Radiology

REVIEWS AND COMMENTARY • EDITORIAL

CRISPR-based Triple-Modality Imaging Ushers in a New Era for Stem Cell Tracking in Stroke

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The advent of regenerative cell therapies has opened new possibilities for treating ischemic stroke, a major cause of morbidity and mortality worldwide. One of the most studied methods is the transplantation of human neural progenitor cells (hNPCs), which shows promise in improving recovery through neuronal replacement and integration in host circuitry. However, a major challenge remains: How can we monitor the fate and function of these transplanted cells in the living brain over time without invasive methods?

In their innovative study, published in this issue of *Radiology*, Li and Zhong et al (1) introduce a compelling multimodal molecular imaging approach to tackle this challenge. They combined clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9 (CRISPR/Cas9) genome editing with triple-fusion reporter gene labeling and a targeted nanocarrier system to improve blood-brain barrier permeability. This approach allowed them to track hNPCs over 8 weeks in a rat model of ischemic stroke. Their research represents an important milestone in the imaging and understanding of cell-based therapies for neurologic disorders. The authors used CRISPR/Cas9 to insert a triple-fusion reporter gene cassette, which included firefly luciferase, herpes simplex virus thymidine kinase, and green fluorescent protein into human embryonic stem cells. They then differentiated these edited cells into triple-fusion hNPCs (TF-hNPCs). The choice of reporters shows a careful mix of methods: bioluminescence imaging for sensitivity, PET for potential translation and quantification, and fluorescence for high-resolution ex vivo validation. In parallel, the team created adenosine A_{2A} receptor–targeting micelles (agonist micelles) that could temporarily and reversibly open the blood-brain barrier. This improved the delivery of PET tracers to the ischemic brain. The combination of reporter gene labeling and delivery vehicle is both effective and necessary. Without a way to circumvent the blood-brain barrier, the usefulness of PET for tracking cells in the central nervous system would be very limited (2).

Li and Zhong et al (1) validated their model in several ways. In vitro, TF-hNPCs showed stable expression of reporter genes and unaltered viability and multipotency, evidenced by their ability to differentiate into multiple neuronal cell types. After being transplanted into the ischemic striatum of rats, these cells were monitored noninvasively with both bioluminescence imaging and fluorine 18 (^{18}F) fluoro-3-hydroxymethylbutylguanine PET for up to 8 weeks (1). Dynamic contrast-enhanced MRI confirmed that the blood-brain barrier opened in specific regions over time after agonist micelle administration. The peak in permeability was measured in the ischemic area 90 minutes after injection. This resulted in much higher tracer accumulation and signal-to-background ratio, detected with PET, in animals that received the tracer and agonist micelles compared with animals that received the tracer alone. Histologic analyses confirmed the in vivo findings, revealing TF-hNPC migration toward the ischemic border, progressive differentiation into mature neurons, and synaptic integration. Furthermore, ^{18}F fluorodeoxyglucose PET showed restored glucose metabolism in ischemic areas. Neurologic scores improved over time, suggesting functional benefits of TF-hNPC transplantation (1).

This study stands out for several reasons. First, using CRISPR/Cas9 for targeted reporter gene integration ensures reproducibility, uniform expression, and minimal disruption to the function of existing genes. This method overcomes the limitations of traditional viral or random integration techniques (3). Second, the triple-fusion construct allows for validation across different imaging methods. This approach improves reliability and compensates for the limitations of any single technique. Third, the authors tackled a major challenge in molecular imaging of the central nervous system: delivering imaging agents across the blood-brain barrier. The authors took advantage of the increased presence of A_{2A} receptors in ischemic blood vessels to achieve targeted and temporary permeability. This targeted approach to blood-brain barrier modulation could have implications for both imaging and therapies (4). Ultimately, by combining imaging data with histopathologic and functional assessments, the authors provide a comprehensive understanding of the fate of transplanted progenitor cells.

While this proof-of-concept is technically strong and provides valuable insights, several key factors limit its application in the clinic. First, nonhuman proteins such as green fluorescent protein and herpes simplex virus thymidine kinase may elicit immune responses in patients (5). Although the present study performed in rats did not show signs of immune-related adverse events, the human immune system is likely to respond to these nonself antigens. This response could affect implanted cell survival and imaging fidelity. Additionally, while PET and bioluminescence imaging are effective tools for long-term studies, neither method resolves individual cells or their microenvironment in vivo. The authors

Author affiliation, funding, and conflicts of interest are listed at the end of this article.

See also the article by Li and Zhong in this issue.

Radiology 2025; 316(3):e252546 • https://doi.org/10.1148/radiol.252546 • Content codes: NM MR

tried to address this issue with postmortem immunohistochemical examination, but a noninvasive way to assess cell phenotype and functional state would be very useful (6). Finally, the complexity of the current approach, which includes genetic editing, stem cell differentiation, micelle synthesis, and tracer delivery, may slow its acceptance in clinical settings. Simplifying this platform or using label-free imaging methods (7) will be crucial for wider use.

As for future directions, one opportunity would be to replace immunogenic reporters with humanized sequences or natural transcriptional barcodes that show specific cell states. For instance, using PET-based metabolic reporters or cell surface markers that can be detected with radiolabeled antibodies would minimize cell manipulations and possible undesirable outcomes. Additionally, as we learn more about the ischemic environment, personalized imaging strategies triggered by molecular signals (such as A_{2A} receptor expression) may become possible. Biologically, this work creates opportunities for systemic studies of cell therapy mechanisms. Visualizing cell engraftment and differentiation in real time will help researchers identify which stem cell factors—such as survival, differentiation, and proliferation—are the best predictors of functional recovery after implantation. This can help mitigate risk and guide the design of next-generation stem cell therapies. From a clinical viewpoint, the approach presented here may be especially useful for preclinical safety and effectiveness studies, particularly those needing detailed in vivo cell tracking. With further development, simpler reporter systems could support patient-level monitoring, allowing early detection of graft failure or unexpected developments.

In summary, Li and Zhong et al (1) present a relevant and thorough study that advances the field of regenerative imaging. They combine CRISPR-engineered reporter cells with a new probe delivery system to create a platform for long-term multimodal monitoring of hNPCs in the ischemic brain. Although there are still challenges to overcome before this method can be used in clinical settings, its impact on imaging science and the development of regenerative therapies is significant. As we work to fully realize the potential of stem cell therapies for neurologic diseases, being able to observe cells in action will be crucial. This study brings us one step closer to that goal.

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Received August 14, 2025; revision requested August 21; revision received August 21; accepted August 25.

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Funding: Author declared no funding for this work.

Disclosures of conflicts of interest: F.C. Pilot grant for the Research Corporation for Science Advancement (SA-ABI-2023-039b); grants from the National Institutes of Health (R03 EB035177, KL2 1KL2TR001444); U.S. patent application 18/054,387; nominated to the Academy for Radiology and Biomedical Imaging Research's Council of Early Investigators in Imaging Class of 2024; member of the International Society for Magnetic Resonance in Medicine (ISMRM), World Molecular Imaging Society, and Society for Immunotherapy of Cancer; session chair, Imaging and Diagnostics Session at BIC; session chair, member-initiated symposium on Manganese Revisited at 2025 ISMRM annual meeting; elected chair, ISMRM Molecular & Cellular Imaging Study Group (2024), guest editor, Special Issue on Molecular and Cellular Imaging, *Magnetic Resonance in Medicine* (2023–2024); moderator, ISMRM virtual workshop titled "Imaging Inflammation" (2024), elected vice-chair, ISMRM Molecular & Cellular Imaging Study Group (2023), moderator, ISMRM Virtual Workshop titled "Methodologies in Molecular MR" (2023), elected secretary, ISMRM Molecular & Cellular Imaging Study Group (2022).

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