancer, multiple myeloma, and AIDS-related Kaposi sarcoma. Albumin bound paclitaxel (Abraxane) is currently approved for the treatment of metastatic pancreas and breast cancers, and advanced non-small cell lung cancer. Much of the literature specific to DVT is in its infancy, and consequently inferences will be made from atherosclerosis, cancer, and other diseases.

Nanoparticles

A complete review of nanoparticle engineering is beyond the scope of this review, and the focus here will be on those ways in which nanoparticle structure might impact treatment of DVT. In essence, a nanoparticle is a nonbiological structure of submicron size that can be custom synthesized. NPs have been described in many materials, including entirely inorganic drug carriers (gold, silver, silica, and carbon) or polymeric compounds, liposomes, and dendrimers, which can be designed to mimic biological constructs such as micelles, exosomes, or circulating microparticles. Of primary importance in the design of NPs is assuring biocompatibility and minimal toxicity, while maintaining clinically efficacy and maximizing the unique advantages of the nanostructure.

► **Table 1** outlines common variables used in nanoparticle engineering, and the more common solutions to these variables.

Nanoparticle Material

Inorganic NPs are constructed as crystals of relatively nonreactive inorganic elements. Gold,^{15,16} silver,¹⁷ silica,^{18,19} and carbon²⁰ have all been used. To prevent agglomeration in the circulation, an inorganic nanoparticle core is surrounded by a

Table 1 Nanoparticle engineering

Variable	Example
Material	Gold, Silver, Silica, Carbon
Inorganic	Bilayer of amphipathic lipids
Liposome	poly(lactic-co-glycolic acid) (PLGA),
Polymeric	polycaprolacton (PCL)
Dendrimer	Poly(Amidoamine) (PAMAM)
Viral	Cowpea Mosaic Virus (CPMV)
Size	10–200 nM
Shape	Spherical Discoid Cylindrical Star-shaped
Structure	Solid Hollow
Targeting Molecule	Antibody Ligand Peptide Nucleic Acid

shell of organic molecules—peptides, polymers, or lipid micelles.^{21,22} Consequently, these can be considered hybrid-NPs with the other materials discussed later.

Liposomal NPs are organic lipid-bilayer-based NPs constructed from naturally occurring amphipathic molecules, and consequently have a lower likelihood of toxicity. Liposomal NPs resemble physiologically occurring micelles, and are created as an emulsion. Polymeric NPs are constructed from biodegradable, biocompatible molecules such as poly (lactic-co-glycolic) acid (PLGA), polycaprolactone (PCL), and others. PLGA is a polyester, which is biocompatible and biodegradable, with a low toxicity, and is degraded into nontoxic metabolizable and excretable byproducts. Polymeric NPs can be constructed either as solid structures or as a bilayer of amphipathic molecules similar to a liposome.

Dendrimers are a type of complex molecules that have a branching pattern of functional units from a central core.²³ Like liposomes, dendrimers have an open central core, in which therapeutics can be incorporated. Unlike liposomes, dendrimers are composed of a single molecule and can thus have extensive surface modifications as their specific application requires.²⁴ As liposomal nanostructure mimics micelles or microparticles, dendrimer nanostructure mimics that of globular proteins.²⁴ Poly(Amidoamine) (PAMAM) dendrimers are among the most commonly used for medical applications, and demonstrate excellent biocompatibility and low toxicity.²⁴

Finally, engineered viral particles have been used for NP applications and drug delivery. These are attractive due to their biological origins and consequent low toxicity and biocompatibility, and are considered by some to be nature's nanoparticles.²⁵ The Cowpea mosaic virus (CPMV) is commonly used, as it has a natural binding capability to cell-surface vimentin and has been targeted to vascular lesions by exploiting this capability.²⁵ Concerns remain, however, regarding the safety of viral therapies.²⁶

Nanostructure

Nanoparticles can be synthesized in nearly any size or shape, which is an important consideration when designing nanoparticle therapies for DVT. Both size and shape affect NP behavior in the circulation, due to hemodynamic factors and shear stresses. Larger nanoparticles may be more likely to accumulate in small vessels due to mechanical trapping,²⁷ and potentially have more binding capacity than smaller particles with the same density of coating due to steric effects and increased likelihood of multivalent binding.²⁸ Moreover, size and shape affect uptake of NPs into cells, and spherical particles are also more efficiently internalized by endothelial cells than tubular or disc-shaped ones.²⁹ Mechanical conformability of NP is similarly important, as shear stress in the vessel lumen affects the detachment force of NPs from their targets, and affects the ability of NPs to navigate through small flow channels or capillaries.³⁰

Nanoparticle Clearance

Clearance of NPs can occur via hepatic degradation, renal filtration, or by opsonization and elimination via the reticu-

loendothelial system (RES). Both the size and the shape of NPs have been shown to influence rate of clearance. Smaller particles, especially those less than 10 nm, are primarily eliminated by renal filtration.

Binding of polyethylene glycol (PEG) to the surface of the nanoparticle has been shown to reduce opsonization and increase time that the drug is present in the circulation and thus increase the likelihood that the particle will reach its desired target.³¹ While effective, one limitation is that PEGylated NPs demonstrate increasing rates of clearance upon repeated administration.³² PEGylated nanoparticles have been demonstrated, however, to have the potential for catastrophic anaphylactic and pseudoallergic complement-system-related reactions; the nature and minimization of this risk must be addressed as these NP therapeutics evolve.³³

Another technique to affect clearance is modifying nanoparticle surfaces with glycoproteins such as CD47.³¹ CD47 is a cell surface glycoprotein involved in the self-recognition process during leukocyte phagocytosis, which interacts with monocyte-macrophage signal regulatory protein- α , an anti-inflammatory regulator. Binding of CD47 to the surface of the nanoparticle may reduce phagocytosis by members of the monocyte-macrophage lineage, and consequent clearance of the NPs by the RES.³¹

Nanoparticle Uptake

Another important consideration in the design of NPs is the mechanism of uptake that required for many types of nanoparticle-delivered pharmacotherapy. Endothelial cells are capable of several different cellular uptake mechanisms, including receptor-mediated endocytosis, uptake via caveolae, and phagocytosis.³⁴

The nature of uptake and intracellular trafficking affect the time of delivery of therapeutics to its target within the cell.^{19,29,35,36} This then must be taken into account when designing NPs for specific targets—targeting thrombus-endothelial interaction may require a small, non-internalized NP, whereas targeting the endothelium itself, for administration of a therapeutic which would limit vein wall fibrosis, may require differently designed NPs.

Nanoparticle Targeting

Targeting of NPs to the site of desired action is either passive or active. In passive targeting, nanoparticles accumulate due to mechanical forces such as hydrostatic pressure and transudation. Smaller NPs have been shown to passively accumulate in sites of inflammation, due to increased vascular permeability in those regions. This is exemplified by accumulation of NPs within tumors due to leaky capillary beds within the cancer.³⁷

Active targeting directs NPs to the site of desired effect, which can be done by covalent bonding of a locator molecule to the surface of the nanoparticle itself.^{38,39} These bound locator molecules can be antibody fragments, cellular ligands, or small peptides⁴⁰ with an affinity for the predetermined target at intended site of activity at which the drug is to be released.

The binding avidity of NPs—determined by affinity and density of ligand on the surface of the NP—can affect the efficacy of NP therapy. Moreover, the density of the target molecule on the surface of the target cell affects uptake of NP through increased likelihood of multivalent binding.^{41,42} Increased avidity may enhance off-target effects such as binding of NP with greater strength to nonactivated endothelium.

An important consideration in the engineering of targeted NP therapies are off-target effects and unexpected, on-target effects, also called adverse inhibition. Off-target effects would include action of the therapeutic at other than the site of desired action. Drug leakage from the nanoparticle into the systemic circulation—an effect of NP porosity and duration of circulation prior to arrival at the target site—will naturally have similar adverse effects to systemic administration of the same drug.

Ideal nanoparticle targets must be safe and specific to the greatest extent possible. In regard to safety, the binding of NPs to target molecules may affect signaling pathways mediated via those molecules causing on-target, but unexpected effects such as initiating thrombosis instead of promoting lysis. For example, anti-P-selectin antibody has been shown to have beneficial effects on thrombus resolution and vein wall fibrosis.⁴³ Thrombomodulin targeting, on the other hand, has been shown to paradoxically increase the likelihood of thrombosis in pulmonary endothelium by interfering with its thrombin-inhibiting action.⁴⁴

Potential Targets

Many potential targets have been described, and these have varying degrees of specificity for the thrombus itself. Molecular targets must be accessible from the blood stream in peripherally administered NP therapies, and to minimize systemic effects, the ideal nanoparticle therapy will have a relatively high level of specificity to the area of desired effect. A challenge when designing nanoparticle therapies is that all vascular beds do not express the same surface receptors, each cell in a particular bed may not present the same receptors at the same concentrations, and many receptors are present on multiple cell types.^{37,45}

Two general categories of targets will be discussed on the treatment of DVT with NPs: endothelial targets and targets within the intraluminal thrombus. ►Table 2 outlines some of the potential target molecules for nanoparticle therapy for deep venous thrombosis.

Endothelial Targets

Thrombosis requires paired activation of thrombotic and inflammatory pathways. Development of pathologic thrombus is a complex process, in which numerous systemic, local, and genetic factors contribute to an imbalance in physiologic pro- and antithrombotic and inflammatory cascades. Circulating microparticles, platelets, and endothelial cells are the primary effectors of this process, though leukocytes contribute as well, particularly at later time points. Normal hemo-

Table 2 Potential target molecules for nanoparticle therapy for DVT

<i>Constitutively Expressed Endothelial Targets</i>
ICAM-1 PECAM-1
<i>Expressed on Activated Endothelium</i>
VCAM-1 P-selectin E-selectin
<i>Targets in the Intraluminal Thrombus</i>
von Willebrand Factor GPIb Receptor GPIIa/IIIb Receptor Fibronectin Fibrin Factor XIII

stasis is initiated by disruption of the endothelial lining, triggering endothelial activation and exposure of negatively charged collagen to the blood. These events in turn activate platelets in the bloodstream, induce leukocyte rolling and extravasation, and set off cascades of serine proteases (the intrinsic and extrinsic pathways) culminating in the cleavage of fibrinogen to fibrin, and crosslinking of fibrin by factor XIII. Numerous feed-forward mechanisms drive this process toward thrombus development, and these pathways interlock at several points with antithrombotic and fibrinolytic pathways. Imbalance at any of these steps can drive the normal thrombotic process to pathologic thrombosis.

Constitutively Expressed Targets

Activation of the endothelium after vessel wall injury causes translocation of the cellular adhesion molecules to the cell surface. These molecules are transmembrane glycoproteins involved in the tight adhesion of circulating leukocytes to the endothelium, and have been demonstrated to play a role in the thrombotic process by allowing adhesion of inflammatory cells to the damaged endothelium. Vascular cell adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), and intercellular adhesion molecule-1 (ICAM1) targeted NPs have been created, using antibodies bound to the surface of the nanoparticle.^{46–50} PECAM is present on platelets, though in much lower concentrations than on the endothelium. PECAM is constitutively presented on the surface endothelial cells—whether activated or nonactivated. Because of this, PECAM allows for targeting to endothelium—but not specifically to activated endothelium. Like PECAM-1, ICAM-1 is constitutively expressed and present in significant quantity on activated and nonactivated endothelium, though ICAM-1 is upregulated in inflamed endothelium.³⁶

Furthermore, for constitutively expressed endothelial markers, such as PECAM-1 and ICAM-1, NPs targeted to the endothelium can accumulate in the lung because of the enormous surface area of endothelium present there.⁴¹ For

example, tissue plasminogen (tPA) has been shown to traffic to the lung when using an anti-ICAM antibody.^{51,52} Despite this, successful targeting has been shown of PECAM-1-conjugated antibodies infused peripherally to cardiac and mesenteric endothelium,⁵³ and anti-ICAM-1 NPs with decreased avidity may be more likely to specifically target to diseased endothelium.⁵⁴ This is an important consideration in the engineering of nanoparticles for the treatment of DVT, and potentially makes these less attractive targets DVT therapy specifically as accumulation of nanoparticles at sites other than the diseased vessel could put patients at risk for hemorrhagic complications. However, NPs targeted to a pan-endothelial marker may be preferable for the creation of NPs for DVT prophylaxis.

Anti-ICAM NPs have been targeted successfully to ICAM-1, and by adjusting the size of the NP, internalized or non-internalized NPs can be created.⁵¹ This may have implications for differential drug delivery either to endothelium or to intraluminal thrombus. For example, it allows an NP to bind to a target on the endothelium, rather than being internalized by the cell, to remain within the vessel lumen at the thrombus-endothelium interface.

Targets Upregulated in Activated Endothelium

VCAM-1 is another glycoprotein of the cellular adhesion molecule class, which is upregulated with endothelial activation. Unlike ICAM-1 and PECAM-1, VCAM is not expressed at high levels constitutively on endothelial cells. Pan et al created a VCAM-1-targeted perfluorocarbon nanoparticle with a peptide sequence that acts as a VCAM-1 ligand for targeting to atherosclerotic lesions and breast cancer in a mouse model.⁴⁰ Nanoparticles targeted to VCAM-1 bound four times more efficiently to atherosclerotic aortas than nontargeted nanoparticles.⁴⁰

P- and E-selectin are *selectins*, a class of molecules that translocate to the surface on activated endothelium, and are involved in the process of leukocyte “rolling”—where circulating leukocytes are slowed down by selectins allowing for their tight binding to CAMs. P-selectin has been extensively studied as a primary regulator of thrombosis, and marker of vein wall injury. Unlike ICAM and PECAM, selectins are not constitutively expressed on the endothelium, and are present only in an activated state. As they are present only in a limited way on nonactivated endothelium,³⁶ and are induced during the thrombotic process, they are natural targets for NPs. Consequently, these molecules are attractive as targets for DVT therapies. However, these are expressed, even in activated endothelium at relatively lower levels. Moreover, P-selectin is expressed on activated platelets, and soluble P-selectin is secreted in significant amounts into the intraluminal thrombus, where it facilitates platelet activation and micro-particle binding. Consequently, P-selectin must be considered a relatively specific target for areas of diseased vessel—but not entirely specific to the endothelium. Peripherally circulating soluble P-selectin is elevated in patients with DVT,⁵⁵ and this would need to be considered when engineering NP therapies aimed at this target. When considering on-target, unexpected effects, fortunately there are data which indicate that inhibi-

tion of P-selectin has beneficial effects on thrombus resolution and vein wall fibrosis^{43,56}—whether a similar effect would be demonstrated with P-selectin-targeted NPs remains to be investigated.

E-selectin-targeted liposomes have been created, and were demonstrated to bind specifically to activated endothelial cells in vitro under simulated shear stress,³⁴ and due to the inducible presence of selectins on cell surfaces, this is a promising target for NP therapy.³⁴

Intraluminal-Thrombus Targets

Targets within the intraluminal thrombus include several molecules that are secreted by local activation of platelets and endothelial cells, or insoluble molecules of the coagulation cascade. Intraluminal thrombus targets have the potential to deliver fibrinolytic or anticoagulant therapies while minimizing systemic effects. Though little research has been done specific to thrombus-targeted therapies in DVT, there are several targets and targeting molecules that have been investigated, from which inferences can be made.

Platelets are present within the growing thrombus, and express a different set of surface receptors when in the activated state compared with circulating platelets. Anti-platelet therapies are commonly in use for cardiovascular disease, but have demonstrated a concerning frequency of hemorrhagic side effects. NPs targeting these markers would have the potential to specifically target areas of ongoing thrombosis. Von Willebrand factor (vWF) is contained in platelet α -granules and in the Weibel-Palade bodies of the endothelium. It is present in relatively low levels outside of areas of inflamed vasculature. The specific bond between platelet glycoprotein Ib (GPIb) receptor and vWF has been exploited for targeting of polystyrene and PLGA NPs, which allows relatively high specificity to the intraluminal thrombus.³⁰ Similarly, liposomal NPs loaded with tPA were targeted to activated platelets by conjugation of a peptide mimicking the c-terminus of fibrinogen, which binds the platelet GPIIb/IIIb receptor.⁵⁷

As the final step of the coagulation cascade, soluble fibrinogen is cleaved to insoluble fibrin and crosslinked by factor XIII. These molecules make for attractive targets, because they are present specifically at the site of the thrombus. Arginine-glycine-aspartate (RGD) tripeptides are small peptides that can be bound to NP surfaces for targeting intraluminal thrombus. This RGD peptide sequence mimics a similar sequence of human fibrinogen at residues 572–575,⁵⁸ which binds vWF, fibronectin, and collagen.⁵⁹ GPRPPGSGKGC is a peptide sequence that similarly binds fibrin, which has been used to deliver iron oxide nanoparticles to thrombus.⁶⁰

Other peptides with fibrin sequence homology have been used for this purpose as well.^{61,62} Anti-fibrin antibodies have been used to successfully target a recombinant tPA to thrombi as well.⁶³ Chung et al investigated a PLGA NP with surface bound RGD tripeptide for delivery of plasminogen activators into thrombi created in vitro, and demonstrated effective thrombolysis with this system.⁵⁹ There may be beneficial on-

target, unexpected effects with the use of these peptide locator molecules, as fibrinogen homologue peptides have been shown to inhibit platelet binding to fibrin and platelet activation.^{58,61} Factor XIII-targeted NPs have been created using a NP-bound peptide-chain based on α_2 -antiplasmin.⁶⁴

Thrombin is a circulating coagulant factor that when activated cleaves soluble fibrinogen to insoluble fibrin, and is the target of several current systemic anticoagulants, including the hirudins, argatroban, and dabigatran. D-phenylalanyl-L-prolyl-L-arginyl-chloromethyl ketone (PPACK) is an effective and irreversible thrombin inhibitor, but has the major limitation of an extremely short half-life in vivo. Myerson et al complexed PPACK to a perfluorocarbon nanoparticle as a novel anticoagulant, to extend its half-life.⁶⁵ The authors demonstrated a beneficial antithrombotic effect, decreased platelet deposition, and minimal systemic effect on bleeding time in a mouse model of carotid thrombosis.⁶⁵ PPACK has been targeted to renal vasculature as well to limit thrombosis in an animal model of renal transplantation, and has been shown to limit in-stent thrombosis in vitro.^{66,67} PPACK may also have off-target, beneficial effects through activation of protease-activated receptors.⁶⁸

One potential limitation in the use of thrombus-targeted NPs is successful delivery into an occlusive thrombus within the vessel; consequently, the administration of NPs directly into thrombus via catheter-directed techniques has been considered. Urokinase-bearing NPs have been combined with catheter-directed thrombolysis in a rabbit model of venous thrombosis, and demonstrated improved thrombolytic function compared with free urokinase.⁶⁹

Conclusion

Nanoparticle therapies have shown promise in the treatment of several diseases, and are FDA approved for the treatment of certain cancers. Increasingly, nanoparticle therapies will be moving from bench to bedside as experience with engineering NPs matures and knowledge of the specific interactions of the NP with the body grows, and NP therapy for DVT is no exception. Further research is needed to clarify exactly how these therapies will function—and which combination of target or targets, materials, shapes, and therapeutic will be most beneficial to patients.

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