New Drugs and Technologies

Molecular Magnetic Resonance Imaging in Cardiovascular Medicine

David E. Sosnovik, MD; Matthias Nahrendorf, MD; Ralph Weissleder, MD, PhD

The ability of magnetic resonance imaging (MRI) to evaluate cardiovascular pathophysiology at several levels is one of the strengths of the technique. As shown in Figure 1, cardiac phenotype can be assessed at the whole-organ level by cine MRI, at the regional level with strain, perfusion, and viability imaging, and at the metabolic level with spectroscopic techniques.1,2 However, MRI of biological processes at the cellular and subcellular level (molecular MRI) has been studied most extensively in other disease states such as cancer.3,4 Molecular MRI techniques are, however, being increasingly developed for cardiovascular applications and, if fully developed, have the potential to make a significant impact on the practice of cardiovascular medicine.

The advantages of an MRI-based approach to molecular imaging reflect the general attributes of the technique. MRI is noninvasive, tomographic, nonionizing, and able to generate images with high spatial resolution and excellent soft tissue contrast. In the initial portion of the present review, the technology that underlies molecular MRI agents is reviewed. The application of these agents to the imaging of myocardial processes, cancer, atherosclerosis, and stem cell therapy in the heart is then discussed. Finally, the potential of multispectral and hybrid imaging techniques is explored.

Basic Considerations

Imaging Agents

Chelates of the paramagnetic metal gadolinium have been used as extracellular MR contrast agents for more than a decade. The detection threshold of these chelates is in the micromolar range and thus significantly superior to the millimolar detection limit of iodinated contrast agents. However, because many molecular targets of interest in the cardiovascular system are expressed in the low nanomolar range, the detection sensitivity of routinely used gadolinium chelates is, with rare exceptions, inadequate for molecular MRI. Conventional extracellular gadolinium chelates also have extremely short intravascular half-lives, which limits their utility for most molecular imaging applications.

Several novel gadolinium constructs have thus been developed to address these limitations. These include gadolinium chelates with a strong affinity for albumin (MS-325, Epix Pharmaceuticals, Lexington, Mass.),5 gadolinium-containing high-density lipoprotein–like nanoparticles,6,7 gadolinium-containing micelles,8 and gadolinium-containing liposomes.9–11 In addition to longer intravascular half-lives, these novel gadolinium constructs also have higher longitudinal relaxivities (R1). The relaxivity of an MR contrast agent reflects its ability to interact with adjacent protons and strongly influences its detectability. The higher the R1 of an agent, the brighter tissue in its vicinity becomes, whereas the higher the transverse relaxivity (R2), the darker the tissue becomes. The R1 of most clinically approved gadolinium chelates is 4 s⁻¹mM⁻¹.12 The R1 values of the novel gadolinium constructs mentioned above, however, tend to range from 10 to 20 s⁻¹mM⁻¹ and in the case of some micelles even twice that.8

Magnetic iron oxide nanoparticles (MNP) constitute another large class of molecular MRI agents and are based on the ability of these superparamagnetic nanoparticles to modulate the uniformity of a magnetic field.12,13 MNPs have a central core of iron oxide, which measures 3 to 5 nm in diameter surrounded by a dextran, starch, or polymer coat.14,15 A citrate-coated iron oxide nanoparticle has also recently been developed and used as a blood pool agent.16 Selected MNPs have been used extensively in the clinical arena to image the liver and lymphatic system,17 and their established safety record thus makes them a highly appealing platform for molecular MRI. A selected list of representative MNPs is provided in the Table.

The first generation of MNPs, such as Feridex (Advanced Magnetics, Cambridge, Mass.), contained thin dextran coats and had the propensity to form aggregates in vivo that were rapidly cleared from the bloodstream. Subsequent MNPs have been synthesized with more extensive polymer coatings and remain monodisperse.14 The term monodisperse iron oxide (MION) is thus often applied to these agents. A highly stabilized and cross-linked derivative of MION, known as CLIO, has recently been developed for targeted molecular imaging applications.15,18 CLIO is also aminated, which allows a large variety of ligands to be conjugated to the nanoparticle with relative ease and a high degree of stability. Near infrared fluorochromes, for instance, can be attached to the amine groups on the probe to form a dual-modality
magnetofluorescent nanoparticle. In addition, many copies of the targeting ligand can subsequently be attached to the CLIO-fluorochrome conjugate to form a multivalent targeting ligand magnetofluorescent nanoparticle. Examples of such recently used ligands include annexin for apoptosis imaging and a peptide specific for the vascular cell adhesion molecule 1 (VCAM-1).

Intravenously injected MNPs are eventually removed from the circulation by the reticuloendothelial system. The blood half-life of MION and CLIO in humans is 24 hours, and in mice it is 11 hours. The small size (approximately 30 nm in diameter) and long circulation half-lives of these agents allow them to penetrate tissues of interest such as atherosclerotic plaques or the interstitial space of injured pancreas and myocardium. In addition, the high relaxivities of these probes allow them to detect sparsely expressed targets, such as VCAM-1, with adequate sensitivity. The R1 of MION-47 at 0.5 Tesla is 19 s⁻¹mM⁻¹, whereas its R2 is approximately 50 s⁻¹mM⁻¹. The R2 value of CLIO and other more recently developed iron oxides, however, can reach 150 s⁻¹mM⁻¹.

Strategies for Targeted Imaging
An MR contrast agent can be targeted to a moiety on the surface of a cell through 1 of 2 mechanisms. The first involves the attachment to the nanoparticle of a ligand directed against a known target on the cell surface, such as αvβ3 integrin, phosphatidylserine, or VCAM-1. The second approach involves modification of the nanoparticle surface with small molecules to modulate its uptake. Phage display, for instance, has been used to identify peptides specific for the adhesion molecule VCAM-1. High-throughput screening of a library of surface-modified peptides for targeted imaging libraries plays an increasingly important role in probe discovery. Phage display, for instance, has been used to identify peptides specific for the adhesion molecule VCAM-1.

Selected Superparamagnetic Iron Oxide Nanoparticles

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Ferumoxides (Feridex)</td>
<td>Approved liver imaging agent</td>
</tr>
<tr>
<td>Ferrixan (Resovist)</td>
<td>Approved liver imaging agent</td>
</tr>
<tr>
<td>Ferumoxtran (Combidex, AMI 227)</td>
<td>Completed phase 3 trials</td>
</tr>
<tr>
<td>Ferumoxyl (AMI 228)</td>
<td>Studied for iron replacement prescription</td>
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<tr>
<td>Monocrystalline iron oxide (MION-47)</td>
<td>Experimental nanoparticle highly similar to Ferumoxtran</td>
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<tr>
<td>Monocrystalline iron oxide (MION-48)</td>
<td>Experimental nanoparticle with extremely high transverse relaxivity (R2)</td>
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<tr>
<td>Cross-linked iron oxide (CLIO)</td>
<td>Experimental nanoparticle developed for targeted imaging</td>
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<tr>
<td>Very small superparamagnetic iron oxides (VSOP)</td>
<td>Citrate-coated iron oxide nanoparticle</td>
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CLIO nanoparticles has revealed agents that are highly specific for either resting or activated macrophages. Probes may thus be targeted not only to cell type but also to cell state.

The earliest targeted MR probes consisted of antibody ligands conjugated to the MR contrast agent. Subsequent probes have used antibody fragments, small proteins, peptides, or small molecules, usually in a multivalent fashion to enhance probe sensitivity. The size and physical properties of certain MNPs, such CLIO, also promote cellular internalization after ligand binding to an appropriate receptor, such as VCAM-1. The subsequent trapping and compartmentalization of the internalized nanoparticles within lysosomes produces strong biological amplification of the signal in a manner analogous to that of a magnetic relaxation switch, described below. This process plays a significant role in the enhancement of the sensitivity of the agent for a sparsely expressed target such as VCAM-1.

The stability and nonionizing nature of magnetofluorescent imaging agents allows probe discovery and development to be performed in a highly rigorous and rationalized approach. The presence of a fluorochrome on the probe allows high-throughput screening, flow cytometry, and fluorescence microscopy to be performed in vitro. In addition, ex vivo imaging of probe biodistribution can be performed with fluorescence reflectance imaging and fluorescence microscopy with higher throughput and less expense than MRI. In the final step of the process, in vivo imaging of the validated probe can be performed by MRI. An example of this approach that highlights the study of atherosclerotic plaque inflammation is demonstrated in Figure 2.

**MR-Detectable Nanoswitches and Biosensors**

Targeted molecular MR contrast agents are designed to image targets with a fairly constant level of expression during image acquisition. However, another class of magnetic nanoparticles, designed specifically to image dynamic events, has recently been described. These agents, termed magnetic relaxation switches, consist of MNPs that undergo reversible assembly and disassembly in the presence of a specific enzyme or chemical compound. The change in size of the nanoassembly produces a change in R2, which can then be detected by T2-weighted MRI. Magnetic relaxation switches have previously been developed to detect proteases, oligonucleotides, viral particles, and enantiomeric impurities, and could be synthesized to detect a wide variety of diagnostic markers used in clinical cardiology. One such
potential switch could be designed to detect troponin, brain natriuretic peptide, and C-reactive protein levels in multiplexed format and in whole blood. This could potentially allow point-of-care testing with a bench-top relaxometer to be performed more rapidly and reliably than is currently possible.

Magnetic relaxation switches have also been used to determine the concentration of analytes such as glucose and calcium. A construct that consists of CLIO-glucose and concavalin was able to accurately determine glucose concentration over a clinically meaningful range (Figure 3). The mechanism of action of the switch is described in Figure 3. The ability of this switch to accurately measure glucose concentration across a semipermeable membrane raises the possibility of its use as an implantable biosensor in the future. It should further be possible for a library of biosensors to be included within a membrane, which would allow a panel of cardiovascular metabolites to be sensed continuously and noninvasively by MRI in vivo.

Enzyme-Activatable Probes
The concept of enzyme-mediated nanoparticle aggregation has also been extended to novel gadolinium conjugates. A gadolinium-serotonin chelate, able to sense myeloperoxidase activity, has been developed on the basis of this concept. When oxidized by myeloperoxidase, the peptide serotonin forms oligomers with a significantly higher R1 than the parent compound. These high-relaxivity oligomers also undergo protein binding, which further increases their R1 (Figure 4). The presence of myeloperoxidase in a mouse model of myositis could be imaged in vivo with this probe, as shown in Figure 4. Although further study will be needed, it is possible that myeloperoxidase activity in the myocardium and atherosclerotic plaques will be able to be imaged in vivo with this probe.

Clinical Applications
Myocardial Injury
The hyperacute phase of myocardial injury is characterized by increased capillary permeability and hyperemia. The wash-in kinetics of MNPs, which are normally confined to the intravascular space, can thus be used to demarcate the area of myocardium at risk. In the absence of active ligand binding, however, these MNPs are subsequently washed out of acutely injured myocardium, which creates suitable con-
ditions for targeted imaging. The retention of MNPs in the myocardium during the acute phase of myocardial injury is thus likely to reflect active ligand binding.

Several important biological processes occur during acute myocardial injury, such as cardiomyocyte apoptosis, cardiomyocyte necrosis, and free radical release. Annexin V is a protein that binds to phosphatidylserine on the surface of apoptotic cells, and pioneering work has previously been done with a radiolabeled annexin probe in patients with acute coronary syndromes and cardiac transplant rejection. The advantages of an MR-based approach to apoptosis imaging, however, include the ability to acquire high-resolution images of the process and correlate regional probe distribution with indices of myocardial function, perfusion, and viability.

The imaging of cardiomyocyte apoptosis by in vivo MRI has recently been performed in a mouse model of transient coronary ligation. No significant changes were seen in myocardial signal intensity when mice were injected with an unlabeled control probe. However, as shown in Figure 5, injection of an identical dose of the annexin-labeled probe AnxCLIO-Cy5.5 produced significant negative contrast enhancement in the injured myocardium. A small (40 nm in diameter) annexin-coated gadolinium-containing liposome has also recently been used to successfully image cardiomyocyte apoptosis in the isolated perfused rat heart ex vivo. The utility of this construct for in vivo imaging, however, will require further study.

Apoptosis is the dominant form of cell loss during the first few hours after complete coronary artery occlusion, after which necrosis predominates. Cardiomyocyte necrosis has been imaged by MRI in the rat heart ex vivo with an antimyosin antibody conjugated to MION (Figure 5). The use of this probe in conjunction with annexin-based probes could thus provide powerful insights into the pathogenesis of cell death during acute myocardial injury.

Several conditions in the myocardium, such as healing infarcts and transplant rejection, are characterized by macrophage infiltration and can be imaged with the use of macrophage-avid MNPs. Surface modification of the nanoparticle can also refine the specificity of the MNP for either resting or activated macrophages. Long-circulating MNPs have been shown to accumulate in several models of tissue inflammation and have already been used to successfully image transplant rejection in a rat model.

![Image](http://circ.ahajournals.org/)

**Figure 4.** In vivo MRI of myeloperoxidase activity in mice. A, Exposure of the gadolinium-serotonin chelate to myeloperoxidase causes it to undergo oligomerization and protein binding. The protein-bound oligomers have a higher R1 than the parent compound. B, Human myeloperoxidase embedded into a matrix gel and implanted into the right flank of a mouse is able to activate the gadolinium-serotonin probe, whereas an implanted matrix gel without myeloperoxidase (left flank) does not. C, A transient increase in signal intensity (arrow) is seen when gadolinium-diethylenetriamine pentaacetate is injected into a mouse with myositis. D, A sustained increase in signal intensity is seen when the gadolinium-serotonin chelate is used. MPO indicates myeloperoxidase; Gd, gadolinium.

**Figure 5.** Molecular MRI of myocardial injury. A, Superparamagnetic MION does not accumulate in the transplanted heart of a rat treated with cyclosporine. B, Significant signal loss from monodisperse iron oxide accumulation, however, is seen in the transplanted heart of an untreated rat. Adapted from Kanno et al with permission of the publisher. Copyright © 2001, The American Heart Association, Inc. C, Imaging of cardiomyocyte necrosis in a rat heart ex vivo with an antimyosin-labeled monodisperse iron oxide probe. The accumulation of the probe in the distribution of the ligated coronary artery produces negative contrast enhancement. D, Imaging of cardiomyocyte apoptosis with the nanoparticle AnxCLIO-Cy5.5 in the mouse heart in vivo. Negative contrast enhancement (arrows) is seen in the anterior wall of the left ventricle after transient coronary artery occlusion, which is caused by the uptake of the probe by apoptotic cardiomyocytes. No changes in signal intensity were seen with the injection of an equivalent dose of the unlabeled probe, CLIO-Cy5.5. E, Uptake of a gadolinium-annexin construct in an isolated perfused rat heart model ex vivo. Probe accumulation is seen after the creation of transient ischemia.
Atherosclerosis and Vascular Disease

Molecular MRI approaches to the imaging of atherosclerosis have focused on the detection of plaque lipid content, plaque inflammation, plaque thrombosis, and plaque angiogenesis. Gadofluorine is a novel chelate of gadolinium, which is more lipophilic and has a longer circulation half-life than conventional chelates. This compound has been shown in a rabbit model to accumulate preferentially in lipid-rich atherosclerotic plaques (Figure 6). A liposome that contains gadolinium and is targeted to the \( \alpha_\beta \) integrin has also been used in a rabbit model of atherosclerosis to image plaque angiogenesis (Figure 6). Furthermore, incorporation of the antiangiogenic agent fumagillin into the liposome was shown by MRI to decrease subsequent uptake of the probe (Figure 6). The concept of using a targeted magnetic nanoparticle in both a diagnostic and therapeutic capacity has thus been demonstrated.

High-density lipoprotein–like nanoparticles that contain gadolinium have also been shown to accumulate in atherosclerotic plaques (Figure 6). Several variants of these nanoparticles have been synthesized and their rate of uptake by plaques in apolipoprotein E–deficient (ApoE) mice seems to be determined by the lipid and macrophage content of the plaque. Plaques rich in macrophages tended to take up the probe more quickly than those with a low macrophage content. Plaque macrophage content has also been imaged ex vivo in explanted aortas from ApoE mice with a micelle that contains gadolinium and is decorated with an antibody to the macrophage scavenger receptor.

The largest experience in the imaging of plaque macrophage content, however, has been with MNPs. Long-circulating MNPs are able to penetrate an atherosclerotic plaque and are then taken up by its cellular components. Plaque inflammation has been imaged with MNPs in mice, large animal models, and in human carotid arteries in vivo. The use of the MNP CLIO-Cy5.5 in ApoE mice showed that although the majority (65%) of CLIO-Cy5.5 within the plaque was taken up by macrophages, activated smooth muscle and endothelial cells also accumulated the probe. In large animal models and humans, a good correlation between in vivo visualization of the MNP and the histological presence of plaque macrophages has also consistently been found.

Two gadolinium-based probes have been developed to image fibrin and detect thromboses and vulnerable atherosclerotic plaques. A fibrin-specific peptide has been conjugated to a chelate that contains gadolinium to yield a low molecular weight probe. The high levels of fibrin in thrombi allow high target to background ratios to be obtained with this probe, and both acute and subacute thrombi have thus been successfully imaged in vivo in a variety of large animal models (Figure 6). A fibrin-targeted liposome that contains gadolinium and is able to image thrombosis in a dog model in vivo by MRI has also been developed.

One of the principle aims of molecular imaging is the detection of early disease. Thus, significant interest exists in the development of probes to detect expression of the adhesion molecule VCAM-1 on vascular endothelium. Several generations of VCAM-1–targeted MNPs have now been developed. The first of these agents used a cyclic peptide to bind to VCAM-1, whereas the most recent used a phage-derived linear peptide. The experience with the linear peptide-based probe, however, is perhaps the most illustrative of the potential of molecular MRI. VCAM-1 expression could some results in a significant decrease in plaque neovascularization, which is not seen when the agent is absent in the liposome (Figure 6). Reproduced from Winter et al. with permission of the publisher. Copyright © 2006, The American Heart Association, Inc.

Figure 6. Imaging of atherosclerosis with paramagnetic gadolinium-based probes. Detection of lipid-rich atherosclerotic plaques can be accomplished with agents such as gadofluorine (A) and a high-density lipoprotein–like nanoparticle that contains gadolinium (B); A is reproduced with permission from Frias et al. Copyright © 2004, American Chemical Society. B is reproduced from Sirol et al. with permission of the publisher. Copyright © 2004, the American Heart Association, Inc. C and D, Angiogenesis in atherosclerotic plaques has been detected with a liposome that contains gadolinium targeted to the \( \alpha_\beta \) integrin. Incorporation of the antiangiogenic agent fumagillin into the liposome results in a significant decrease in plaque neovascularization (E), which is not seen when the agent is absent in the liposome (F); reproduced from Winter et al. with permission of the publisher. Copyright © 2006, The American Heart Association, Inc. G, Imaging of plaque thrombosis in vivo with a fibrin-targeted gadolinium chelate; reproduced from Botnar et al. with permission of the publisher. Copyright © 2004, The American Heart Association, Inc.
The effect of statin therapy on VCAM-1 expression could be documented in vivo by demonstration of decreased accumulation of the probe in the aortic roots of statin-treated mice (Figure 7). This VCAM-1–sensing MNP thus not only demonstrated adequate sensitivity to detect a sparsely expressed molecular marker early in the disease process, but also adequate dynamic range to detect a treatment effect.

**Stem Cell Imaging**

The MNP Feridex has been used to label and image stem cells injected directly into the myocardium (Figure 8). However, when cells colabeled with Feridex and 111indium-oxine were injected peripherally, homing to the heart could be detected only by single-photon emission computed tomography and not by MRI (Figure 9). This is in contrast to the experience in the brain, where even single iron oxide–labeled cells have been successfully detected. At present, therefore, the main utility of imaging iron oxide–labeled cells in the heart lies in its ability to precisely delineate the infarct, guide the intramyocardial injection of the cells, and track their movement over time.

Studies that use bioluminescence and positron emission tomography reporter probes have suggested that stem cells injected into infarcted myocardium may not survive long. Although cell labeling with Feridex cannot determine survival, several MRI-based reporter genes have been developed to image transgene expression (Figure 8). A galactosidase-cleavable chelate of gadolinium is able, only after enzyme activation, to interact with the surrounding protons and affect the MR signal (Figure 8). Transgenes that encode for surface receptors to bind and internalize MNPs have also been developed. Tumor cells that expressed an engineered transferrin receptor or a biotinylated surface receptor could be detected by CLIO-transferrin and CLIO-streptavidin, respectively (Figure 8). More recently, transfection with a gene that encodes for the synthesis of ferritin from endogenous iron pools in living cells has been used (Figure 8). The sensitivity and impact of these agents on stem cell integrity, however, will need to be carefully considered and compared with established positron emission tomography and bioluminescence-based reporters.

**Future Developments**

**Multispectral and Hybrid Techniques**

One of the traditional disadvantages of MRI has been its inability to image more than one molecular signature simul-
taneously. This is in contrast to dual-tracer single-photon emission computed tomography and fluorescence techniques,13,77 which, in addition to being highly sensitive, are also multispectral. Multinuclear MRI with proton- and fluorine-based readouts offers a mechanism to overcome this limitation by imaging a particular cardiovascular target with a proton-based approach and a complementary target with a fluorine-labeled probe.78 An alternative approach that generates multiple MR signatures from only the proton pool has also been developed. This technique exploits the transfer of magnetization between 2 pools of protons with an offset in their precession frequencies and forms the basis of the chemical exchange saturation transfer (CEST) techniques.79,80 The potential of the CEST approach has already been demonstrated in a cell-labeling experiment in which 2 distinct populations of cells could be selectively imaged.79

Despite the broad capabilities of MRI, the use of hybrid imaging techniques has significant appeal. The combination of MRI and fluorescence tomography has already proven extremely useful in the small-animal setting,77 and MRI and single-photon emission computed tomography data sets have been co-registered in dogs to allow labeled stem cells to be tracked to areas of myocardial infarction (Figure 9).68 Coregistration of carotid MRI and fluorodeoxyglucose–positron emission tomography data sets also allowed plaque morphology and metabolism to be accurately correlated (Figure 9).81

In addition, dual modality MR–positron emission tomography scanners are currently being developed for both small animal and clinical use82 and could become an extremely powerful platform for molecular imaging.

**Conclusion**

Molecular imaging of cardiovascular disease is likely to play an increasingly important role as the era of genomic and personalized medicine dawns. The properties of magnetic resonance are well suited to molecular imaging of the cardiovascular system and the potential of this approach has already been strongly demonstrated in numerous animal models of cardiovascular disease. In addition, first-generation polymer-coated iron oxides have already been approved by the US Food and Drug Administration for clinical use, and subsequent generations of iron oxides (Combidex, Advanced Magnetics, Cambridge, Mass.) and the gadolinium chelate MS-325 have already completed phase 3 clinical trials.17,83

In the near future, molecular MRI is therefore likely to become a routine technique in basic science investigation and to streamline the development of novel pharmaceuticals. In the clinical realm, molecular MRI has the potential to facilitate the early detection of cardiovascular disease, refine risk assessment, aid in the selection of individualized thera-
pies, and subsequently monitor the efficacy of such therapies with excellent sensitivity. Molecular MRI thus offers the possibility of complete bench-to-bedside translation with a single molecular, anatomic, and functional imaging modality and has the potential to become a transformative technology in cardiovascular medicine.

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**References**


**Disclosures**

None.

**Figure 9. Potential value of hybrid imaging in molecular MRI.** A through C, Single-photon emission computed tomographic imaging of the transglutaminase Factor XIII in a healing myocardial infarct. Autoradiography of a Factor XIII-targeted 111indium probe shows a local increase in activity in the infarcted myocardium, which correlates well with the zone of hypocontractile myocardium by cine MRI (B) and TTC staining (C) of the infarcted tissue. D, Fusion of MRI and single-photon emission computed tomography data sets that show the homing of 111indium-labeled mesenchymal stem cells to a region of infarcted myocardium, delineated by delayed hyperenhancement MRI; reproduced from Kraitchman et al68 with permission from the publisher. Copyright © 2005, The American Heart Association, Inc.


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