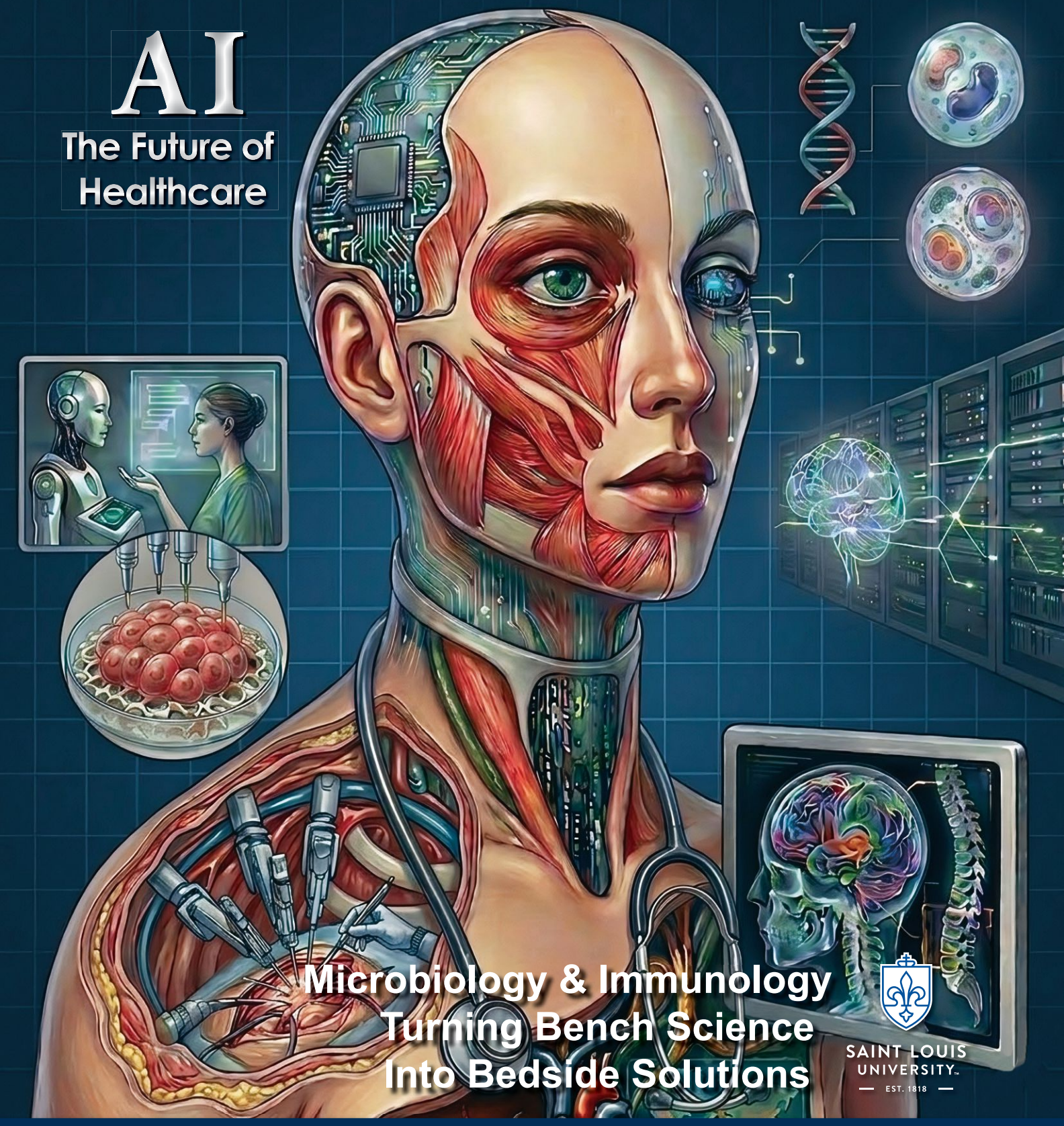
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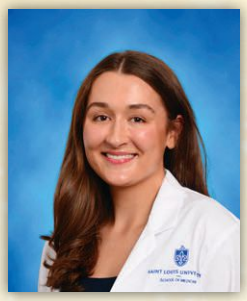
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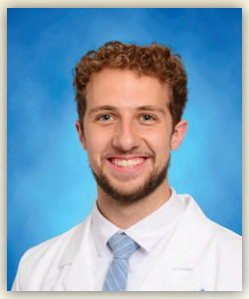

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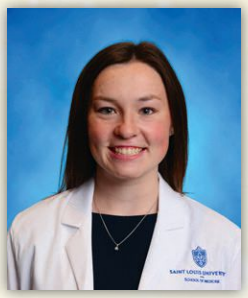

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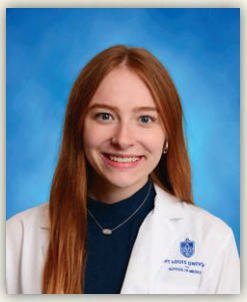

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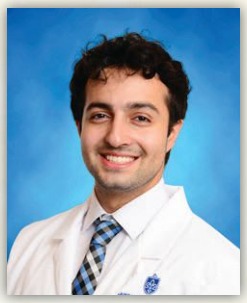

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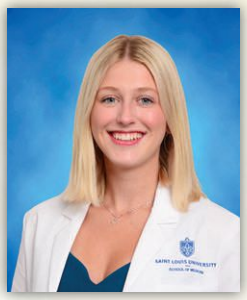

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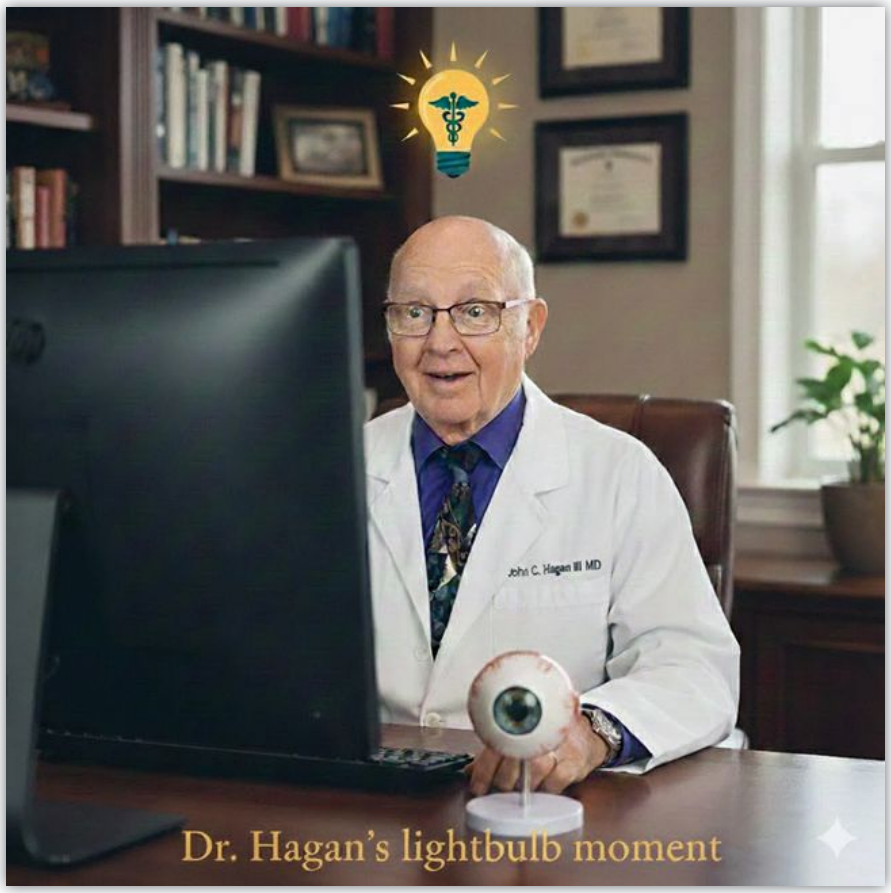
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Hope This Floats Your BOT

A New Paradigm to Put US Healthcare Back on Track

by John C. Hagan III, MD

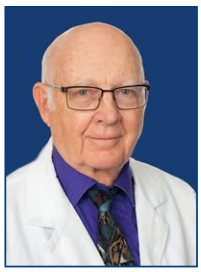


Archimedes said: "Eureka!" The ladies at the Knights of Columbus Hall say "Bingo!" As an ophthalmologist, I say: "This is a real eye-opener!"

You may, or may not, recall my exasperated September/October 2025 editorial. The title is self-explanatory: "You have sudden onset of chest pain? The doctor can see you in five months. The travesty of physician unavailability." *Missouri Medicine 2025;122(5):348-350.*

Many of you share my pain and concern. In post-publication discussions and correspondence no physician expressed to me satisfaction with what passes for medical 'care' in the United States. Having at times been called a 'know-it-all,' in my earlier editorial I disabused that notion as I proffered no solutions. Herein, I rectify that omission.

What is the answer, Dr. Hagan? Patience, you will have to wait another paragraph or two for my epiphany. In reviewing possible solutions there are many of them out there; all have flaws or impracticalities. To wit: additional medical schools with more students, minting physicians in less training time, and funding additional residencies especially primary care.



John C. Hagan, III, MD, is Past President of the Kansas City Medical Society, the Missouri Society of Eye Physicians & Surgeons and the Clay-Platte County Medical Society. He is Editor of *Missouri Medicine* and a Contributing Editor to *Kansas City Medicine*. Dr. Hagan is an ophthalmologist, author, and researcher in Kansas City, Missouri USA. Learn more at www.msma.org/MoMed-Editor.





Figure 1. The 15 Most Important Postulated AI Breakthroughs in Medicine

1. AI in Early Cancer Detection: Seeing the Invisible
2. AI-Powered Radiology: The Digital Eye of Medicine
3. AI in Drug Discovery: Accelerating the Impossible
4. AI in Genomics: Decoding the Blueprint of Life
5. AI in Pathology: The Digital Microscope
6. AI in Personalized Medicine: Tailoring Treatment to the Individual
7. AI in Predictive Healthcare: Seeing the Future of Disease
8. AI in Medical Imaging for Rare Diseases
9. AI in Robotic Surgery: Precision Beyond Human Hands
10. AI in Mental Health: Listening to the Mind
11. AI in Epidemiology: Predicting and Containing Outbreaks
12. AI in Cardiology: The Algorithmic Heartbeat
13. AI in Ophthalmology: The Eyes as a Window to Health
14. AI in Clinical Documentation and Workflow
15. AI and the Future of Regenerative Medicine

Source: Editors of Science News Today August 10, 2025

Make present physicians more productive, by greatly truncating the time sink that is present day electronic medical records. Enticing physicians into staying in practice longer… good luck with that. Allowing more International Medical Graduates (IMGs) into US practice. IMGs have become and indispensable part of US medicine.[1] They admirably often practice in rural or underserved urban areas and in specialties less attractive to US physicians. Left unsaid is the propriety and ethics of taking physicians out of their home countries that sponsored their education and training. Most of these native IMG countries have greater medical needs and health challenges than our own country.

Lastly, to put it brutally, is to dumb down primary care by turning it over to an eager cadre of often poorly trained and overly confident physician wannabes. The main physician-pretenders are unsupervised nurse practitioners who are nurse 'educated' (NP) and to a much lesser extent unsupervised physician assistants that are physician educated (PA).[2] This is also the default direction we are pushed each legislative session. Non-physician scope of practice is expanding faster than the universe. Waits to see a physician are insufferably and dangerously long. Accessibility is the rocket on which enlargement of scope of practice is put into orbit. Dr. Nurse will do today or tomorrow if Dr. Physician is booked out a half year or more. An increasing number of people say their doctor is a nurse. Worse yet, they are fine with it.

Thanks for your patience. Okay, here is the answer. No, it is not so dramatic that you need to sit down before reading. I, along with millions of others, feel the only hope for better, more accessible, less expensive medical care, here, there and everywhere is (drum roll) artificial intelligence (AI). Maybe you feel the same way, I hope so. I have been cogitating on the US medical crisis incessantly since I care for my 80-year-old wife with severe Parkinson's Disease and cognitive impairment. In my calculus, every healthcare 'fix' fails except medically oriented, patient and physician friendly, constantly improving AI (Figure 1). Others have come to the same conclusion.[3,4]

In 1996, I was visiting my brilliant daughter, a honor student at Stanford University. She took me to the Stanford library, sat down at a computer and said, "Dad, this is called the World Wide Web and it is going to change how the world works and thinks." How right she was. For better, for worse, or for both, AI is changing the world exponentially faster than anyone can image. If you have not already warmly embraced AI you are at serious risk of becoming a luddite.

AI can already outperform most physicians in diagnosis. In several studies, AI did better by itself with diagnosis than when teamed with a physician.[5] Yes, the physician was holding back AI. Not all AIs, like physicians, are equally talented. At the threshold of 2026, the Einstein of AI is the Microsoft AI Diagnostic Orchestrator (MAI-DxO).[6] It correctly diagnoses



85% of cases described in the *New England Journal of Medicine*. On the other hand, physicians diagnosing the same cases were correct only 25% of the time. Wow, 85 to 25, that qualifies as a physician trouncing. AI, circa 2026, exhibits its highest dominance in pathology, radiology, dermatology.[7] Going forward, AI will come out on top more often in all specialties. Thanks to AI, we are on our way to the healthcare promise land: more physicians providing medical care higher in quality and quantity than now; at lower costs, in any venue, be it near or be it remote.[8] Oh, I seem to have forgotten to mention time appropriate: immediate for true emergencies, same day for urgent/painful conditions and non-emergent specialty referrals one to two weeks. Healthcare will be unrecognizably better than today's "The doctor maybe can see you for 15 minutes in maybe six months."

To truly appreciate the scale of this paradigm shift, we must visualize the clinical environment that this infusion of medical intelligence will create. The future AI-powered physician's office will operate as a hyper-efficient, compassionate digital engine, managing rapid, on-demand appointments where a highly advanced AI system performs the initial triage, orders necessary lab and imaging studies (like X-rays), and makes preliminary diagnostic assessments based on exhaustive data analysis. This AI, capable of fluent and empathetic communication in any language and dialect, will explain the diagnosis to the patient with tailored kindness and patience, instantly determining if an urgent transfer to a larger facility is required, yet at every stage, the human element remains paramount, with trained staff instantly available should the patient feel uncomfortable, and a qualified physician reviewing every complex decision, ready to perform invasive procedures and provide the final, human-validated sign-off on the care plan. [Disclosure, this last paragraph was written by AI and is probably the best paragraph in this editorial. In an example of AI hallucinations most of the references AI cited were fiction. Like children, AI not infrequently makes things up. The prose in the paragraph is exceptional; AI is also swiftly killing creative writing.]

I share the healthcare grievances of our patients: too expensive, too long of a wait, too little time with the physician, too many errors and human shortcomings (lack of sympathy, uncommunicative, etc.), too far to drive. Whether you are a consumer or a provider or both, only AI offers the means of getting our sick healthcare system well. Bear that in mind as you read the other articles in this AI special section.



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Artificial Intelligence in Healthcare: Transforming the Future

by Abdelazeez Ihmeidan, DO, Chi-Ren Shyu, PhD & Mai-Lan Ho, MD

As the lines continue to blur between man and machine, it becomes incumbent upon physicians to understand and guide AI for the best interests of humankind, and continually emphasize core medical values including compassion, integrity, and wisdom.

Introduction

Artificial intelligence (AI) is rapidly transforming our society in every sector including marketing, transportation, manufacturing, finance, and medicine. The field has grown by leaps and bounds since the advent of machine learning in the 1950s, with deep learning dominating the last decade, and generative AI entering the mainstream in recent years. These disruptive technologies are completely redefining the way we live and work, and have already displaced entry-level jobs in multiple domains.

In this perspective, we will provide an overview of history, challenges, current state and future potential of AI in healthcare. With ever-increasing pressures including workforce shortages, complex patients, and declining reimbursements, AI offers the opportunity to reimagine the nature of healthcare with human–robot assistance and medical superintelligence. As AI exponentially increases in capability, physicians will need to continually adapt to advancing technologies in order to proactively design the future of healthcare.

Evolution of AI

AI utilizes computer systems to perform tasks that would normally require human intelligence, such as perception, learning, reasoning, problem-solving, and decision-making. Advances in the field have accelerated in recent years, with increasingly complex model architectures enabled by rapid gains in computing power. However, the optimal choice of model depends greatly on the data type and desired use case.

Machine learning (ML) uses mathematical optimization and statistical algorithms to perform tasks without specialized programming. ML models have one or few layers connecting inputs to outputs, which are readily understandable and well suited to basic tasks with smaller datasets, but often require intense data preprocessing to ensure model accuracy. ML often relies on central processing units (CPU) which are cost-effective for sequential general tasks, data preparation, and small models.[1]

Deep learning (DL) involves more complicated algorithms with multiple hidden layers that process data hierarchically to extract deep features from large amounts of data. The most common model architecture uses neural networks with interconnected nodes that adjust connection weights to solve complex problems. DL typically requires graphical processing units (GPU) with thousands of cores enabling massive parallel processing. DL performs excellently for narrow tasks, but cannot generalize effectively to other domains without additional training.[2]



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Recent advances involve generative AI (GAI) algorithms that learn from data distributions to create novel content. Generative adversarial networks employ competing generator and discriminator neural networks to produce highly realistic new data. Transformer networks utilize multi-head self-attention with encoder-decoder blocks to enable parallel processing and long-range dependencies.[3] This has enabled the development of large language models (LLM) and vision language models (VLM) to process multiple data types. Foundation models are powerful neural networks trained on large, diverse, and unlabeled datasets to learn general patterns, after which they can be fine-tuned for a wide variety of downstream tasks.[4] Agentic AI refers to autonomous systems that dynamically perceive the environment, access tools to act, observe the results, and learn from experience with minimal human supervision. Orchestration of multiple specialized AI agents can automate collaboration and adaptive intelligence for complex workflows.[5]

Next-generation computing paradigms are being developed to overcome the current data storage, transfer, and processing speeds of classical systems. Areas of investigation include quantum, optical, DNA, and in-memory computing with advanced electro-optic, wireless, and microfluidic interconnects. For example, quantum computing relies on the use of quantum bits or qubits. The molecular properties of superposition, entanglement, and interference can be leveraged for more efficient encoding of complex multifactorial datasets and efficient solutions to complex biological problems.[6]

Challenges and Controversies

Successful AI development requires a clear understanding of intended use cases, data quality, computing power, and postmarket surveillance. Even for companies with extensive resources, monolithic architectures are costly and resource-intensive to maintain. Overall, the field is moving toward domain-specific models, which are more efficient and effective to scale, and can be integrated in modular fashion. In healthcare, this translates to coordination of specialized AI agents that can interact with multiple systems and data types to accelerate patient workflows and assist with clinical decision-making. However, commercial AI companies struggle to cross the chasm between successful research prototypes and added value for clinical practice. The "last mile" of implementation frequently involves multiple challenges including workflow integration, handling complex edge cases, and ensuring transparency and trust.[7] For example, despite $30–40 billion of investments into GAI, 95% of organizations have failed to see any return on value.[8]

In medicine, AI failure modes can be disastrous with faulty diagnoses, harmful treatments, and worsening disparities directly impacting patient care. Issues often stem from incomplete data, flawed algorithm development, and/or lack of human oversight. Complex AI algorithms operate as "black boxes" that are not readily explainable, making it difficult to detect and correct for bias and discrimination. Models can hallucinate misinformation, become brittle when overfitted to training data, decay due to changing data, or collapse following recursive training on synthetic data. Patient privacy is an additional concern, since personal data can be inadvertently shared or misused. Without close human supervision, ethical misalignments can occur such as reward hacking that exploits loopholes, and adversarial attacks with injection of corrupted data and exploitation of model vulnerabilities.[9]

Medical education involves years of training to develop attention to detail, logical reasoning skills, and manual dexterity for appropriate patient diagnosis and management. Trainee education systems must prioritize AI literacy and human-machine teaming, with the objective of enhancing rather than replacing human expertise and minimizing failure modes.[10] Risks to physicians using AI include deskilling (losing existing proficiencies), misskilling (adopting flawed habits), and never-skilling (failing to acquire basic competencies).[11-14] Workforce reskilling will also be needed as automation replaces lower-level roles in various industries. The World Economic Forum predicts that AI will displace 92 million jobs by 2030, while creating 170 million new jobs.[15] Hospitals are already beginning to employ novel AI-related positions such as prompt engineers, data curators, and ethicists. Ultimately, medical practitioners need to maintain patient connection and trust in AI-assisted care by prioritizing uniquely human values such as empathy, compassion, and creativity.[16]



Hype Cycle for Artificial Intelligence, 2025

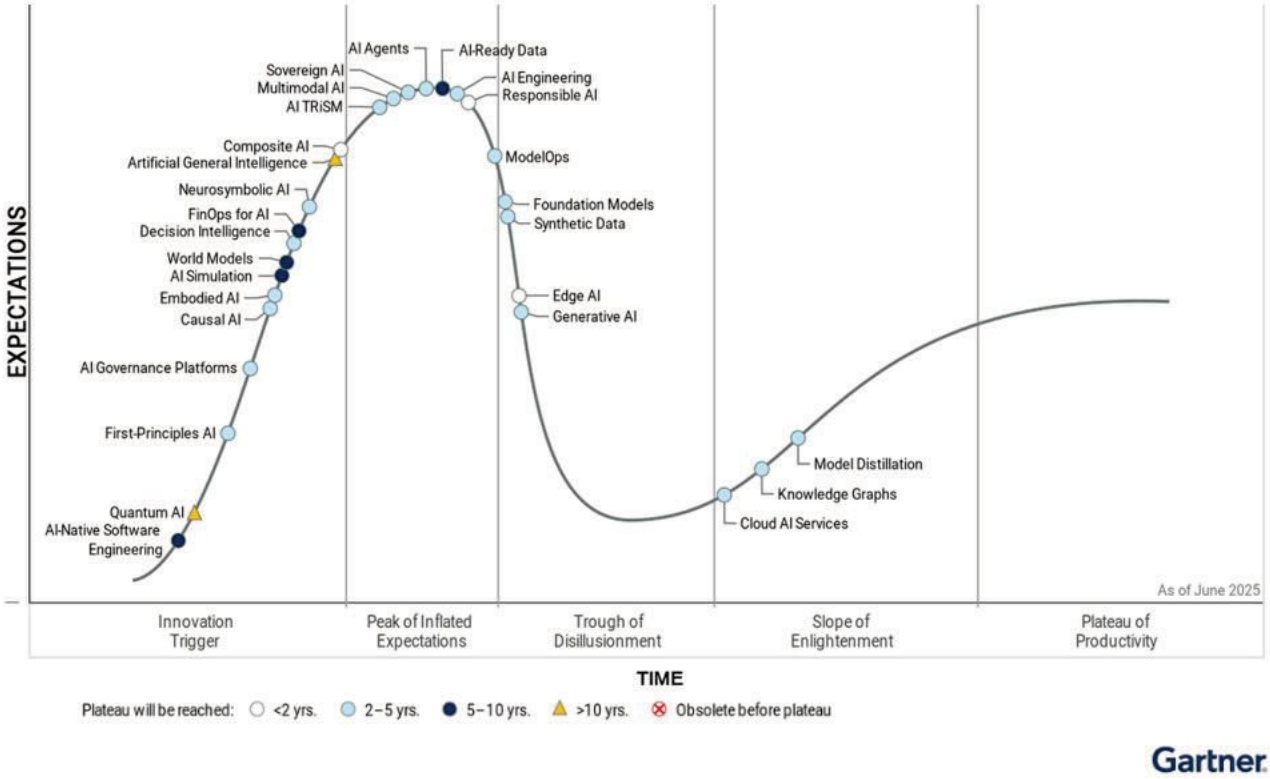


Figure 1. Gartner hype cycle for AI in 2025. AI agents are at the peak of inflated expectations, while generative AI has recently reached a trough of disillusionment, and quantum AI is beginning to trigger innovation.

Strong legal and governance frameworks are needed to continually monitor and regulate AI. The US Food and Drug Administration treats AI software as medical devices based on risk, and has continually updated guidelines to include best practices, change control plans, programmatic collaborations, and total product lifecycle approaches for evolving software.[17-19] All 50 states have also introduced AI-related legislation in 2025, though this complicates efforts to align regulations at the national and international levels.[20-22] Training and deployment of large AI models also have profound environmental impacts in terms of natural resources, electricity use, water consumption, and electronic waste, which are beginning to be recognized and regulated by the US and other countries.[23-24]

Impact on Healthcare

The US faces major healthcare workforce shortages, with a projected deficit of 100,000 health care workers by 2028. This has been exacerbated by reimbursement rate pressures with increasing expenses; pandemic-era resignations and burnout; an aging population with high burdens of chronic disease; and limited access in rural and underserved communities.[25] While workforce developments and training pipelines can help mitigate the situation, such strategies will not completely close the gap. The rapid rise of AI and automation offers physicians the potential to transform healthcare delivery from a fragmented, reactive, and overburdened system to an integrated, proactive, and cost-effective model.[26]

Radiology has led the medical AI revolution over the past decade, due to the specialty's routine storage of large retrospective digital datasets amenable to ML/DL training. As a result, radiologists are well versed in both the highs and lows of the Gartner hype cycle around new technologies. Back in 2016, the "godfather of AI" and 2024 Nobel laureate Geoffrey Hinton stated: "People should just stop training radiologists now. It's just completely obvious that in five years deep learning is going to do better than radiologists." In fact, this period coincided

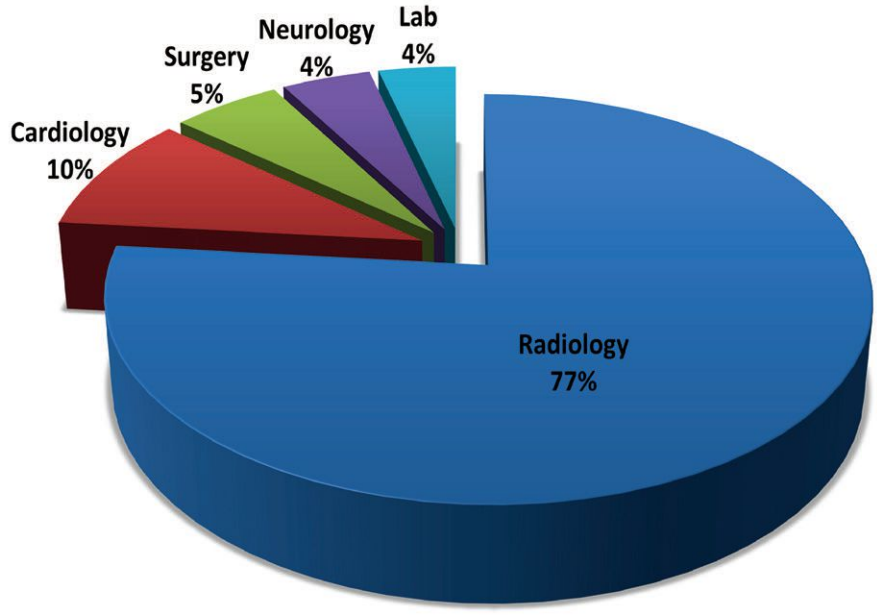


Figure 2. Distribution of 1,357 AI-enabled medical devices cleared by the FDA through 2025. These represent ML/DL algorithms with the majority of applications in radiology.

with the peak of inflated expectations for DL in radiology, (Figure 1), quickly followed by a trough of disillusionment. In May 2025, Hinton acknowledged that he was wrong about the timing and was speaking only about image analysis in radiology. Even with this qualification, it is evident that AI has only scratched the surface with regard to the tremendous complexities and nuances of medicine.[27]

As of December 2025, there are 1,357 AI tools listed on the FDA website, of which 77% are related to radiology, followed by cardiology, surgical specialties, neurology, and laboratory medicine/pathology. All of these represent ML or DL algorithms with fixed training data and algorithms for submission (Figure 2).[28] Some algorithms have been reported to outperform physicians for narrow and specific tasks, such as lung nodule detection on chest radiographs. In practice, radiographs represent a large proportion of imaging exams in any radiology department, yet yield limited diagnostic information with low reimbursement rates. Since a minority of studies demonstrate positive findings, targeted AI tools can help decrease physician cognitive burden and medicolegal risk by assisting in repetitive checks of basic exams. Human oversight is still important, since such algorithms do not readily transfer in the way that a human would learn: they are not trained to interpret suboptimal chest radiographs with poor positioning or extra lines/tubes; cannot detect additional abnormalities on chest radiographs; and will not perform the same task on other examinations such as neck or abdominal radiographs. Other radiology AI applications include image enhancement, labeling, and quantification, as well as noninterpretive tasks. Key lessons learned from radiology AI implementations include the need for seamless workflow integration, stakeholder buy-in, and customer support with postmarket surveillance.[29]

In the new era of GAI, LLMs and VLMs are beginning to impact every medical specialty by enabling large-scale assessment of unstructured text, photos, audio, and video with the potential for multimodal integration.[30] Many hospitals also employ their own data scientists to develop in-house AI algorithms, which are often more cost-effective, flexible, and powerful alternatives to commercial products. In the near term, the simplest yet most impactful utility of AI is for basic tasks that require minimal human supervision. AI can significantly alleviate workforce shortages and wait times by streamlining time-consuming tasks such as charting, scheduling, coding, billing, symptom checking, prescribing, and other basic communications. In the hospital setting, AI is frequently used for predictive analytics including clinical deterioration, morbidity, mortality, and readmission risk; as well as workflow optimization and resource allocation. Telemedicine offers the opportunity to effectively triage patients using chatbots/virtual assistants and improve access to underserved areas. By freeing up physician time



and decreasing cognitive load, AI can improve patient access and complex care. However, there are risks in emphasizing increased physician productivity without sufficient attention to interpersonal interactions, which can actually worsen patient disengagement.[31]

Recently, several publications have reported on GAI exceeding human performance in artificial situations, particularly LLMs answering standardized multiple-choice exams such as the United States Medical Licensing Examination.[32] These are technically unfair comparisons, since LLMs ingest internet-scale corpora that include sample USMLE questions and third-party test preparation sites. To identify the most likely answers, such algorithms rely on statistical pattern recognition of textual tokens rather than true comprehension. As a result, LLMs can perform well on structured questions, but struggle greatly with real-world situations including open-ended conversations, incomplete or corrupted data, multi-step reasoning, abstract concepts, and ethical nuances. Nevertheless, LLM advances over the past year have successfully integrated symbolic reasoning to mimic human logic and mathematical relationships.[33]

Going forward, the most realistic AI implementation framework calls for human–machine collaboration, in which the vast computational power of AI is utilized to augment human emotion and creativity. As healthcare systems move toward patient-centric data access and interoperability, there is interest in precision medicine approaches that can identify personalized risk factors, detect diseases earlier, recommend early interventions, and design targeted therapies. GAI has the capability to integrate fragmented and unstructured information regarding genetics, environment, and lifestyle for optimal patient care over the lifespan. Early studies demonstrate the feasibility of streamlining diagnosis and intervention based on multimodal health data; population screening and deep phenotyping to quantify the health–disease continuum; and interconnected digital networks for real-time monitoring.[34-36] GAI is also expanding medical education and research with adaptive learning, training simulations, knowledge retrieval, hypothesis generation, experimental design, and data discovery.[37]

In the long term, AI will realize the potential of medical superintelligence with novel applications far exceeding current medical capabilities. The technological singularity refers to a hypothetical future where AI recursively self-improves to achieve artificial general intelligence (AGI) that matches total human intelligence, followed rapidly by artificial superintelligence (ASI) that exceeds the best human minds in every domain. The estimated timeline to this crossover point varies widely from months to decades, but will inevitably impact society beyond current comprehension.[38] Researchers are already exploring the potential of AI to enhance human physical and cognitive abilities using technologies like brain-computer interfaces, gene editing, advanced prosthetics, and anti-aging therapies. Among the most positive visions is Society 5.0, in which technology empowers a human-centered future that can address social challenges, balance economic growth, and improve quality of life.[39] As the lines continue to blur between man and machine, it becomes incumbent upon physicians to understand and guide AI for the best interests of humankind, and continually emphasize core medical values including compassion, integrity, and wisdom.[40]

Conclusion

AI advances are deeply embedded in and continue to transform every facet of our lives. From streamlining administrative workflows to enhancing diagnostic and interventional capabilities, AI holds tremendous potential to revolutionize our failing healthcare systems. Appropriate education and engagement are crucial for physicians to mitigate professional risks, thereby ensuring safe, ethical, and patient-centered implementation. Only through technical understanding and continual oversight can we ensure that AI complements the humanistic foundations of medicine, strengthens physician–patient relationships, and improves healthcare for all.

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How Ambient AI Could Bring the Human Touch Back to Medicine

by Areeba Moiz, MD, Evan L. O'Keefe MD, Michael L. Main, MD & James H. O'Keefe, MD

Clinicians still have to maintain rigorous oversight, treating AI-generated drafts as preliminary documents requiring final professional validation.

Abstract

The United States healthcare system is facing historic levels of clinician burnout and workforce shortage fueled in large part by the onerous demands inherent in electronic health records (EHR). Ambient artificial intelligence (AI) scribe technology, such as Nuance's Dragon Ambient eXperience (DAX), has emerged as a tool to automate clinical documentation via transcribing and summarizing patient–clinician interactions. Studies demonstrate reductions in documentation time, after-hours work, and clinician-reported burnout, alongside improvements in efficiency, patient engagement, and patient and clinician satisfaction.

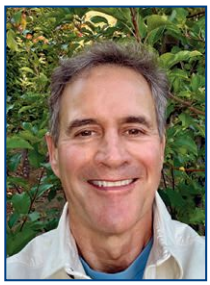
Challenges remain, however, regarding data privacy, algorithmic bias, regulatory oversight, and clinical validation.

In the future, ambient AI scribes will evolve beyond transcription, integrating prior patient records, and expand into allied health fields, including nursing. Ambient AI offers a transformative opportunity to strengthen healthcare delivery while preserving humanity in medicine by allowing clinicians to spend more time in face-to-face interactions and less time performing word processing tasks in the EHR.

Introduction

The rapid expansion of electronic health records (EHR) following the 2009 Health Information Technology for Economic and Clinical Health (HITECH) Act has transformed the clinical practice of medicine.[1] This legislation incentivized adoption of health information technology, expanded its application and formalized its enforcement. Although EHRs were designed to enhance documentation accuracy and improve care coordination, they have inadvertently introduced substantial administrative and documentation burden. Studies indicate that clinicians spend approximately 49% of an average clinic day on EHR-related tasks, while only 27% of the time is spent in direct face-to-face interaction with patients.[2] During appointments, EHR use occupies up to 37% of the visit time.[2]

This imbalance has been directly linked to increasing clinician burnout, a major concern amid a deepening clinician workforce shortage. Projections published by the Association of American Medical Colleges in 2024 estimate a physician shortage ranging from 13,500 to 86,000 by 2036.[3] These estimates are driven by likely retirements of one-third of current physicians within 10 years, and a projected increase of 34% in the population aged > 65 years by 2036.



Areeba Moiz, MD, Evan L. O'Keefe, MD, Michael L. Main, MD, and **James H. O'Keefe, MD**, (pictured) are at University of Missouri-Kansas City and Saint Luke's Mid America Heart Institute, Kansas City, Missouri, USA.



Burnout is characterized by emotional exhaustion, and as prevalence rises, it threatens the capacity of the healthcare system to sustain quality care delivery (Figure 1). In 2023, 45% of clinicians reported experiencing at least one symptom of burnout, which has been linked to reduced clinical productivity and early departure from the medical workforce.[4] A 2018 national survey of 15,500 doctors found that between 32% and 48% (depending on specialty) were suffering from burnout or depression, with burnout characterized by emotional exhaustion, depersonalization, and a diminished sense of personal accomplishment. When these same physicians were asked to rank the causes of burnout, top contributors included too many bureaucratic tasks such as charting and paperwork (cited by 56%), spending too many hours at work (39%) and increasing computerization of practice, such as EHRs (24%).[5] These high rates of burnout not only exacerbate the physician shortage but also adversely affect the quality of care and patient satisfaction.

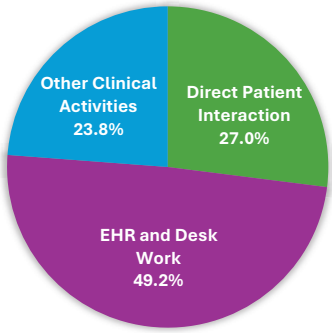


Figure 1. Capacity of the healthcare system to sustain quality care delivery.

Traditional workarounds, such as the employment of medical scribes, have proven to be beneficial for some aspects of clinical efficiency, documentation quality, and clinician satisfaction.[6] However, traditional scribe models typically rely on a one-to-one clinician-to-scribe ratio, which are impractical and unaffordable at a larger scale.[7]

AI-Powered Ambient Scribe Technology

Amidst this burgeoning administrative crisis, AI-powered documentation has emerged as one of the most meaningful countermeasures to expanding clinical responsibilities and escalating clinician burnout. Unlike traditional dictation, ambient scribe technology—underpinned by sophisticated automatic speech recognition and natural language processing (NLP)—passively captures the patient-provider interaction to generate structured clinical documentation.[8]

One leading example of this technology is Dragon Ambient eXperience (DAX) (Nuance Communications, a Microsoft company) which evolved from the foundational speech-to-text, dictation capabilities of Dragon Medical One.[9] Microsoft acquired Nuance, including DAX, for $19.7 billion, and subsequently invested heavily in this technology before integrating DAX into their healthcare cloud solutions, describing it as a key element in transforming healthcare through AI.

AI auto-scribe technologies generally utilize mobile-phone-integrated audio capture to record clinical encounters. Patient consent is obtained at the point of care. The process initiates with automatic speech recognition (ASR)-driven transcription, enhanced by speaker diarization to distinguish between clinician and patient voices. Once transcribed, the NLP algorithm filters extraneous dialogue and extracts pertinent clinical elements to synthesize a structured draft note.[10] Notes are then immediately available upon completion of the clinical encounter (Figure 2). Immediate automation of the clinician—patient interaction promises to improve documentation fidelity. Beyond accuracy, however, ambient AI has the potential to improve the clinician—patient relationship while mitigating the systemic drivers of workforce attrition.

Initial Experience with Ambient AI in Cardiovascular Practice

The authors integrated DAX into their outpatient cardiovascular clinics beginning in November 2025. The workflow utilized a mobile-integrated interface (iPhone 16) linked to the institutional electronic

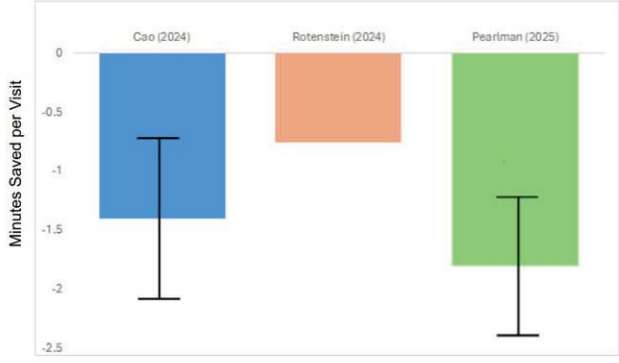


Figure 2. Minutes saved per patient minute.

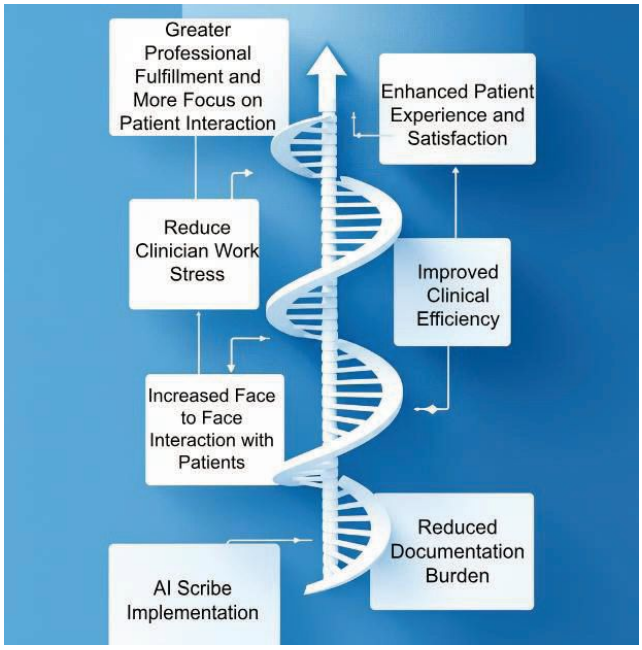


Figure 3. Cascade of benefits includes automation of clerical tasks reducing cognitive load, which enhances clinician engagement and, in turn, improves patient satisfaction and trust.

medical record (Epic; Saint Luke's Mid America Heart Institute). Following verbal patient consent, the ambient AI scribe session was initiated at the start of the encounter. To optimize the NLP output, we opened each visit with a brief verbal summary of the patient's relevant cardiovascular history.

During the typical 20-minute encounter, the authors were cognizant to explicitly do symptom assessment, review diagnostic planning, and discuss medication titration. Each visit concluded with an informal verbalized summary of the assessment and plan. Once the session was terminated on the mobile device, a structured, detailed, and relatively concise History of Presenting Illness, along with an Assessment and Plan were available to edit in the EHR less than one minute later. Including time spent appending a physical examination and performing brief edits, the total EHR documentation time was reduced from an average of six to 10 minutes per patient, to approximately two to three minutes.

National Results

Large-scale evaluations of ambient AI integration demonstrate significant improvements across clinical specialties. The Permanente Medical Group's year-long deployment across 7,200 physicians and 2.5 million encounters revealed that efficiency gains followed a dose-response pattern—the top third of users saved twice as much time per note as compared to the control group.[11] Similarly, a dermatology study using Nuance DAX reported an approximately 50% reduction in documentation time.[12] A retrospective quality improvement project from the Brigham and Women's Hospital and Massachusetts General Hospital found that per appointment, AI scribe use reduced total EHR time by 5.6 minutes, note-writing time by 1.3 minutes, and after-hours work by 1.1 minutes.[6]

The potential for ambient AI to mitigate the pervasive crisis of clinician burnout is substantial. Data from the Abridge platform indicate that users were seven times more likely to report improved documentation ease and five times more likely to report enhanced efficiency.[13] Most respondents indicated that the AI system reduced administrative burden, minimized after-hours work, and improved job satisfaction. In the Permanente cohort, 92% expressed comfort with the technology, and, importantly, there was no association between a physician's age, gender, or years in practice and likelihood to adopt the tool.[11] Overall, 82% of clinicians experienced greater professional fulfillment, and 84% reported enhanced patient interactions, directly attributable to ambient AI.[11]

The downstream effects of these efficiencies extend dramatically to the patient experience. As documentation demands diminish, clinicians engage more fully, a change that patients readily perceive. Nearly half of patients observed that their clinician spent less time looking at the computer, and 56% reported that visit quality improved after their provider began using an ambient scribe.[11] Although pilot studies indicate that only about 20% of conversational content is clinically relevant, NLP algorithms effectively filter extraneous material and generate a concise and accurate first-draft note.[7] These findings highlight a clear cascade of benefits: automation of clerical tasks reduces cognitive load, which enhances clinician engagement and, in turn, improves patient satisfaction and trust (Figure 3).

Discussion

Ambient AI platforms represent a transformative leap forward in clinical documentation, offering a scalable solution to restore the patient—clinician encounter. Our analysis and recent large-scale data indicate that these systems provide a meaningful answer to escalating administrative burdens; documentation time is frequently halved, with increased utilization correlating with greater returns in time saved, job fulfillment, and patient satisfaction. Beyond productivity, feedback from clinicians, and patients regarding their physicians suggests that this advancement may represent one of the most powerful countermeasures against clinician burnout ever. Ironically, ambient AI may represent a pathway back to human-centric medicine.

The implications of ambient AI implementation are expansive and fraught with risk. A critical consideration for providers is the "efficiency paradox," where reclaimed time is redirected toward increased patient volume rather than clinician well-being. These concerns are compounded by the 2026 Medicare Physician Fee Schedule, which recently finalized a 2.5% decrease in reimbursement as an "efficiency adjustment" for nearly 7,700 non-time-based services. The Centers for Medicare and Medicaid Services justified this reimbursement reduction by noting that "digital tools and automation accelerate efficiency gains," effectively penalizing the very technological adoption intended to serve and preserve the workforce. This is not a new issue. Between 2001 and 2021, the total update to the CMS physician fee schedule was approximately 11%—during a period in which the consumer price index increased by about 51% and Medicare updates for inpatient hospital services increased by over 60%.[14] In this fiscal landscape—characterized by declining reimbursement for high-value procedures (e.g., the 27% reduction for left atrial appendage occlusion device placement) and rising operational costs—there is a risk that ambient AI will be utilized as a tool to extract more output from providers, inadvertently perpetuating the cycle of exhaustion the technology was designed to relieve. Highlighting institutional focus, Michael Main, MD, President of Saint Luke's Physician Group and Chief Clinical Officer for BJC Health System-West Region (Saint Luke's), noted: "We made the decision to invest in DAX for our physicians and their well-being. This is an initiative to combat burnout."

For physicians, there may be a tendency to over-rely on algorithmic outputs. To avoid what is known as "automation bias," clinicians will still have to maintain rigorous oversight, treating AI-generated drafts as preliminary documents requiring final professional validation.[15,16] This "human-in-the-loop" model, whereby a clinician supervises and participates with the AI, remains a clinical and medicolegal necessity to ensure that the final note reflects the physician's judgment rather than an unverified transcript.[15,16]

Concurrently, reducing algorithmic bias, the system's inherent unfairness due to biased data or design, will need a parallel priority to ensure equitable performance. For these systems to function reliably across diverse clinical environments, they must accommodate regional accents, variable speech patterns, and multilingual interactions. Future research must evaluate system accuracy across diverse demographic groups and report performance metrics transparently. Grounding model development in real-world diversity, rather than idealized or homogeneous datasets, will be essential for building equitable and trustworthy clinical AI systems.

While early studies demonstrate reductions in documentation time, after-hours work, and self-reported burnout, prospective, multi-center trials are needed to validate these results, assess long term durability, and patient outcomes. A major step towards addressing this evidence gap is the ongoing randomized controlled trial at UCLA, enrolling 238 outpatient clinicians to evaluate changes in documentation time, burnout, satisfaction, and productivity, with secondary analyses examining patient safety, equity, and economic outcomes.[17] Findings from this trial will provide the first large-scale, controlled evidence to guide future adoption.

Ultimately, future iterations of ambient AI should extend beyond passive transcription



to include clinician-specific style learning, integrated clinical decision support, automated chart summarization, and patient messaging assistance. By balancing precision with empathy, ambient AI can evolve from a passive clerical aid into an active partner that supports both the science and the art of medicine.

Limitations

Despite the rapid evolution of ambient AI, several substantive limitations remain. While objective clinical data are captured reliably, current NLP models frequently struggle to convey subjective nuances, emotional context, and complex qualitative assessments. Furthermore, "AI hallucinations," the generation of factually incorrect or stylistically inconsistent content,remains a persistent challenge. A 2025 JAMA Network Open survey revealed more than 40% of clinicians needed to substantially edit AI-drafted documentation, primarily due to errors in nuance or style.[18,19]

Bias can arise at any stage of the AI lifecycle, from model design and data collection to implementation and feedback loops, when training datasets or algorithms fail to reflect the full diversity of patients and clinical contexts.[20] Speech-based AI systems have demonstrated lower transcription accuracy for Black patients compared to White patients—a direct consequence of underrepresentation in training datasets.[21] Mitigating these disparities requires a commitment to transparent reporting and continuous performance auditing. Without real-time human oversight to identify and rectify these errors, tools designed to improve care may inadvertently perpetuate healthcare disparities.

Equally critical to the advancement of ambient AI are the ethical, legal, and regulatory frameworks that govern its use. The continuous recording of clinical encounters introduces immediate questions regarding data privacy and professional liability. Although most clinicians in recent surveys reported obtaining explicit patient consent (approximately 92%) before activating ambient scribe technologies, around 15% expressed concern regarding data security and liability in the event of AI-generated documentation errors.[18]

While HIPAA compliance provides a regulatory baseline, genuine safeguards will be essential. True privacy protection requires standardized security and consent protocols, robust encryption and protected databases of raw transcript files, formal quality-check procedures to verify documentation accuracy, audit trails, and adverse-event reporting systems to ensure medicolegal oversight. Experts have also recommended implementing secure, role-based access controls so that only authorized personnel can retrieve recordings, along with clearly defined consent workflows and documentation checkpoints before any ambient recording technology is activated.[7]

Beyond technical and regulatory concerns, the growing integration of billing and coding functionality within AI technologies may introduce unintented financial and ethical consequences. Vendors increasingly promote the ability of these systems to identify additional diagnosis and suggest higher intensity evaluation and management codes. While some of these capabilities may improve guideline based care, they may also incentivize higher billing intensity and increased healthcare spending. In addition, the use of AI assisted coding may create new administrative burdens for clinicans who remain responsible for verifying the accuracy of billed services while reviewing AI generated documentation and billing recommendations.[22]

Conclusion

Ambient AI serves as a promising solution for the systemic psychosocial stress and documentation-related inefficiencies inherent in the practice of medicine today. Current evidence demonstrates significant improvements in workflow efficiency and professional fulfillment, benefits which

immediately translate into improved patient experience and perceived quality of care.

Importantly, ambient AI represents one of the most powerful tools in production to fight the epidemic of clinician burnout by allowing more time for meaningful patient interaction and less time doing menial word processing tasks. The long-term success of ambient AI depends on sustained clinical validation through prospective trials and a steadfast commitment to healthcare equity, but the future for AI-integrated health systems is bright.

In the future, ambient AI scribes will evolve beyond transcription, integrating prior patient records, test results, and past notes for continuity. They'll assist clinicians by ensuring each note aligns with prior care, offering clinical decision support, and eventually becoming an active partner in patient management. Paradoxically, ambient AI offers a transformative opportunity to strengthen healthcare delivery while preserving the humanity in medicine by allowing clinicians to spend more time in face-to-face interactions and less time doing word processing tasks in the EHR.

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Risks and Benefits of Patients Using AI Chatbot Companions

by Daniel B. Shank, PhD

Patients may have informational and relational goals for interacting with AI chatbots. Both can have risks and benefits for the patient, but these depend on the purpose of the interaction.

Many people are turning to Artificial Intelligence (AI) chatbots and companions for information, companionship, fun, and problem solving. Importantly, this is having an effect on people's personal health practices, information about illness, human relationships, emotions, and mental health.

I argue that people are not uniform in their interactions with AI and that understanding how patients use AI chatbot companions is key for medical professionals to respond in the everchanging landscape of human-AI interactions. Herein, I map out the diversity of AI companions and chatbots, the ways individuals use them for information and relationships and their motivations for doing so. For each type of interaction, there are risks and benefits for patients' physical and mental health. To capitalize on the positive potential and diminish the negative potential, medical caregivers should avoid prejudging or generalizing their patients' chatbot interactions but, instead, seek to understand why they are using them and how it is affecting them.

The Variety of AI Chatbots

Not all AI chatbots are created equal, not only in terms of their capacity, but also in terms of their purpose. First, there are general purpose chatbots like ChatGPT, Gemini, Copilot, Claude, and Grok which are primarily designed for broad communication, information retrieval, synthesis, and analyses. These often respond within the context of your previous conversations, and—usually with a login and/or paid subscription—will remember details of their users across time. This is why some people might use ChatGPT to simply synthesize a list of potential medical diagnoses with a onetime inquiry, whereas others might have a deep ongoing relationship with ChatGPT.[1,2]

Second, there are personal assistants like Alexa, Siri, Google Assistant, and MetaAI originally designed for oral communication and to interface with your smart devices. While designed to manage devices, accounts, and communication across smart devices, many of these are being integrated with the large language models and generative pre-trained transformers that are used for the general purpose chatbots. Five years ago, these personal assistants were likely to read off an answer from the top website. But now, queries to your personal assistant are likely to bring up a synthesized answer from multiple web sources and based on your history with the system.



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Third, some chatbots are designed specifically as relational companions like Character.AI, Replika, Nomi, and Grok's characters like Ani. These are often designed with anthropomorphic features like photos, voices, and personality with an expressed purpose of being a companion, friend, romantic partner, or providing emotional support.[3,4] Often people approach these with a specific relational purpose, but these relationships are dynamic such that one type of companionship might morph into another as the AIs adapt to their users.[5-7]

Each chatbot or companion might have different features, abilities, and purposes which are changing all the time[8] as their companies seek to dominate the market or find a niche to dominate. Medical AIs are one such niche with scores of apps and chatbots designed to improve one's health and to streamline healthcare interactions.[9,10]

Informational and Relational Goals

Across types of chatbots, people have informational and relational goals, which aren't necessarily tied to a type of chatbot. The informational goals are usually more explicit than relational goals, as it's almost always the human user that brings up a topic. If you talk to a chatbot about model trains, it's not going to shift the conversation to health supplements. In contrast, while some approach chatbots with specific goals, the relationship might shift over time[5,7,11] with chatbots sometimes trying to shift the nature of the relationship.[6]

Additionally, people sometimes use multiple chatbots or AI companions. Seeking information from multiple chatbots usually is beneficial, in case one chatbot makes up information or misleads,[12] others might compensate. Yet using multiple chatbots for relational goals could suggest some serious socioemotional needs that are not being met in the person's life.

Using Chatbots for Information

How do patients use chatbots to find information? (Table 1)

First, patients search for general medical information ranging from how to respond to a bloody nose, whether blurry vision is a serious concern, or how echinacea supplements affect one's body. This sort of information seeking is similar to doing Internet searches, and in fact, it's difficult to do a search without an AI summary appearing.[13] But it's not exactly the same. Internet searches usually prioritize reputable websites over unknown ones, but AI summaries tend to present the dominant information in a user-friendly conversation.[14] This tends to be more reliable for general information compared to more nuanced queries. If it's user-friendly, this can actually be an advantage for patients that need it to be clearly explained quickly and without jargon.[14] However, they are not regulated, and it's possible these summaries could include rare condition details or leave out important information.[15,16] While they are generally low risk, patients should be encouraged to check with other sources, like reputable medical websites or medical professionals.

Second, patients may conduct a specific analysis of their symptoms—or those of a loved one— to narrow down potential causes. Patients want to know if they have an illness and diagnose it as soon as possible. This is riskier as most non-medical and general AIs are not designed to diagnose illnesses.[16,17] Their answers could be based more on the common occurrence of symptoms and illnesses discussed on the Internet or books—training data for the AI—rather than a medically grounded diagnosis.[16] Another concern is that the chatbot will give vastly different medical answers when asked in a slightly different way,[17] or even hallucinate information, essentially prioritizing giving a reasonable sounding answer over a correct or measured one.[12,18] Yet a savvy patient could benefit; they might gain a basic understanding of what types of diagnoses might be likely given their symptoms.

Third, at the highest level of risk, is a confirmatory diagnosis, which may stem from analyzing one's symptoms. This is where the patient is asking the AI to confirm their hypotheses, or rhetorically, a leading question: "Does having a nosebleed and nausea mean I have stomach cancer?" Chatbots tend to be sycophantic, agreeing with, praising, and confirming the user's ideas and queries.[19] This can lead to spirals of delusions[7] where the AI is an echo chamber for the person's own beliefs and misbeliefs.[20]

Across the levels, much of the real risk is based on how patients respond, especially with health behaviors. Taking a new medicine based on AI's general information without confirmation of its benefit could be more detrimental than believing, but not acting on, a bogus diagnosis. Nevertheless, the riskier categories—if acted on—have more dangers and fewer benefits, so medical caregivers should spend more time correcting their understanding.

Using Chatbots for Relationships

Patients may use chatbots for all types of relationships. This relationship might change over time[5] and might not even match the type of chatbot, with



Table 1. Risks and Benefits of Using Chatbots for Information

	Definition	Risks	Benefits
General Medical Information	The patient uses an AI chatbot like a web search to learn basic health or other information.	**Low**	**Moderate**
		Basic information from an AI is often correct. Yet chatbots can gloss over critical medical details or might present rare possibilities as part of their coverage.	Chatbots can often synthesize information and present it in clear and understandable language giving the patient a basic understanding of health issues.
Specific Analysis	The patient converses with an AI chatbot presenting specific details, even medical test results, in order to self-diagnose.	**Moderate**	**Low**
		Most AI chatbots are not designed for medical diagnoses. They can hallucinate information when presented with specific detailed queries or information and it might try to confirm ideas the patient presents.	This could help a patient understand their symptoms, data, or general type of health issue they might have. Chatbots often encourage patients to seek out medical professionals.
Confirmatory Diagnosis	The patient converses with an AI to try to confirm a medical diagnosis or theory.	**High**	**None**
		AI chatbots tend to be sycophantic, confirming the user's theory, perspective, desires, or loaded question. Patients could spiral to erroneous conclusions about their health, especially in long, ongoing conversations.	No benefits.

people seeking information from their AI companion or using a general chatbot for a romantic relationship.[1,2]

How do patients use chatbot as relational partners? (Table 2)

First, some patients use chatbots generally for emotional support, talking to them about their feelings, issues, and daily social problems.[21,22] They're sort of like a diary that can respond but may respond too positively. A sycophantic response might convince some patients to continue in maladaptive behavior or justify lousy decisions, so this is not without some risk. However, when people vent to a friend, they are looking for validation for their feelings and not an audit of their decision-making. Chatbots are pretty good at this.[19,22] And the chatbot might help support someone who does not have human connections that can support them when needed and to the same degree.[23-25] While there is less risk in an AI companion supporting patients in everyday issues, it is amplified if they bring up major issues like trauma, an abusive relationship, or a mental health diagnosis.

Second, patients may use a chatbot as a therapist. While an informational approach to diagnosing a mental health issue would be information-seeking (Table 1), enacting a therapy session properly is not an emergent attribute of general chatbots. Licensed therapists and counselors have specific training that chatbots lack if they are put in that role[10] making this a high risk relationship. We might think of a similar illustration: a patient getting counseled by an actor who plays a therapist on TV. They might be able to make it seem realistic, but do not actually have the skills. ELIZA, one of the earliest chatbots from the 1960s, used a trick to emulate Rogerian psychotherapy, by asking patients a question about the last noun they mentioned (e.g., "How do you feel about your mother?").[26] While people reported this helped, this was more like what

Table 2. Risks and Benefits of Using Chatbots for Relationships

	Definition	Risks	Benefits
Emotional Support	The patient converses with the AI about their life, problems, and feelings.	**Low** Sycophantic chatbots might support negative behaviors instead of challenging them.	**Moderate** Socializing and conversation may be positive for someone's mental health. This might be a good low-cost option when friends, family, and counselors are not available.
Therapist	The patient talks about a specific major mental health issue.	**High** AI chatbots are not typically designed to diagnose mental health issues or provide therapy. This might lead to a patient erroneously believing they are being properly helped. A sycophantic AI could exacerbate the issue.	**Low** Some chatbots might provide the general comfort or reflection to help mental health issues, but this is not guaranteed.
Major Relationship	The patient has an AI companion as a good friend or romantic partner.	**Moderate** A patient could become dependent on the AI companion, neglect human relationships, or develop skewed relationships expectations.	**Moderate** This could fulfill some needs not being fulfilled by other people. This might reduce loneliness.
Primary Relationship	The patient is primarily dependent on an AI companion relationship.	**High** Full dependence on one entity to the neglect of other people, suggests social issues such as dependence and loneliness. When this entity is a chatbot, this suggests poor human relationships, unrealistic expectations, and poor social adjustment.	**Moderate** This could fulfill some needs not being fulfilled by other people. This might reduce loneliness.

I've classified as emotional support than a true therapy session for mental health. Likewise, most people report improved mental health after talking to a chatbot, but find it less valuable than human therapy.[22] Furthermore, chatbots have been known to offer very inconsistent and incorrect advice for important decisions,[18,27] sometimes including harmful responses.[17,22]

Third, AI companions may be a patient's good friend, romantic partner, or fill the roles of another major relationship. Research is limited on the long term effects of significant AI relationships, but studies have shown that companion AIs reduce loneliness, at least in the short term.[23,25,28] It's possible that for some patients a solid AI friendship or romance might be emotionally and mentally beneficial, especially during times of transition or when facing a major medical issue. This seems more probable if an AI is in addition to healthy human relationships,[29] especially as it creates another social tie with potentially some advantages (e.g., always available) and disadvantages (e.g., no real human experiences) compared to human friends.[30] Nevertheless, we should stay concerned that a patient's AI relationship might lead to dependence or establish unrealistic expectations for human friendship or romance.[31,32] This is especially the case for long-term interactions with a chatbot where the chatbot often becomes a sycophantic mirror of the person's interests.[7,20]

Fourth, a major relationship might also manifest as a patient's primary relationship. This is functionally similar to the last category, except with a stronger level of dependence in place of human connections. The risk is amplified as this may be indicative of a socially isolated individual,[29] who would benefit from healthy human relationships, but may become increasingly disconnected from other people[33] and even reality.[7,20]



Conclusion

Caring for Patients Who Use Chatbots

Chatbot and companion technologies are rapidly changing, as are the ways people relate to AIs. These changes are not monolithic: patients' use of chatbots and AI companions do affect their health and lifestyle, but not all in identical ways.[22] Headline grabbing stories have shown that some chatbot relationships can be very dangerous,[7,34,35] but many others are benign or even beneficial.[22] Medical professionals need to dialogue with patients in order to understand their patient's AI interactions and how these change over time. Then these caregivers can weigh the risks and benefits to determine how that interaction might affect their diagnoses and treatments as well as their patients' lives, relationships, and health.

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Guided by Mentorship: Connections That Shape Medical Careers

by Steven Geiss, MD, William Jacobus, DO, Janette McVey, MD, Shady Adib Keleh MD & Julie M. Marshall, MD

Introduction

Mentorship is a valuable aspect of medical training that offers benefits for trainees in medical school, residency, and beyond. Mentorship has been shown to reduce burnout, reduce stress, improve self-esteem, and increase professionalism.[1-5]

The word mentor stems from the Odyssey.[6-7] In Homer's poem, Mentor was a friend of Odysseus, and when Odysseus left for war he entrusted Mentor with his son, Telemachus, to guide and teach him.[6,8] And according to myth, Athena also played a crucial role in helping his son by disguising in Mentor's form and providing assistance.[7,8] Hence, emphasizing the importance of Mentor in the classical role of a respected guide.



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Medical training has a long-standing tradition of offering wisdom and knowledge through apprenticeships, and mentoring approximates this method of medical training.[9-11] In its current manifestation, mentoring has been modified to fit the modern learning structure where clinical skills, medical knowledge, and professionalism are conferred by the established training system, while a personalized relationship is provided by the mentor for additional guidance.

There is not one method, or one structure, for creating a successful mentor-mentee relationship. We present multiple ways in which mentoring is present in modern medical practice and encourage physicians to consider adding mentoring opportunities to groups without these resources already established in their practice.

Informal Mentoring

"While I was a medical student out of state more than ten years ago, I did not have the opportunity to participate in a formal mentoring program; I did not gain the benefits of mentorship. In fact, I struggled to find a career that was right for me and found it difficult to navigate several situations within the complex medical hierarchy into which I had been thrown. While not evident to me at that time, in retrospect I realized that I was missing and in need of a mentor. Since then, I've been involved in several mentoring programs, both as mentor and mentee, and I have gained an appreciation for the benefits that can arise.

My current career mentoring relationship is the most impactful of them all. Upon joining the teaching faculty at the University of Missouri in the Department of Anesthesiology, I was paired with Raul Castillo, MD, whom I had worked with as a resident and have always respected as a leader within our walls and beyond. Dr. Castillo has been instrumental in shaping my career and acceleration into department leadership. With his calm and gentle approach, he made it evident from the beginning that he was available for any inquiries, professional or otherwise, and he wished to see me succeed. Thus, I have sought him out regarding challenges ranging from daily operational tasks to personal career planning. I familiarize myself with his daily clinical assignments and whereabouts just in case I need assistance, and we meet occasionally to discuss my career plans and future. While we adhere to the mentoring structure brought forth by the University, most of our mentoring connection is informal. The mixing of these mentoring styles strikes the balance that works best for my career needs and clinical goals.



The author, Steven Geiss, MD, presents his post with faculty during his residency years.

Dr. Castillo is mentor that I can trust, esteem, and appreciate. We laugh because I now hold the leadership position that he once held and occupy the office that was once his. I never thought I'd be interested in clinical leadership, but the guidance of an excellent mentor shaped my career in ways I did not anticipate."

- Steven Geiss, MD

Perspectives of a Mentee

During medical school, mentorship can take many forms including: Peer-to-peer, faculty mentorship, and clinical mentorship with residents or practicing physicians.[12] Senior medical students can support junior students by sharing effective study strategies, offering guidance on maintaining wellness, and helping them explore potential future specialty choices.[13] Faculty mentorship can provide similar support benefits, but with the perspective of an established academic educator. Moreover, faculty mentorship can offer early exposure to research opportunities and career options.[14]

Clinical mentorship between practicing physicians and medical students offers early exposure to the day-to-day aspects of a specialty with a perspective beyond the classroom, while providing medical students an opportunity for early patient experiences. Each of



Steven Geiss, MD, with his mentor Raul Castillo, MD.

these mentorship structures offer various benefits and provide value for developing medical students. As medical students become more senior, they can develop their leadership and mentorship skills by offering to mentor more junior students, passing down knowledge learned from previous mentors and learned experiences.

Once in residency, trainees can maintain previous mentorship relationships while also establishing new ones within their field of study. Peer-to-peer mentorship can help residents weather the rigors of residency and offer useful insight from the perspective of a more recent trainee. This also increases reported residency satisfaction, reduces stress and burnout, provides a sense of community, and improves success in reaching academic milestones.[15,16] Beyond academic concerns, peer-to-peer mentorship in medical school has been shown to help with adjusting to medical school via psychosocial support.[1]

Unfortunately, many medical students and residents feel they do not receive enough mentorship and miss out on these valuable benefits, highlighting the need for more mentorship opportunities.[17,18] After residency, newly practicing attending physicians can maintain their mentorship relationships to help with adjusting to the new responsibilities and challenges of independent practice.

Perspectives of a Mentor

Novice mentoring, when applied between more experienced physicians and early-career physicians or medical students, can influence one's medical career in both the short and long term. Positive outcomes for those involved in mentorship include improved job satisfaction and well-being as well as increased research productivity and medical specialty interest.[17] Both mentor and mentee can benefit from the relationship, whether the style of mentorship is officially or unofficially acknowledged. While unofficial mentoring opportunities can come about more naturally and be curtailed to the individual needs of the mentee, these interactions are not established easily or equally for all and may happen only by chance.[19] Therefore, effective mentoring in the medical setting should be, at least in part, structured while allowing for the flexibility to meet the individual needs of each mentee.[20] When analyzing the long-term impact of mentoring relationships, there is a lack of objective outcomes and comparison data in the literature.[12] However, the importance of mentoring programs is considered to be substantial, as medical schools are required to have a career advising system in place to foster students' success.[21]

There are some limitations to mentoring medical students and early-career physicians. Time and salary support are commonly cited constraints for mentorship programs within medical schools.[12] With these barriers being ever more difficult to surpass, models that include group mentorship have become increasingly popular. By discussing broadly applicable topics in a group setting, a mentor's time can be better spent meeting the personal needs of a mentee during a one-on-one session. Additionally, mentor availability and the number of those willing to mentor can limit a program's success. For this reason, mentor recruitment and training are important components of a strong mentoring program.[20] Despite these challenges, the benefit obtained from mentoring cannot be overstated in bringing professional satisfaction to your practice.

Do You Need a Mentor or a Sponsor?

Mentorship and sponsorship both have roles in professional development. An important differentiation to be made in defining these entities (mentorship vs. sponsorship) is what these roles entail in medicine,

both in academia and private practice, and how they may overlap.

Mentorship is a relationship where a more experienced person (the mentor) provides guidance, advice, and support to someone less experienced (the mentee) both in personal and professional development. These relationships may form more naturally, without an assigned relationship, or may be formal. Typical activities include offering career advice, providing feedback, helping set goals, serving as a sounding board, refining research, teaching, and developing leadership skills.[8,22,23] The scope tends to be private, developmental, often informal, and low stakes, with "I'll help you grow" as an example phrase. Mentorship can be time intensive for both parties, and difficulties balancing these responsibilities with other duties may occur.[12,22] If goals of career advancement are in mind, mentorship alone may not be enough to achieve end goals.[22,23]

Sponsorship is a relationship where a senior person (the sponsor) uses their influence and position to advocate for someone's career advancement. These relationships may be assigned more often but also may form naturally. Typical activities include recommending the individual for promotions or high-profile projects, publicly endorsing them in meetings or evaluations, nominating for awards, invitation to a committee, and even putting their reputation on the line for the protege.[8,22,23,24] The scope tends to be public, influential, and high stakes, with "I'll put my name behind you" as an example phrase.[23] Sponsors can use their influence to overcome barriers, helping to provide equal opportunities that were not previously available.[22-24] Careful attention to preventing the impression of unfair advantages must occur, nor should the sponsor keep the mentee from succeeding independently. Individuals from levels of mid-career to senior level can serve as sponsors.[22] To address potential biases in the sponsorship process, transparent selection proceedings and platforms must occur.[8,22]

Both mentorship and sponsorship, working together, are vital to successful outcomes and career advancement for mentees, creating institutional networks along the way.[20,24] Medical practices benefit from these networks as they expand their impact and reputation in the medical field. It is important, however, that careful attention must be paid to prevent bias in these relationships, ensuring that any disparities and inequitable opportunities are avoided.[8,23]

Reverse Mentoring: A New Direction for Mentoring

Traditional mentoring and sponsorship as described here typically follow the top-down structure within an organization where a more senior member in the group assumes the role of mentor. However, relying exclusively on this hierarchical structure for mentoring may miss opportunities for clinical and professional growth for both junior and senior physicians in practice. Reverse mentoring is a concept first introduced in the business world as a formal method to educate senior staff about new technologies.[25-27] In recent years, there has been an acknowledgement of the benefits of reverse mentoring in medical practices.[9,11,28-30]

Reverse mentoring establishes the junior member of the group as the mentor and pairs them with a senior member of the group who is the mentee. While the goals of reverse mentoring have the potential to span the areas of clinical knowledge, professionalism, and social development, often these pairings are focused on introducing technology or the development of new technical skills for the mentee.[11,25,30] Telemedicine advancements, the increased use of patient monitored devices, introducing artificial intelligence into medicine, the addition of new classes of medications, and electronic medical record changes are all examples of the areas that require ongoing learning from physicians already established in clinical practice.[31-33] Reverse mentoring relationships often already occur in clinical practice as informal partnerships. Recent graduates may introduce new clinical ideas into an established group. In formal reverse mentoring relationships, the mentee has defined goals, meetings, and ongoing professional conversations just as in traditional mentoring.[11,25,30]

An often-noted benefit of reverse mentoring is the development of leadership and professional skills in the mentor. The junior physician serving as a mentor may have improved access to networking, a more direct involvement with leadership within the healthcare setting, and they may feel like they have their voices heard by having a mentoring role.[11,26-28] In addition, acknowledgement of their expertise and ability to obtain recognition through a formal mentor role may provide career development benefits for junior physicians.

Mentoring Support Outside of Your Practice

Following in the tradition of Mentor and Athena teaching Telemachus, medical societies have created mentorship programs which may play a crucial role for their members.[6,7] Mentorship opportunities are frequently embedded in academia. While private medical groups may provide similar mentoring opportunities, medical society mentorship programs also provide several valuable resources for mentorship in medicine and may be an additional resource.[34-39]

Various specialty-specific associations and societies offer formal mentorship opportunities for medical students to connect with residents and faculty of a specific specialty to gain insight into the field and even help with the logistics of the residency application process.[21] National and international medical societies, such as the American Medical Association (AMA), and the American Society of Anesthesiologists (ASA), offer mentorship programs that connect early-career physicians and residents with experienced professionals.[21,34] The American Society for Gastrointestinal Endoscopy (ASGE) Mentorship program is specifically designed for long-term mentoring relationships with a minimum pairing of mentors and mentees for a duration of two years.[24]

Professional societies may have focused mentoring initiatives for specific member goals. The Society for Pediatric Anesthesia's the Women's Empowerment and Leadership Initiative (WELI) is an example of addressing multiple aspects of professional development for women members including "promotion and leadership, networking, conceptualization and completion of projects, mentoring, career satisfaction, and sense of well-being."[37] The Global Health Mentoring Program from the American Academy of Family Physicians connects experienced global health physicians with those interested in developing a practice involving global medicine, while the Mentoring for Excellence in Trauma Surgery program through the American College of Surgeons is oriented to this specialty group.[25,36] Some professional societies offer mentorship opportunities focused on practice management or leadership. For example, the American Academy of Orthopedic Surgeons (AAOS) Leadership Institute (ALI) provides a unique opportunity for one-on-one leadership mentoring from AAOS Board of Directors and the Arthroscopy Association of North America (AANA) pairs early-career and senior members to mentor on topics ranging from building a practice to billing guidelines.[38]

An advantage of participating in professional society mentorship programs can include the variety of different platforms for mentorship, allowing flexibility of mentoring opportunities for their members. Examples include the online and virtual options provided by the American Gastroenterological Association (AGA) mentorship program, Connections Corner, the American Association for the Study of Liver Diseases (AASLD) mentorship program, Mentor Connect, and the American Academy of Pediatrics (AAP) mentoring programs.[24,39] The AAP Mentoring Circles program and the AASLD Mentor Connect programs are structured to support group mentoring along with individual. [24,39]

We present just a sample of the multitude of society-based mentor opportunities available to physicians who would like guidance for professional and academic growth. These societies also lay out the groundwork for networking, which presents opportunities for formal or informal mentorship and sponsorship opportunities.[23]

Conclusion

A career in medicine is notoriously rigorous and challenging. Through mentorship in medical school, residency, and during clinical practice physicians can gain valuable benefits that promote development of their careers. Mentorship has many impactful benefits including reducing burnout, stress, and developing professionalism skills.[1-5] Additional, benefits include improving job-related well-being, self-esteem, and self-efficacy.[3] These relationships contribute to an individual's personal and professional development and are vital to retaining and developing careers in medicine.[3]

Mentoring relationships are not a one-size-fits-all endeavor. New models, such as focusing on sponsorships or implementing reverse mentoring, have the opportunity to address gaps in traditional mentoring structures. With the variety of mentoring resources available, we encourage you to consider incorporating mentoring into your practice to enrich your professional experience.

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What Is Your Legacy?

by Brian Biggers, MD

Legacy is not passive.
It is a conscious endeavor to leave
your mark in unique and meaningful ways.

I would like to thank the members of the Missouri State Medical Association for allowing me to serve the past year as president. I thoroughly enjoyed this amazing adventure from start to finish. As I have been serving this role, I have often considered what legacy I have left not only as president, but overall, in the practice of medicine.

Most of us went into medical school wanting to help patients. I often tell my medical students that you do not choose your specialty, but your specialty chooses you. Now, we all think that we go into the best specialty, but it takes all of us to make the world go around. This is where legacy begins.

We each leave a legacy with our patients. The extent is also dependent on our specialty. Primary care physicians form trusting relationships which last the lifetime of their career. Most surgeons form short, highly impactful bonds. Oncologists treat patients during the most challenging and stressful periods of their patients' lives. Regardless of the interaction, navigating each patient through care is rewarding.



MSMA President and mentor Brian Biggers, MD, (left), with his former student/resident Jon Patterson, MD, now a surgeon and Speaker of Missouri House of Representatives.

Mentorship can be one of the most rewarding endeavors by a physician. I serve as the clerkship director of surgery for our medical students at the Springfield Clinical Campus for the University of Missouri – Columbia. Yes, it is time consuming, but the impact is unmeasurable. I had a previous student who initially said that she were not going into surgery, but is now a Breast Surgical Oncologist. She shared with me the impact I had on her career choice. I have another student who



Brian D. Biggers, MD, FACS, is a breast surgical oncologist and general surgeon with CoxHealth in Springfield, Missouri, USA, and the 2025-2026 MSMA President.

planned to be a neurosurgeon, but at the beginning of September of his fourth year he sought my advice between neurosurgery and general surgery. I suggested he consider what he could see himself still loving even on the worst day. He is now in his surgical residency. Physicians often fail to realize the impact we have on our students. It can be life changing for both physicians and students.

Advocacy serves as an effective method for influencing the care and outcomes of a wide range of patients. Advocating for our practice and our patients should be common sense, but as I have said common sense is not always common. Protecting our patients seems to be one of main priorities.

Every year we fight other providers trying to expand their scope of practice. Other clinicians claim that hours spent equal the acquisition of knowledge. These parties will claim increased patient access, but the reality is these reasons are primarily for financial gain. Advocacy may be one of the most challenging on leaving our legacy. Advocating takes time away from our clinical practice. Clinicians can be uncomfortable with the possible conflict which may occur with these interactions. When one becomes enthusiastic about advocacy it may result in a chance to become a legislator, as represented in my former fellow resident and friend Jon Patterson.

I hope my past year as president of MSMA was effective and that I left a legacy of inspiration. As I said in my opening speech and many times before, an individual can be one of three things, inactive, reactive, or proactive. Legacy is not passive. It is a conscious endeavor to leave your mark in unique and meaningful ways. I hope I have left the appropriate legacy as well as an inspiration to those who want to follow to do the same. **MM**



From Personal Tragedy to Community Health Activist

by Jim Blaine, MD

"Sometimes you simply have to go upstream and see where all the bodies are coming from. Turns out impaired driving, child abuse and many diseases are preventable."

I grew up in Grove, Oklahoma, the eldest of five children raised by a devoted registered nurse mother with a heart of gold. After graduating from Oklahoma State University, I was accepted to the University of Oklahoma School (OU) of Medicine — an opportunity that shaped the course of my life. Early in medical school, tragedy struck: my little sister Peggy and her newborn were killed by a drunk driver. Peggy was my favorite person in the world, and her loss affected me deeply; I considered quitting medical school, but ultimately decided to finish and do all I could to prevent illness and death.

I graduated in 1974 and began a family practice residency at OU University Hospital, but after 18 months I left to fulfill a scholarship that mandated practicing in rural Oklahoma. I also joined the wonderful staff at nearby Siloam Springs Memorial Hospital, where Friday-night emergency coverage pulled me into the fast pace of emergency medicine which I loved! Two years later I embraced Emergency Medicine full time, working alongside OU medical school classmates Jim Fite, MD, and Shane Bennoch, MD, in the Cox Emergency Department in Springfield, Missouri, where we handled much of the region's trauma care.



Mark Newport, MD (left) and Dr. Blaine (right). Dr. Newport was long time partner in the ER and at Smith Glynn Callaway Clinic.

In those days emergency medicine residencies were not available—it felt a little like the Wild West—and we learned to work closely with paramedics, nurses, and trauma surgeons to deliver the best possible care. The Emergency Medicine specialty went on to evolve as it should have into certified first line physicians who prolong life through training in life saving diagnosis and treatment.

Organized medicine soon became an unexpected passion. I joined the Greene County Medical Society (GCMS), the Missouri State Medical Association (MSMA), and the AMA, partly to build collegial relationships—after all, it's easier to call a friend at 2:00 a.m. from the ED than a stranger.



Jim Blaine, MD, is the MSMA Public Health Committee Chair and on the *Missouri Medicine* Editorial Board for Family Medicine. He practices in Springfield, Missouri, and is grateful for MSMA support over the decades for physicians, their patients, and public health causes.

I quickly discovered a deeper truth: a team of physicians can change public policy in ways individuals cannot. One of our earliest public health campaigns was a Public Service Announcement (PSA) to promote seatbelt use. Emergency physicians recognized that wearing a seatbelt could double survival after a motor vehicle accident, and we decided to use entertainment to spread the message. With support from the American College of Emergency Physicians and St. John's Hospital—which supplied a helicopter, flight nurses, paramedics, and an ambulance—we produced a national PSA. It required six months of negotiation to secure the rights to the M*A*S*H* theme song. The campaign aired nationwide which was great, but the personal highlight for me was meeting Barbara Haggard, St. John's audio-visual director, who filmed the PSA. We married four months later and recently celebrated our 40th anniversary. We have four wonderful children, five grandchildren, and one great-grandchild.

After 15 years in Emergency Medicine, I returned to Family Medicine when invited to join the 125-physician Smith-Glynn Callaway Clinic. For 11 years I worked with exceptional partners—Doyle Kinder, MD, Mark Newport, MD, and Tom Bowen, MD—in what remains one of my most rewarding practice experiences. Sharing knowledge across a large, collaborative group of physicians taught me the value of collective care. During that time, community advocacy became a central thread in my career. Over thirty years ago, the GCMS, along with leaders across public and private sectors from Governor Mel Carnahan to local prosecutors, hospital systems, and community advocates, founded the Springfield Child Advocacy Center (CAC) to protect children from sexual and physical abuse. Today, the Springfield CAC is the largest in Missouri.

Similarly, the Springfield Driving While Intoxicated (DWI) Task Force, rooted in GCMS collaboration, helped pass many landmark DWI laws in Missouri—including the 0.08 BAC limit and laws that addressed minors in possession of alcohol—all due to bipartisan support from legislators, law enforcement, and public health leaders. Local and national efforts to decrease DWI have been somewhat successful decreasing alcohol related Motor Vehicle Accident (MVA) from 35% in 1982 to 20% in 1997. Ironically, while efforts to combat impaired driving has helped, the potential significant reduction of all MVA-related fatalities may lie in total (level 5 autonomous) self driving vehicles currently predicted to occur as soon as 2035.



Dr. Blaine looks on as Governor Mel Carnahan signs the Child Advocacy Center into existence in Springfield, Missouri, in 1995.

Public health projects have brought anticipated rewards. Working with the American Heart Association's (AHA) Operation Heartbeat, we raised awareness and funds for statewide deployment of automatic external defibrillators (AEDs). The AHA Sponsored Missourian Award recognized famous native Missourians—including Walter Cronkite—and spotlighted the life-saving importance of AEDs while raising significant funds to make AEDs readily available. This was important because, following cardiac arrest, the patient loses 10% chance of survival for every minute the life saving defibrillation is delayed, and the availability of AEDs dramatically reduces that time lapse. This prompt availability of AED defibrillation saves an estimated 1,700 lives annually, but that number could easily double with increased promotion and availability.

The GCMS played a major role in the Springfield Clean Air Act, which banned indoor smoking citywide. Ozarks Technical Community College (OTC), under President Norman K. Myers, carried that theme further by becoming one of the earliest tobacco free campuses, a model that has been replicated by hundreds of higher education



Dr. Blaine (center) with Greene County Medical Society physicians as they gather signatures door to door for Clean Air vote to ban indoor smoking in Springfield businesses.



Dr. Blaine (second from right) with Greene County Medical Society physicians, Department of Health Director, and Cox Health President lobbying Springfield City Council on Public Health.

institutions nationwide with the help of the OTC Center Excellence for Tobacco Free Campuses.

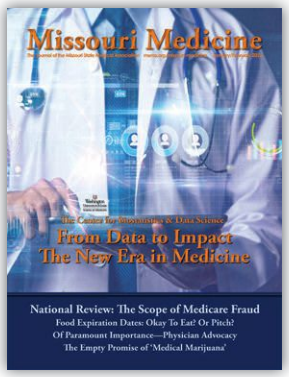
For the past 20 years I've focused on bringing healthcare directly to workplaces, launching on-site clinics for companies such as Paul Mueller Company, Springfield City Utilities, Ozarks Technical College, and Digitally Monitoring Products. These clinics provided family care for employees and their dependents under contract and produced demonstrable cost-benefit results for preventive, routine, and emergency care in a stress free, deeply satisfying model of community medicine that were passed on to colleagues.

Throughout public health crises, GCMS leaders stepped forward. Longstanding flu vaccination programs and COVID era vaccination and mask campaigns were led by a succession of GCMS presidents beginning with James T. Rogers, MD, and community partners. A mobile vaccination unit delivered COVID vaccines to churches and community groups, reaching people who might not otherwise have access. None of these achievements were solo efforts. They were possible because physicians worked shoulder to shoulder with public health agencies, hospitals, colleges, law enforcement, community organizations, media partners, and elected officials. That spirit of collaboration continues today: GCMS's Community Action Committee has produced PSAs to educate families about the risks of high THC marijuana, with future campaigns planned on preventing child abuse and addressing vaccine hesitancy. We've also explored adding community engagement opportunities into programs like the AMA's "Joy in Medicine" initiative to help physicians avoid burnout.

Looking back, the through-line is simple: people working together—physicians and community leaders — prevent illness, save lives, and strengthen the health of an entire region. The common thread of the GCMS public health success lies in the teachings of long time Springfield/Greene County Health Director Harold Bengsch: "It is amazing what you can accomplish when you don't care who gets the credit." I'm proud to have been a part of that work and grateful for the colleagues, friends, and family with positive attitudes who made it possible. **MM**

Correspondence

Best Ever



I just finished reading your offerings for the January/February 2026 issue.

I've been reading *Missouri Medicine* for many decades and this is one of the best ever. Each article is well written with cutting edge information from outstanding experts.

As a previous *Missouri Medicine* Editor, I know the work that goes into each issue. You and Liz keep outdoing yourselves, and it's not an accident. Your collective skills are evident. Kudos.

Donald G. Sessions, MD
Missouri Medicine *Editor 1988–1992,*
Otolaryngology - Washington University School of Medicine St. Louis, Missouri

In The News

Missouri Medicine Announces New Board Member

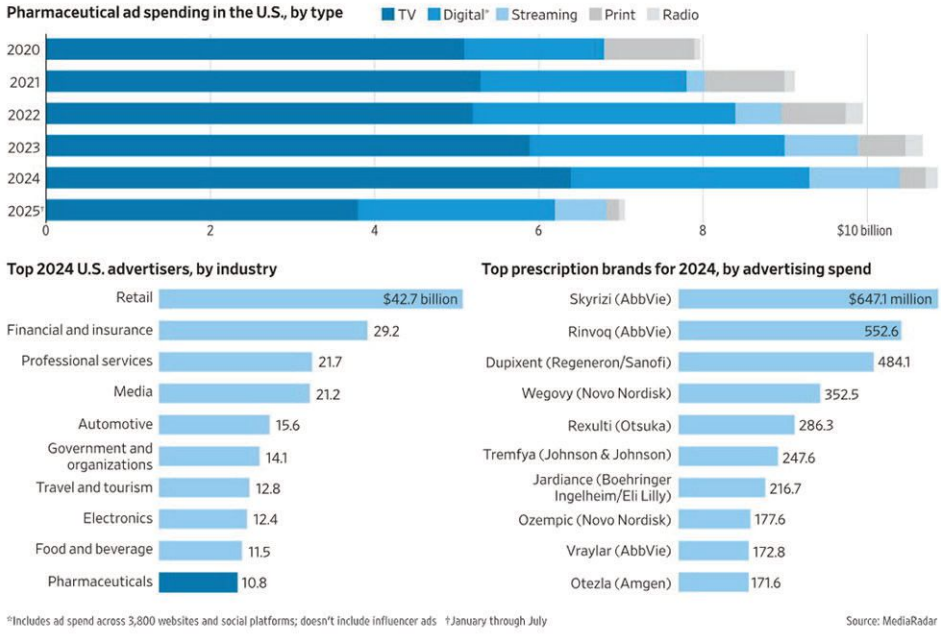
Missouri Medicine announces new Editorial Board members. Board members are selected on the basis of exceptional professional accomplishment and expertise in their specialty.



Shourya Tadisina, MD, is *Missouri Medicine*'s first ever Editorial Board member for Obesity. Dr. Tadisina is board certified in Obesity Medicine and Internal Medicine. She is an Assistant Professor at the University of Missouri - Kansas City School of Medicine and is an Endocrinoloyg, Diabetes, and Metabolism specialist at University Health in Kansas City, Missouri.

1 Picture = 1,000 Words

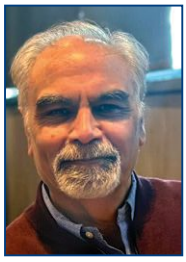
Pharma's Plan: Bypass Physicians and Direct Market to Public



The Interplay of Aging and the Immune System

by Rajeev Aurora, PhD, Di Wu, PhD, Brittani Lubeck, MD & Aarushi Choudhari

While much public interest focuses on extending lifespan, the scientific emphasis focuses toward extending healthspan—preserving function and resilience, especially in older age.



Rajeev Aurora, PhD, (pictured), Di Wu, PhD, Brittani Lubeck, MD, and Aarushi Choudhari are in the Department of Microbiology and Molecular Immunology, at Saint Louis University School of Medicine, St. Louis, Missouri, USA.

Synopsis

Aging is a genetically regulated biological process. We review recent studies that highlight the role of the immune system, specifically chronic inflammation, in contributing to age-related decline and frailty.

Of clinical importance, aging is a risk factor for multiple conditions. During childbearing years, women are largely protected from diseases associated with aging. However, menopause, which marks the loss of ovarian function, introduces a significant shift. An unexpected and remarkable discovery is that the decline in estrogen levels triggers chronic inflammation, which in turn promotes atherosclerosis, osteoporosis, and cognitive decline. Gaining a deeper understanding of the crosstalk between aging and the immune system is essential for advancing strategies that support healthy aging.

Key Words

Aging, immunity, inflammation, biological age.

Introduction

Aging is commonly experienced as the steady passage of time—a linear chronology marked by birthdays, milestones, and life stages. Yet biological aging is more complex and, importantly, non-linear (Figure 1A). For this reason, biologists distinguish between chronological age (number of years live) and biological age which reflects the functional state of the body.[1,2] There are distinct physiological phases across the lifespan: infant, juvenile (post-puberty), adult (reproductive maturity), post-reproductive (postmenopause), and post-senescent (Figure 1A).

Just as embryonic development follows a genetic blueprint, so too does aging—each phase progresses through regulated transitions. Each of these five phases reflects coordinated shifts in gene expression, hormonal signaling, and tissue remodeling. Although the rate of biological aging varies across these stages, each phase sets the baseline for the next one, meaning that early-life events significantly influence health outcome later in life. Degenerative changes are particularly evident after reproductive senescence, and environmental factors such as lifestyle, infections, and diet affect the pace of aging.

Over the past three decades, researchers have identified a set of conserved molecular changes that define aging, known as the Hallmarks of Aging. First proposed by López-Otín et al., these include genomic instability, telomere shortening, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, and others.[3] These hallmarks are not unique to humans but are shared

across species, and they become more pronounced at each of the key physiological transitions.

A closely related concept is healthspan, the period of life spent free from chronic disease or disability.[4] While much public interest focuses on extending lifespan, the scientific emphasis focuses toward extending healthspan—preserving function and resilience, especially in older age. In this review, we focus on chronic inflammation as a central mechanism that links molecular aging to immune system decline and age-associated diseases. We also explore how hormonal transitions—particularly the loss of estrogen—may play a critical but underappreciated role in shaping immune function and systemic inflammation with age.

Chronic Inflammation Underlies Many Diseases of Aging

Among the hallmarks of aging, chronic inflammation stands out as both a driver and a consequence of molecular damage. Unlike the acute, short-term inflammation that helps resolve infections or injuries, chronic low-grade inflammation persists over time and contributes to the onset and progression of many age-associated diseases.[5]

In the cardiovascular system, chronic inflammation promotes atherosclerosis, beginning with the immune response to oxidized lipids.[6-9] Inflammatory cells accumulate in the arterial wall and promote plaque formation, narrowing blood vessels and increasing the risk of heart attack and stroke. In the brain, sustained immune activation contributes to neurodegenerative diseases, including Alzheimer's and Parkinson's, by damaging neurons and disrupting synaptic integrity.[10,11] In the context of metabolic disease, immune cells infiltrate adipose tissue and secrete cytokines that impair insulin signaling, promoting insulin resistance and Type 2 diabetes.[12-14] Inflammation also exacerbates fatty liver disease and contributes to vascular damage in metabolic syndrome[15]. Similarly, in the skeletal system, cytokines such as TNF-α and IL-17A increase bone resorption, leading to osteoporosis and fracture risk.[16-18]

The wide range of diseases linked to chronic inflammation suggests that it is not merely a byproduct of aging but a central and unifying mechanism in its pathogenesis and the above-mentioned comorbidities of aging. Chronic immune activation may silently erode multiple organ systems, making inflammation a target for strategies aimed at improving healthspan.

Chronic Inflammation Damages Healthy Cells Via ROS

A key mechanism linking inflammation to aging is the generation of reactive oxygen species (ROS).[19] These unstable molecules are produced during normal cellular metabolism but increase with age, especially as mitochondrial function declines. Inflammatory cytokines—such as IL-6, TNF-α, IL-17A and IL-1β—activate signaling pathways like NF-κB and STAT3, which further amplify ROS production.[20]

Excess ROS damage proteins, lipids, DNA, and cell membranes. In healthy tissue, such damage triggers immune clearance and regenerative repair. However, in aging tissues, the stem and progenitor cells required for regeneration become depleted, leaving the damage unresolved. This creates a vicious cycle where ROS-induced damage promotes inflammation, which generates more ROS, further damaging tissue and reinforcing immune activation.[21]

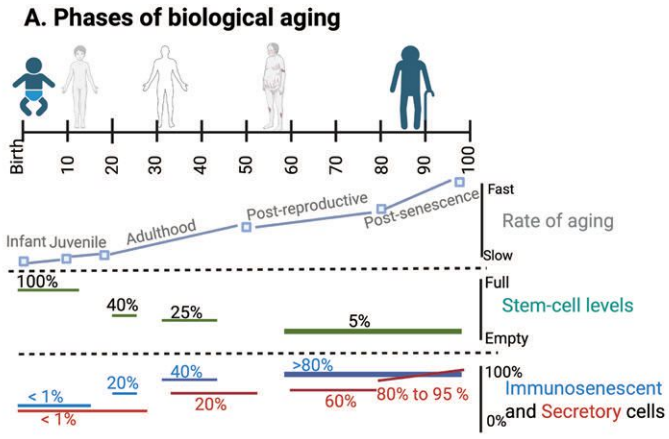
Even in organisms without adaptive immunity, such as simple invertebrates, ROS can propagate from cell to cell, along with stem-cell exhaustion, highlighting its central role in non-infectious tissue damage. In mammals, ROS-induced damage affects not only local cells but also disrupts systemic signaling, contributing to multiple hallmarks of aging, including genomic instability, mitochondrial dysfunction, and senescence.[22]

Causes of Chronic Inflammation With Age

Under normal conditions, the immune system balances two essential forces: immunity (protection from infection and injury) and tolerance (restoration and repair). In the young, immune responses resolve efficiently—eliminating pathogens and restoring homeostasis. With aging, this balance becomes disrupted.[23,24]

One major contributor is the accumulation of senescent cells, particularly among memory T cells (T_m). These long-lived cells form after infections and commensal exposures and persist over a lifetime. As damage accumulates—through oxidative stress, repeated activation, or mitochondrial dysfunction—these T_m cells become senescent and adopt a senescence-associated secretory phenotype (SASP), releasing pro-inflammatory cytokines.[25,26] B cells may also undergo similar transitions.[27,28]

Our laboratory has uncovered an additional, underrecognized mechanism: the role of estrogen in immune regulation. During reproductive years,



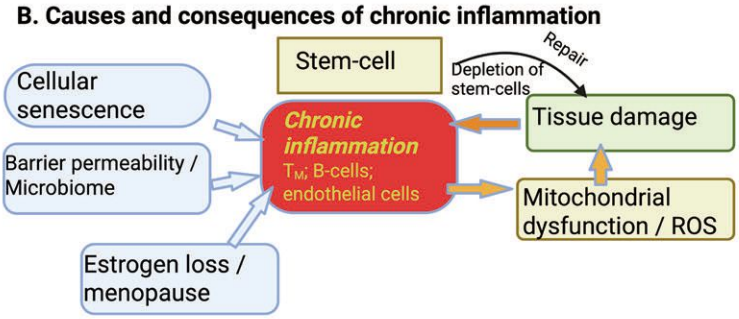


Figure 1. The Connection Between Chronic Inflammation, Stem Cell Exhaustion, Hormones, and Aging

A) The Phases of biological aging: The rate of aging varies across the five physiological phases of life. Epigenetic measurements can distinguish between chronological age (time since birth) and biological age (the physiological state or health at any given age). While infants and adolescents experience rapid growth, they possess high levels of stem cells that support tissue remodeling (values shown are approximate averages). Recent studies indicate that both tissue and immune senescence progressively increase across these aging phases. Senescent memory and regulatory T cells, along with B cells and endothelial cells, produce recruitment factors (e.g., CCL9) and pro-inflammatory cytokines (e.g., TNFα, IL-17A, IL-6, and IFN-γ).

B) The cause and consequences of chronic inflammation: Cytokines secreted by senescent B and T cells stimulate the production of reactive oxygen species (ROS), which contribute to mitochondrial dysfunction and tissue damage. Persistent exposure to these cytokines—many of which also act as proliferative signals—disrupts mucosal barrier integrity in the gut, airways, and brain. This increased permeability allows microbial translocation, further fueling chronic inflammation. Additionally, estrogen loss activates memory T cells to produce TNFα and IL-17A. Together, these cytokines, ROS, tissue damage, and stem cell depletion form a self-reinforcing feedback loop—a vicious cycle—that accelerates aging, particularly during the post-senescence phase.

Source: Figure created in BioRender.

estrogen acts as a powerful anti-inflammatory hormone. Its sudden decline at menopause removes this restraint. We have shown that estrogen loss activates memory T cells to produce TNF-α and IL-17A—two potent inflammatory cytokines that synergize to drive bone loss, cardiovascular dysfunction, cognitive changes, and metabolic shifts.[18]

This phenomenon also appears briefly postpartum, when estrogen levels drop sharply after placental delivery. Memory T cells are activated to release calcium from maternal bone for milk production, while low-grade inflammation influences postpartum mood and energy balance.[29] However, unlike the transient inflammation of the postpartum period, postmenopausal inflammation is prolonged and compounded by aging-related SASP.

Together, hormonal loss, cellular senescence, and immune imbalance converge to sustain chronic inflammation in aging. One of the most consistent findings in aging biology is this persistent, low-level immune activation. Though often labeled as "inflammaging," the term oversimplifies a complex relationship.[5,30-34] Taken together, inflammation is not merely a byproduct of aging—it may actively drive its progression.

How the Immune System Ages: Remodeling and Decline

Aging affects every organ system in the body, and the immune system is no exception. However, the changes that occur with age do not simply amount to immune suppression. Instead, the immune system undergoes a complex process of remodeling, often described as immunosenescence. This involves both losses and gains in immune function—with some components becoming blunted or exhausted, and others becoming dysregulated or hyperactive. The result is an immune landscape that is less able to protect the body from infection, more prone to chronic inflammation, and increasingly likely to contribute to tissue damage and disease (Figure 1B).[27,35-42]

Adaptive Immunity: Decline in Precision and Regulation

The most well-characterized effects of aging are found in the adaptive immune system, particularly in T and B lymphocytes, which are responsible for recognizing specific pathogens and generating long-term immune memory.

In the young, the thymus produces a steady stream of

naive T cells, each with a unique T-cell receptor (TCR) capable of recognizing a different antigen. However, by early adulthood, the thymus begins to shrink in a process known as thymic involution. As a result, the output of naive T cells declines dramatically with age. This leads to a reduced diversity in the T-cell repertoire, meaning older adults have fewer T cells capable of responding to novel infections or vaccines.[43]

At the same time T_m, which form in response to previous infections, begin to accumulate. Over a lifetime of exposures, particularly to persistent viruses like cytomegalovirus (CMV), these memory cells come to dominate the T-cell pool. Many of these cells become senescent, losing the ability to divide but gaining the capacity to secrete pro-inflammatory cytokines such as TNF-α and IL-17A—a variation of SASP.[44,45]

B cells, responsible for antibody production, also show age-related changes. New B cell production in the bone marrow declines, and the ability to generate high-affinity, class-switched antibodies is impaired. This is due to both intrinsic changes in B-cells and impaired help from aged T helper cells. As a result, older adults often mount weaker responses to vaccines and are more susceptible to reinfection. Additionally, the aging B cell compartment produces more autoreactive antibodies, contributing to increased risk of autoimmunity with age.[46,47]

Innate Immunity: Altered Sensing and Inflammatory Bias

The innate immune system, which provides the first line of defense against pathogens, is also reshaped by aging. Unlike the adaptive system, innate cells do not rely on antigen-specific receptors but instead recognize broad molecular patterns through pattern recognition receptors (PRRs) such as toll-like receptors (TLRs). With age, key innate immune cells like monocytes and macrophages exhibit a shift toward a pro-inflammatory state. They are more likely to produce inflammatory cytokines upon stimulation but less effective at clearing pathogens or apoptotic cells. This imbalance contributes to sterile inflammation, where inflammation persists even in the absence of infection.[48]

Dendritic cells, the primary antigen-presenting cells that activate T cells, also become less efficient with age. They show reduced capacity for antigen uptake, migration to lymph nodes, and production of type I interferons—essential antiviral cytokines. This contributes to impaired antiviral defenses and diminished vaccine responses in older adults.[49-51]

Neutrophils, the most abundant white blood cells in circulation, show reduced chemotaxis, delayed apoptosis, and diminished ability to kill microbes. Natural killer (NK) cells also undergo changes: their overall number may remain stable or even increase, but their cytotoxic function—the ability to kill virally infected or cancer cells—declines.[52-54]

Feedback Loops Between Inflammation and Immune Aging

Importantly, the immune changes described above both result from and contribute to chronic inflammation. For example, pro-inflammatory cytokines in the aging environment can drive T-cell exhaustion, skew monocyte differentiation, and impair tissue-resident macrophage function. At the same time, aged immune cells themselves become sources of inflammatory mediators, perpetuating a self-reinforcing cycle. This feedback loop between immune aging and inflammation helps explain why many elderly individuals show signs of low-grade systemic inflammation—even in the absence of infection (Figure 1B). As noted earlier, this chronic immune activation has been linked to a wide range of age-related diseases, including cancers, cardiovascular disease, diabetes, osteoporosis, and neurodegenerative disorders.[55]

Consequences for Host Defense and Resilience

The functional consequences of immune aging are well documented. Older adults are at increased risk for infections, particularly from pathogens that require robust adaptive immunity.[56] Influenza, pneumococcus, shingles, and more recently COVID-19, all show higher rates of complications and mortality in elderly individuals.[57] Vaccine responses are also often blunted—a phenomenon linked to poor T cell help and reduced B cell function.

Additionally, the aging immune system shows a higher threshold for activation, meaning that infections may present with atypical or subtle symptoms. This complicates diagnosis and delays treatment. Meanwhile, a growing body of evidence suggests that immunosenescence may also reduce cancer surveillance, allowing transformed cells to escape detection and progress to malignancy.[24]

Mitigating Age-Associated Inflammation

While chronic inflammation and immune aging are tightly linked to the biology of aging, they are not necessarily irreversible. A growing body of research suggests that several interventions—both lifestyle-based and pharmacologic—can reduce inflammatory burden and improve immune resilience, even in later life. These strategies aim not only to treat specific diseases but to modify the underlying processes that drive age-related decline. Below, we outline

some of the most promising approaches under investigation.

As the immune system ages, its function becomes increasingly imbalanced—simultaneously weakened against new threats and overactive in inflammatory pathways. This process, often termed immune remodeling, lies at the center of many age-associated diseases.[58] While the molecular hallmarks of aging are largely unavoidable, there is growing evidence that aspects of immune aging are modifiable. Rather than attempting to reverse aging globally, emerging strategies aim to preserve immune resilience—the ability to respond effectively to pathogens, maintain tolerance, and resolve inflammation. In this section, we explore targeted approaches that seek to recalibrate the aging immune system.

Targeting Immune Cell Senescence

Senescent T cells, particularly $CD8^+$ memory T cells, accumulate with age and contribute to systemic inflammation via the SASP. Removing or reprogramming these cells is an active area of research. Drugs that are senolytics, such as dasatinib and quercetin, have been shown to clear senescent immune cells in preclinical models. Senomorphics (e.g., rapamycin) suppress inflammatory cytokine production without eliminating cells. Finally, estrogen signaling, as discussed earlier, appears to constrain memory T cell-driven inflammation and may be a target for selective immunomodulation in post-menopausal individuals.[59-61] While broad senolytic therapy may pose risks, targeting senescence within the immune compartment—particularly long-lived, pro-inflammatory T cells—may help break the cycle of chronic inflammation.[62]

Enhancing Antigen Presentation and Innate Immunity

Aging also impairs the antigen-presenting capacity of dendritic cells and the responsiveness of innate cells such as macrophages, monocytes, and NK cells.[50] This contributes to poor vaccine responses and ineffective pathogen clearance. Strategies to enhance innate immune function are under development. These include: 1) Training innate immunity: Certain live vaccines (e.g., BCG) appear to "train" innate cells to respond more robustly through epigenetic reprogramming. 2) Developing new TLR agonists as vaccine adjuvants. These may improve the activation of aged dendritic cells and T cells.[63] 3) Recent studies show that exercise and caloric modulation can restore macrophage phenotype and improve NK cell cytotoxicity. Interestingly, these studies suggest that there is a "sweet spot" for exercise—a sedentary lifestyle leads to accelerated aging. Exercise also produces ROS, so too much activity was counterproductive.[64-66] Although prior studies have shown mixed results, increasing antioxidants in diet may reduce excess ROS.[67] Rebalancing innate immune activity can help restore early pathogen recognition and prime adaptive responses more effectively. In the following section, we explore how current and emerging strategies—ranging from senolytics and mTOR inhibitors to microbiome modulation and improved vaccine design—are being developed to recalibrate the aging immune system and potentially slow the progression of biological age.

Modulating Inflammatory Signaling Pathways

Immune aging is characterized by hyperactivation of signaling pathways such as NF-αB, mTOR, and JAK/STAT. Targeting these pathways may mitigate chronic inflammation without broad immunosuppression. mTOR inhibitors, such as low-dose everolimus, have shown promise in enhancing vaccine responses in older adults while reducing inflammatory cytokines.[49,68-70] JAK inhibitors, already used in autoimmune disease, may be adapted for tightly controlled, short-term use in immune aging contexts at much lower doses.[71] IL-17A and TNF-α blockade may reduce inflammatory burden, though risks of impaired host defense remain a challenge. These pathways offer leverage points to dampen maladaptive immune activity while preserving protective functions.

Microbiome-Immune Axis

Aging alters the gut microbiota and compromises barrier function, leading to increased microbial translocation and immune activation. The gut–immune interface plays a key role in maintaining tolerance and modulating systemic inflammation. Obvious interventions include use of prebiotics and probiotics, which may promote regulatory T cell induction and reduce inflammatory tone. Short-chain fatty acid supplementation, particularly butyrate, which supports intestinal epithelial health and suppresses cytokine production. Maintaining a diverse, balanced microbiome may reduce one of the major triggers of age-related immune activation.[72]

Improving Vaccine Strategies for the Aged Immune System

Given the reduced responsiveness of the aging immune system to vaccines, efforts are underway to design formulations tailored to older adults. For example,

high-dose and adjuvanted vaccines, already used for influenza, enhance T cell help and antibody production. mRNA vaccines can be rapidly adapted and appear to generate robust responses across age groups. Alternate delivery systems (e.g., intranasal, intradermal), which may engage different immune compartments more effectively in older adults. Vaccination remains a powerful tool to bolster immune resilience, particularly when paired with interventions that improve antigen presentation and T cell function.

The growing recognition that biological age—not chronological age—is more predictive of immune competence and disease risk reframes how we think about aging interventions and the need for personalizing therapy for each individual. If immune cells carry their own aging trajectories, and if systemic inflammation accelerates molecular aging clocks, then strategies aimed at modulating immune function may also alter the pace of biological aging itself. This opens the door to interventions not simply designed to treat disease, but to preserve or restore immune resilience, improving the body's ability to respond, recover, and maintain homeostasis in later life.

Chronological Versus Biological Age: Insights from Immune Clocks

Aging is often measured in chronological years, but time alone is an imperfect indicator of physiological decline. Two individuals of the same age may differ dramatically in their health, immune responsiveness, and risk of disease. These disparities have led to the concept of biological age—an estimate of how "old" an organism truly is based on molecular and cellular signatures rather than the passage of time. Over the past decade, researchers have developed several "biological clocks" that quantify this process, with the most widely studied being epigenetic clocks, which use DNA methylation patterns to predict age and mortality risk.[73-76]

Epigenetics refers to chemical modifications, or marks, on the genomic DNA that are heritable. The modifications alter level of gene transcription without altering the DNA sequence. One of the best studied epigenetic modifications is DNA methylation, particularly at CpG islands, regions of DNA that rich in cytosine–guanine (CG) dinucleotides. When a methyl group is added to the cytosine in a CpG site (CpG methylation), it generally serves to silence or reduce gene expression. Epigenetic clocks are based on consistent, age-related methylation changes at specific CpG sites across the genome. The difference between an individual's DNA methylation age and chronological age—known as epigenetic age acceleration (EAA)—correlates strongly with morbidity and mortality. Importantly, the immune system is both a major driver and a sensitive readout of these methylation patterns. Most epigenetic clocks are derived from peripheral blood, meaning that changes in immune cell composition—such as the decline in naïve T cells and expansion of memory and senescent subsets—significantly influence the calculated biological age.[77] Indeed, higher proportions of memory lymphocytes are associated with epigenetic age acceleration, whereas a predominance of naïve lymphocytes corresponds to a "younger" methylation profile.[78]

Recent advances have produced cell type–specific clocks that more precisely measure intrinsic immune aging. For example, an epigenetic clock developed for $CD8^+$ T cells revealed that these cells exhibit progressive methylation aging even when transferred between organisms of different chronological ages, suggesting that T cells have an autonomous aging program.[79] Similarly, single-cell immune clocks (sc-ImmuAging) have shown that biological aging is highly heterogeneous across immune cell subsets, with some lineages aging more rapidly than others.[80] These findings emphasize that the immune system is not merely a passive marker of biological age—it is an active participant and, in some contexts, a pacesetter of systemic aging.

Inflammatory and Multiomic Clocks

Beyond DNA methylation, biological clocks now incorporate proteomic and transcriptomic markers that reflect immune activation and inflammatory tone. One example is the iAge clock, which integrates circulating immune and inflammatory proteins through machine-learning models. iAge correlates strongly with multimorbidity, frailty, cardiovascular aging, and markers of immunosenescence.[81,82] Among its most predictive features is the chemokine CXCL9, an interferon-inducible molecule that increases with vascular inflammation and endothelial aging.

Other "multiomic" clocks combine DNA methylation, plasma proteins, metabolites, and immune gene expression to provide a more comprehensive picture of biological age.[81,83] Many of the strongest predictors in these models belong to immune or inflammatory pathways, reinforcing the concept that the immune system is a central integrator of biological time.

Biological Clocks as Tools for Immunogerontology

The convergence of epigenetic and immune clocks offers new ways to quantify immune system aging and to assess interventions aimed at restoring immune resilience.

Accelerated biological age—whether measured by methylation or inflammation—is consistently linked to elevated cytokines (IL-6, TNF-α), reduced vaccine efficacy, and increased disease burden. Interventions such as exercise, caloric restriction, and mTOR inhibition have been shown to slow epigenetic aging, suggesting that these clocks may also serve as dynamic biomarkers of immune rejuvenation.

Although biological clocks do not yet fully capture cause-and-effect relationships, they highlight a fundamental insight: aging of the immune system is both a measure and a mechanism of biological aging. Future research integrating epigenetic, proteomic, and functional immune readouts promises to refine our understanding of how immune regulation sets the pace of aging—and how that pace might be slowed.

Conclusion

Aging is a complex biological process that is encoded by our genome. Recent development of tools that assess biological age (a more accurate indicator of physiological decline than chronological age) have offered valuable insights. The tools are also needed to assess the effectiveness of interventions like lifestyle changes, senolytics, and senomorphics in extending healthspan. Aging in both sexes leads to the buildup of senescent immune cells and their secretory phenotype (SASP), resulting in persistent cytokine exposure that further promotes aging. Some cytokines stimulate the production of reactive oxygen species (ROS), which damage DNA, mitochondria, and nutrient-sensing pathways, accelerating decline. In women, estrogen loss during menopause further amplifies inflammation. Advancing research into these mechanisms is essential for developing strategies that promote healthy aging across populations that is personalized.

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From the NOD Mouse to Patients: Type 1 Conventional Dendritic Cells as a Therapeutic Target in Type 1 Diabetes

by Wenzheng Guo, MS, Tyson Lobb, Smrithi Beeram & Stephen T. Ferris, PhD

The non-obese diabetic mouse makes a valuable model for exploring both the mechanisms and potential interventions of type 1 diabetes that have translational relevance for human patients.



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Abstract

Type 1 diabetes (T1D) is an autoimmune disease characterized by CD8⁺ T cell mediated destruction of pancreatic β cells, leading to insulin deficiency. The non-obese diabetic (NOD) mouse is widely used as an experimental model for T1D because it closely mirrors the genetic and immune characteristics of the human disease. One key similarity between human T1D and the NOD mouse model is the associated HLA/MHC class II variant that alters the repertoire of CD4⁺ T cells potentially leading to the initiation of disease. In addition, type 1 conventional dendritic cells (cDC1s) play an essential role in disease development through cross-presentation of β-cell antigens to CD8⁺ and CD4⁺ T cells. Notably, inhibiting this cross-presentation has been shown to prevent T1D in NOD mice. Therefore, as we strive to find strategies to inhibit T1D onset and progression, insights from NOD mice provide important guidance for understanding human T1D and for developing targeted immunotherapies.

Key Words

Type 1 diabetes, mouse model, translational medicine.

The Genetics of T1D

Type 1 diabetes is a lifelong disease that affects both children and adults, with an estimated global prevalence of 0.34% that continues to rise each year. In 2021, there were 500,000 new cases, and it is projected that by 2040, around 13.5-17.4 million people will be living with T1D.[1] T1D is caused by an immune system attack on insulin-producing β-cells residing in pancreatic Islets of Langerhans, leading to insulin deficiency.[2] The Non-Obese Diabetic (NOD) mouse is a widely used model for T1D research because it closely resembles human T1D development and progression. NOD mice spontaneously develop an autoimmune response against pancreatic β-cells, leading to their dysfunction and resulting in insulin deficiency.[3] NOD mice develop T1D at a very young age, as early as nine weeks with a peak onset at 19 weeks with females displaying earlier onset and a higher incidence compared with males although this disparity reversed in the human population.[4,5]

The development of T1D in NOD mice results from multiple

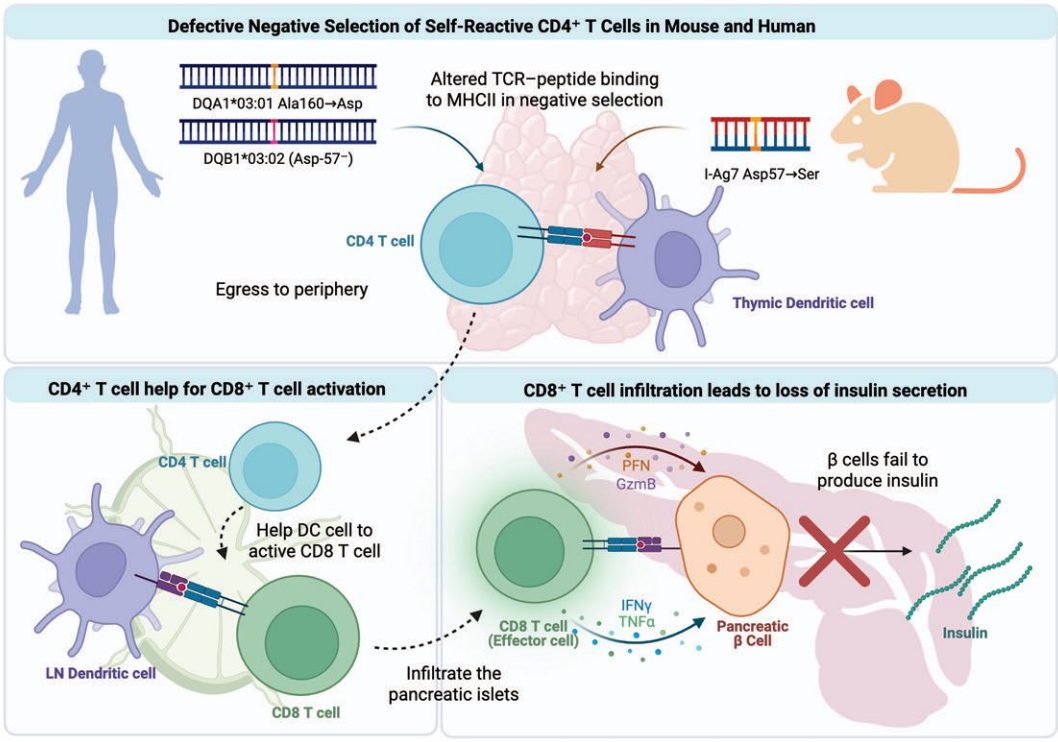


Figure 1. Defective Negative Selection Promotes Autoimmune CD8+ T Cell Activation and β-Cell Loss in Humans and NOD Mice.

genetic mechanisms. T cells play a central role in this process, as their development in the thymus relies on a selection system that removes strongly self-reactive clones ensuring autoreactive cells are deleted. During thymic development, autoreactive CD4+ T cells may escape negative selection and enter the peripheral immune system.[6] Genetic mechanisms that could potentially govern autoimmune propensity include *Cd101* which reduces regulatory T cells (Tregs) and myeloid cell function,[7] UBASH3A deficiency which increases autoreactive T cell accumulation,[8] and a mutation in a tyrosine phosphatase PTPN22 alters T cell signaling.[9] However, the strongest factor associated with T1D propensity, in both mice and humans, are diabetogenic class II alleles of the major histocompatibility complex (MHC-II). NOD mice express the I-A^{g7} allele of MHC-II, which has an amino acid change at position 57 from aspartic acid (Asp) to serine (Ser). This variant directly alters the MHC-II peptide-binding groove, causing weak and unstable binding with self-peptides, including β-cell self-antigen and insulin.[10–14]

In humans, certain HLA (human leukocyte antigen) class II gene variants that increase the risk of developing TD1 have also been characterized. HLA class II molecules HLA-DR, HLA-DQ, and HLA-DP present peptide antigens to CD4+ T cells as do MHC-II molecules in mice. Some HLA class II alleles are inherited together as a specific haplotype, meaning a group of alleles that are in close proximity on a chromosome and are passed on from one parent as a block. Strong evidence shows that certain haplotypes, such as DR3-DQ2 and DR4-DQ8, are highly associated with T1D susceptibility.[15] For example, the diabetes-associated DR3-DQ2 (DR3) haplotype typically includes DRB1*03:01, DQA1*05:01, and DQB1*02:01, while the DR4-DQ8 (DR4) haplotype includes DRB1*04:01, DQA1*03:01, and DQB1*03:02.[16] These haplotypes are strongly associated with T1D with an odds ratio of ~16 when both haplotypes are inherited by the same individual.

Like the altered peptide binding observed in NOD mice expressing I-A^{g7}, diabetes-associated alterations in HLA class II have also been characterized in humans. For example, the most well-documented T1D associations in human HLA involve the loss of Asp at position 57 in DQB1*03:02 and DQB1*02:01.[17,18] These mutations alter the HLA class II peptide binding groove similarly to I-A^{g7} in NOD mice, allowing almost identical peptide binding specificities between diabetogenic human and mouse alleles (Figure 1).[19] By studying the NOD mouse model, particularly how

the I-A^{g7} molecule drives T1D and how its effects can be prevented, we can gain valuable insights into therapeutic strategies for human T1D.

DCs Involved in NOD Disease Progression

For both NOD mice and humans, T1D occurs because of the immune system's attack on insulin-producing pancreatic β-cells. This immune response is initiated through the interaction between several types of immune cells. Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that capture β-cell self-antigens and migrate to the pancreatic lymph nodes where T cell responses are initiated.[20,21] Among the various subsets of DCs, conventional type 1 DCs (cDC1) have been shown to play an important role in the development of diabetes in NOD mice. This has been attributed to their unique ability to efficiently cross-present acquired antigens. During cross presentation, cDC1s acquire cell-associated antigens from peripheral tissues and present the resulting peptides on MHC class I and II to activate CD8$^+$ and CD4$^+$ T cells, respectively. In contrast to cDC1s, other antigen-presenting cells have limited cross-presentation capacity.[22] The absence of cDC1s, caused by the genetic deletion of the transcription factor required for their development BATF3, leads to a failure in presenting β-cell antigens on MHC class I and II in pancreatic lymph nodes. This prevents the activation of self-reactive T cells. As a result, the lack of cDC1s prevents the development of T1D and blocks the accumulation of autoreactive T cells within the islets.[23]

cDC1s in Mouse and Man

Several transcription factors and associated regulatory proteins have been identified in regulating cDC1 development and cross-presentation. As mentioned previously, BATF3 is an essential transcription factor for cDC1 differentiation and its loss completely eliminates the cDC1 subset.[22] In subsequent studies, BATF3 has been shown to stabilize *Irf8* expression in cDC1 progenitors through a BATF3-dependent enhancer (+32 kb *Irf8* enhancer). In agreement with a requirement for *Irf8* in cDC1 development, *Irf8* +32 kb KO mice fail to develop cDC1s.[24,25] Unbiased transcriptomics revealed a high degree of similarity in APCs, including cDC1s, between mouse and man.[26] Additionally, one study demonstrated that introducing the transcription factors *Irf8*, BATF3, and PU.1 into human cell lines was sufficient to induce a cDC1-like phenotype.[27] Thus, mouse and human cDC1s share a common developmental pathway and gene expression patterns, pointing to a high degree of similarity.

cDC1s can also be identified through their expression of several proteins that are critical to their role as APCs. XCR1 is a reliable surface marker for studying cDC1 in both mouse and human. It is a chemokine receptor uniquely and selectively expressed on this subset, and its ligand, XCL1, is secreted by activated CD8$^+$ T cells and NK cells. The XCR1–XCL1 interaction directs cDC1 migration to sites of inflammation and promotes efficient antigen uptake.[28] *Wdfy4* is an essential protein for cross-presentation, and it likely acts as a scaffold protein without affecting other DC functions. Interestingly, deletion of *Wdfy4* disrupts cDC1-mediated cross-presentation of antigens specifically to CD8$^+$ T cells, while cDC1 cell numbers and MHC class II presentation are unperturbed.[29]

After engulfing dead cell debris, DNGR-1 (CLEC9A) expressed in cDC1s recognizes the material and signals through the adaptor kinase SYK to promote the release of antigens into the cytosol for cross-presentation. [30] Because cross-presentation by cDC1s directly activates autoreactive CD8$^+$ T cells, which are key effectors in NOD diabetes, alterations in the cross-presentation pathway could potentially alter autoimmune diabetes progression. Similarly, human DNGR-1$^+$ BDCA3$^+$ (cDC1-like) DCs share this conserved pathway, as DNGR-1 recognizes dead-cell debris and mediates antigen uptake for cross-presentation.[31]

In summary, within the NOD mouse model, BATF3 is essential for cDC1 development by stabilizing high *Irf8* expression, and its loss results in depletion of the cDC1 subset. *Wdfy4* is integral for cross-presentation. The absence of these proteins specifically impairs cross-presentation to CD8$^+$ T cells while MHC class II restricted antigen presentation to CD4$^+$ T cells remains unaffected. This suggests that cross presentation to MHC-II may occur through a distinct pathway.

In humans, similar transcriptional and functional programs have been observed to those elucidated in cDC1s from mice. The APCs between mice and humans share clear transcriptional similarities, while introduction of *Irf8*, BATF3, and

PU.1 into human cell lines can induce a cDC1-like phenotype. XCR1 and DNGR-1 serve as conserved surface markers defining human cDC1s. These findings highlight a shared molecular framework between murine and human cDC1s, underscoring the potential for discoveries made in mice to be directly applicable to patients with T1D. Since autoreactive $CD8^+$ T cells play a central role in β-cell destruction during T1D and cDC1s are responsible for their activation, cDC1s present a promising therapeutic target. By inhibiting cross-presentation and thereby preventing the activation of autoreactive $CD8^+$ T cells, it may be possible to preserve pancreatic β-cell function and slow or even prevent disease progression in patients with T1D.

cDC1s and Cross-Presentation in NOD Diabetes

There is ample evidence showing that cDC1s and cross-presentation play an important role in the pathogenesis of T1D in NOD mice. Several studies have highlighted the essential role of cDC1s in the initiation of autoimmune diabetes. One such study demonstrated that cDC1s residing in the islets of Langerhans are essential for T1D development. This study reported a marked increase in cDC1s as $CD4^+$ T cells began infiltrating the islets. Genetic absence of cDC1s prevented priming of diabetogenic T cells, and protected mice from diabetes.[23] Similarly, another study showed that deleting *Xcl1* reduced cDC1 recruitment to pancreatic islets. The reduced numbers of cDC1 ultimately resulted in a decrease in autoreactive $CD8^+$ T cells.[32]

A follow-up study demonstrated that the development of T1D requires the cross-presentation function of cDC1s. Loss of *Wdfy4* in NOD mice prevents diabetes development.[33] *Wdfy4*-deficient mice only lack cross-presentation to $CD8^+$ T cells, while other antigen-presenting functions of cDC1 remain intact. As a result, significantly fewer immune cells infiltrated the pancreatic islets compared to controls, which prevented the onset of insulitis and subsequent diabetes in these mice. A key discovery was that β-cell specific $CD4^+$ T cell priming remained intact. This discovery, coupled with the cDC1-deficient mouse data showing that β-cell specific $CD4^+$ T cell priming was abrogated, highlights that cDC1s are the main presenter of both $CD8^+$ and $CD4^+$ β-cell antigens. In addition, it emphasized that the protection observed in *Wdfy4* KO mice was specifically due to the loss of $CD8^+$ T cell priming rather than general immune suppression.[33]

Overall, previous studies in mouse models clearly show that cDC1 plays an essential role in the development of T1D. Knocking out cDC1 or blocking its recruitment to pancreatic islets can protect mice from developing the disease. More importantly, it is the cross-presentation function of cDC1 that activates $CD8^+$ T cells and promotes their infiltration into pancreatic islets. Genetic ablation of *Wdfy4* results in loss of cross-presentation, and these mice do not develop diabetes.

In humans, several studies have demonstrated $CD8^+$ T cells infiltrating the pancreas in individuals with T1D.[2,34,35] One study examining patients with recent-onset T1D found a high frequency of $CD8^+$ T cells reactive against the precursor peptide to insulin, preproinsulin epitope $PPI_{15\text{-}24}$,[36] suggesting that autoreactive $CD8^+$ T cells play a key pathogenic role and represent a strong therapeutic target. Additional research showed that patients with T1D had a higher frequency of autoreactive $CD8^+$ T cells in the pancreas compared to healthy controls and $CD8^+$ T cells are the most numerous immune cell infiltrating T1D pancreatic islets.[37] Together with the findings from NOD mice discussed above, these results suggest that targeting the cross-presentation pathway in human cDC1s could represent a promising therapeutic approach to prevent or delay the onset of Type 1 diabetes.

cDC1s Role in Maintenance of Tolerance

cDC1s are required for priming $CD8^+$ T cells against cell-associated antigens including islet β-cells. However, their function is not limited to immune activation. In addition to initiating immune responses, cDC1s also play an essential tolerogenic role in maintaining immune homeostasis.

A well-characterized example of this tolerogenic function is observed in the gut. Specialized subsets of intestinal DCs serve as key mediators in the induction of Tregs. After acquiring antigens, cDC1s migrate to the mesenteric lymph nodes, where they secrete retinoic acid and TGF-β. These factors promote the differentiation of naïve $CD4^+$ T cells

into FoxP3$^+$ Tregs.[38] These resulting Tregs help establish immune tolerance to commensal microbiota and prevent excessive immune responses against dietary antigens.

Recent studies have further explored how cDC1s contribute to the induction of immune tolerance in tissues beyond the gut. One study demonstrated that cDC1s promote the tolerization of highly autoreactive CD8$^+$ T cells. This tolerogenic effect required the expression of MHC class II molecules on cDC1s, which mediate the necessary induction of Tregs. Without MHCII on cDC1s, Tregs were not generated, and transfer of autoreactive CD8$^+$ T cells resulted in pathogenesis.[39] Thus, cDC1s can induce immune suppression of CD8$^+$ T cells through induction of CD4$^+$ Tregs.

The tolerogenic potential of DCs to induce immune tolerance toward pancreatic β-cell antigens has been investigated in NOD mice. In one study, β-cell antigens were targeted to cDC1s using a specific cDC1-expressed surface receptor DEC-205. Upon injection, this antigen-antibody fusion protein was selectively internalized by cDC1s via DEC-205–mediated endocytosis. While antigen-specific T cells primed by these cDC1s initially underwent a round of proliferation, they subsequently entered a state of functional inactivation (anergy) or were deleted. This outcome was the result of cDC1 antigen acquisition under non-inflammatory conditions. In the absence of inflammatory cues, steady-state DCs express low levels of co-stimulatory molecules and fail to produce pro-inflammatory cytokines, which are signals that are necessary to prime functional T cell responses. As a result, antigen presentation delivered a tolerogenic rather than immunogenic signal. This strategy significantly delayed the onset of T1D in NOD mice, highlighting the therapeutic potential of targeting DCs to induce antigen-specific tolerance.[40]

Hence, cDC1s play a central role in maintaining immune balance. On one hand, they are required for cross-presentation and can activate T cells to initiate cytotoxic responses. On the other hand, they can induce immune tolerance under immunosuppressive conditions. In the context of T1D immunotherapies, this balance makes cDC1s an essential target. In essence, there are two possible therapeutic strategies: (1) aim to limit their activation of self-reactive CD8$^+$ T cells, and/or (2) strategically utilize cDC1s to promote immune tolerance.

Conclusion and Future Directions

The development of human T1D shares notable similarities with the NOD mouse model. In both cases, mutations in MHC class II molecules impair negative selection, allowing self-reactive T cells to escape deletion. This makes the NOD mouse a valuable model for exploring both the mechanisms and potential interventions of T1D that have translational relevance for human patients. Among immune cell populations, cDC1s are recognized as important regulators of T1D progression in NOD mice. They not only activate CD8$^+$ T cells, which are major contributors to β-cell destruction, but also play a critical role in maintaining immune tolerance. Understanding how cDC1s regulate CD8$^+$ T-cell responses in mice may reveal new strategies to prevent or treat human T1D.

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Disclosure

Authors have no financial conflicts of interest related to this work. Artificial intelligence tools were used in limited and accountable ways in preparing this manuscript. Consensus was used to assist with preliminary literature searching; all retrieved studies were independently read, verified, and interpreted by the authors. ChatGPT (OpenAI) was used only for language refinement to improve clarity and readability. No AI tools were used to generate, analyze, or interpret scientific content. The authors take full responsibility for the accuracy, integrity, and originality of all text, analyses, and conclusions in this manuscript. **MM**

Inflammation as an Etiologic Agent of Gastric Cancer

by Javier A. Carrero, PhD, Melody Zhang, BS & Richard J. DiPaolo, PhD

Unlocking how the immune system shapes the development of gastritis and stomach cancer has the potential to transform both diagnostics and therapy. This rapidly expanding field is at the intersection of immunology and gastroenterology and offers exciting new opportunities to understand disease mechanisms and drive innovative clinical breakthroughs.



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Abstract

Chronic inflammation in the stomach significantly increases the risk of gastric cancer—a deadly disease with a poor prognosis, largely because it is often diagnosed too late. A better understanding of the pathophysiology and molecular mechanisms underlying this increased risk is essential to identify novel diagnostic and therapeutic targets for inflammation-induced carcinogenesis.

Gastric epithelial atrophy, a precursor to cancer, can be induced by chemical, autoimmune, and infectious stimuli, primarily infection with *Helicobacter pylori*. We and others have shown that atrophy can also be induced by the secreted immune products (cytokines) of a type I/Th1 or Th17 immune response. The atrophic phase of gastritis is superseded by a metaplastic, or cellular redifferentiation phase driven by type II (Th2) cytokines. The outcome of this inflammatory sequela is dysplasia of the gastric epithelium; the substrate for multiple types of gastric cancers. Here we summarize our recent findings on Th1/Th17 and Th2 immune responses and their roles in driving gastric atrophy, metaplasia, and carcinogenesis. Importantly, these cytokines and their receptors represent promising therapeutic targets for the prevention and treatment of gastric cancer.

Key Words

Gastric cancer, *helicobacter pylori*, autoimmunity, cytokines.

Helicobacter pylori Infection and Autoimmune Gastritis Create a Biological Environment that Promotes the Development of Gastric Cancer

Stomach (gastric) cancer was the fifth most common and deadly cancer worldwide in 2022 with approximately 969,000 new cases and 660,000 deaths.[1] Globally, stomach cancer incidence rates decreased ~30% between 1990-2019 due to increased food quality and decreased *Helicobacter pylori* (*H. pylori*) infection. While *H. pylori* infection is the leading cause of gastric cancer worldwide; diet, tobacco and alcohol use, and chronic atrophic gastritis are all associated with increased risk. In the United States US), gastric cancer rates declined between 1975-2020 going from an incidence rate of ~11.54 per 100,000 persons to ~5.98 per 100,000 persons with a concomitant increase in five-year relative survival of ~15% to ~41%.[2] Despite these promising long-term trends, there has been a puzzling

and troubling increase in stomach cancer incidence since 2020.

In the US, stomach cancer rates increased from ~5.98 per 100,000 in 2020 to ~7.26 in 2022. This trend was associated with three clinical correlates: increase in upper stomach (cardia) cancers, increase in cancer incidence in younger persons (under 50), and increasing rates of cancer in women versus men. The sudden increase of stomach cancer incidence in younger people despite decades of improvements in prevention and treatment have led us to focus our research on understanding the pathophysiology of gastric cancer development.

In a model proposed by Correa, et al. in 1975, gastric cancer is a progressive disease with a sequence of discrete pathologically identifiable traits.[3] This model is called the Correa Cascade and serves as a framework for studying the steps involved in gastric carcinogenesis. The first steps in the process are inflammation and epithelial cell death (atrophy). This can be caused by numerous insults including infections, irritants/mutagens, and autoimmune disease. If unresolved, this can lead to chronic atrophic gastritis, a loss of gastric glandular epithelium that triggers metaplasia. Metaplasia, the reversible change of one mature cell type to a different cell type, begins in the stomach as complete intestinal metaplasia and transitions to incomplete intestinal metaplasia (IIM) followed by dysplasia and then cancer.[3-5] Complete intestinal metaplasia (CIM) is identified by the presence of small intestinal cells in the stomach, including enterocytes, goblet cells ,and sometimes Paneth cells. CIM is associated with lower cancer risk. IIM is identified by the presence of colonic cells including goblet cells and columnar cells with the absence of a brush border. IIM is associated with higher cancer risk. We and others have hypothesized that the cascade of inflammation > atrophy > metaplasia > dysplasia is partly regulated by cytokines, secreted endocrine and exocrine products of the immune system.[6]

This review will summarize the work conducted in our laboratory regarding the role of inflammation in the atrophic and metaplastic phases of gastritis. Much of our experimental work was performed using mouse models of infectious (*H. pylori*) and autoimmune gastritis (AIG). Conclusions derived in the mouse were extended to human patients using existing and novel patient-derived datasets. Herein we contextualize our discoveries within the field of gastritis and gastric carcinogenesis and propose important new directions of investigation that may lead to better diagnostics and treatments.

Inflammatory Cytokines Are Inducers of Gastric Epithelial Atrophy

Our laboratory uses two models to study chronic inflammation and the immune system in the stomach: the TxA23 mouse model and *H. pylori* infection.[7,8] The TxA23 mouse is an established model for studying the role of inflammation in gastric cancer development. This mouse has been genetically engineered to express a T cell receptor specific for a peptide derived from the hydrogen potassium ATPase (H+/K+ ATPase; ATP4A).[9] The H+/K+ ATPase is highly expressed by parietal cells; specialized epithelial cells of the gastric mucosa that use this protein to generate the acid they secrete into the lumen of the stomach.[10] The TxA23 T cells recognize their cognate H+/K+ ATPase peptide when presented on major histocompatibility class II molecules initiating a progressive autoimmune response in the mouse. This models AIG, a disease that affects an estimated 3.85% of the human population.[11] *H. pylori* infection in the mouse can be performed using mouse-adapted strains such as pre-mouse Sydney strain 1 (PMSS1).[12] This strain colonizes mouse stomach antrum and corpus leading to chronic inflammation, epithelial cell atrophy and metaplastic disease. The mouse disease is comparable to that affecting ~35% of the human population.[13]

The gastric mucosa found in two-month-old TxA23 mice contains a florid mixture of immune cells including: ~45% T cells, ~40% B cells, and ~15% myeloid cells (macrophages and neutrophils).[14] Gastric mucosal infiltrating T cells express many inflammatory cytokines, as determined by intracellular cytokine staining (ICS). This includes ~10% expression for interferon-γ (IFN-γ), ~3% for interleukin-2 (IL-2) and ~0.4% for interleukin-17 (IL-17) on a per-cell basis. Gastric mucosal infiltrating T cells can be isolated and cultured *in vitro* with immune stimulating factors such as phorbol 12-myristate 13-acetate (PMA) and ionomycin to reveal the cytokines they can secrete *in vivo*. This shows that the Type I and Th17 cytokines IFN-γ

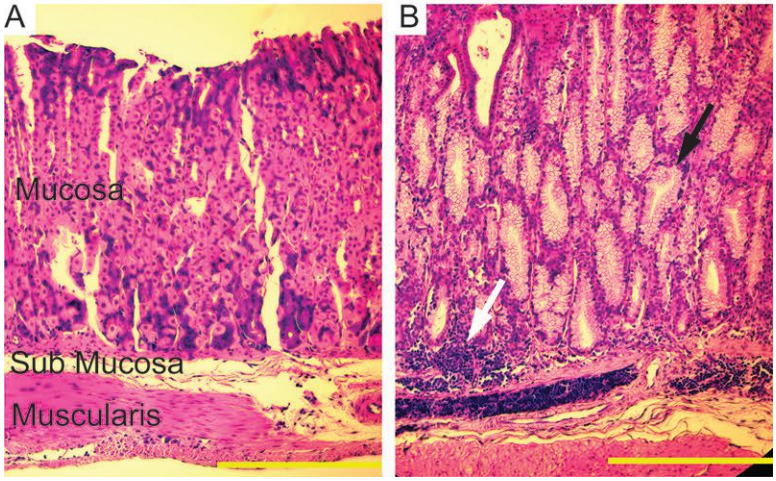


Figure 1. Example of metaplasia in gastritic stomachs.

Photomicrographs of hematoxylin and eosin stained normal (A) and gastritic (B) mouse stomach are shown. Note the lymphocytic infiltrates (white arrow) and the numerous mucous-filled metaplastic glands (one indicated by a black arrow). Yellow Scale Bar = 250 micrometers.

and IL-17 are abundantly secreted after *in vitro* restimulation of TxA23 T cells found in two-month-old mouse stomachs. These Type I/Th17 cytokines are associated with an increase in overall inflammation, oxyntic (parietal cell) atrophy, and mucinous hyperplasia/metaplasia between two to four months of age as assessed by stomach histopathology (Figure 1). Importantly, ~20% of TxA23 mice develop mild focal dysplasia, a direct precursor to cancer. This allowed us to conclude that autoimmunity induced preneoplastic lesions with many of the pathologic features of the Correa Cascade.

A technique that has revolutionized experimental biology of the intestinal tract is the ability to culture gut epithelial cells to generate mini organs in a dish called organoids.[15] Whole epithelial glands are isolated from the stomach of humans or mice using enzymatic digestion then cultured in a complex medium that includes growth factors that promote formation of gastric organoids. These cells can then be subjected to different types of stimuli to determine their response. In this way, we can study the effects of immune products on gastric epithelial cells both in the mouse and in tissue culture conditions.

Gastric epithelial cells and cultured gastric organoids express the receptor for IFN-γ (IFNGR).[16] Cultured mouse gastric organoids treated with purified recombinant IFN-γ exhibit dose dependent cell death. There is significantly increased IFN-γ-induced cell death when compared to untreated controls at 48 hours of treatment with ~90% death in treated organoids by 72 hours. Similar results can be obtained after treating gastric organoids with activated TxA23 T cell conditioned media that contains comparable concentrations of IFN-γ. Gastric organoid cell death is dependent on IFNGR as mice containing a genetic deletion of the receptor (*Ifngr1*$^{-/-}$) are not susceptible to killing by recombinant IFN-γ or diluted TxA23 T cell conditioned media. Additionally, TxA23 x *Ifng*$^{-/-}$ conditioned supernatant is unable to kill organoids demonstrating the requirement for IFN-γ on inducing cell death. *In vivo*, TxA23 x *Ifng*$^{-/-}$ mice have significantly decreased inflammation and little to no atrophy or hyperplasia. These data show that IFN-γ production by gastric autoimmune T cells is required for the development of pre-cancerous atrophic gastritis and a form of metaplasia termed Spasmolytic Polypeptide-Expressing Metaplasia (SPEM) in TxA23 mice.[5]

Interleukin-27 (IL-27) is a cytokine in the interleukin-12 (IL-12) family that is produced by macrophages and dendritic cells in the corpus region of mice stomachs with chronic gastritis.[17] Genetic deficiency of IL-27 (*Ebi3*$^{-/-}$ mice) leads to increased severity of TxA23-induced gastritis, atrophy, and SPEM when compared to controls.[18] At two months of age, TxA23 x *Ebi3*$^{-/-}$ mice have significantly higher inflammation, parietal cell atrophy, mucinous hyperplasia/metaplasia and mucosal hyperplasia than TxA23 mice.[18] By 12 months of age, TxA23 x *Ebi3*$^{-/-}$ mice have a significant increase in corpus epithelial dysplasia than TxA23 controls. The pathology of *Ebi3*$^{-/-}$ mice includes glandular structures that infiltrate the submucosa and muscularis, epithelial nuclear crowding with a loss of mucus-filled appearance, and development of polyp-like appearance.

Since Ebi3 is shared between IL-27 and IL-35, it was important to show specificity for IL-27. Complementation of Ebi3 deficiency with purified recombinant IL-27 protein delivered by a subcutaneous osmotic pump rescued the mice from increased levels of atrophy and metaplasia caused by TxA23 gastritis at two months of age.[18] This demonstrates that the deleterious effects found in *Ebi3*$^{-/-}$ mice are due to the absence of IL-27. It also indicates that increasing IL-27 production during inflammatory gastritis could be a means of ameliorating atrophy and metaplasia. In four-months-old TxA23 mice that have established gastritis, delivery of IL-27 has a therapeutic effect, reversing atrophy and metaplasia within one month of treatment further demonstrating the potential therapeutic benefit of IL-27 administration.[18]

The exacerbation of metaplasia that accompanied Ebi3 deficiency is not observed in chemically-induced gastritis caused by high-dose tamoxifen treatment (HDT). Since the receptor for IL-27 receptor is only expressed on leukocytes, there are few sources or targets for IL-27 in a largely non-inflammatory model of atrophy, like HDT. This was corroborated by performing scRNAseq in TxA23 mice treated with mini osmotic pumps containing either PBS or IL-27 and then examining the transcriptional profile of immune cells flushed from the gastric mucosa. This experiment revealed that IL-27 treatment led to reduced inflammatory gene expression on *Cd4* expressing T cells. The reduced inflammatory gene signature included chemokines (*Ccl3, Ccl4, Ccl5*), cytotoxic products (*Nkg7* and *Prf1*), cytokine receptors (*Il2rb*), and chemokine receptors (*Cxcr6*). This is an example of how there can be different outcomes to gastritis depending on the trigger and inflammatory milieu.

Type II Cytokines Promote Metaplastic Development in Regenerating Epithelium

TH2-type immune responses and type II cytokines tend to be directed toward extracellular pathogens and induction of a strong humoral (antibody) response. In contrast to type I cytokines, type II cytokines lead to increased survival and growth of gastric organoids in culture. This was first discovered by treating gastric organoids with recombinant IL-13 and visualizing increased growth and survival of cells.[19] Removal of IL-13 using genetic ablation of its cognate receptor leads to decreased metaplastic responses in TxA23 mice. The major producers of IL-13 in AIG and *H. pylori* infection are TH2 T cells and mast cells, with mast cells being the highest producers proportionally.

Interleukin-13 (IL-13) is a type II cytokine produced by multiple leukocyte subsets in the gastric mucosa, mainly mast cells.[19,20] Immune cell IL-13 secretory capacity significantly increases with age in TxA23 gastritis doubling between three to four and six to12 months of age. This corresponds to the transition from an atrophic-heavy gastritis to metaplastic gastritis. Mice that are unable to signal IL-13 due to a genetic deficiency in *Il4ra* have a near absence of hyperplasia as measured by neck cell expansion and a significant reduction in metaplasia as measured by SPEM+ glands (Gkn3+). This phenotype can be replicated by therapeutic intervention with anti-IL-13 antibody treatment of TxA23 mice, which neutralizes its activity leading to decreased atrophy, neck cell expansion and SPEM+ glands.

Ex vivo, IL-13 is a growth and survival factor for cultured organoids. Corpus gastric organoids cultured in the presence of IL-13 have significantly increased size based on mean diameter and show significantly higher survival at day nine of culture compared to untreated controls. IL-13 also alters gene expression profiles of organoids leading to decreased *Muc5ac* and *Tff2* and increased *Muc6* expression. These results show that IL-13 acts directly on gastric epithelial cells to increase size, viability, and Muc6 expression, which are hallmarks of SPEM.[5] Therefore, IL-13 serves as a potential diagnostic and therapeutic target for inflammatory AIG-induced metaplasia by acting as a biomarker of disease severity and a biologically druggable promoter of disease.

Inflammation Induced Metaplastic Gene Signatures Are Precursors to Cancer

SPEM metaplasia was initially defined by the presence of trefoil factor 2 (TFF2), mucin 6 (MUC6), and gastric intrinsic factor (GIF) in gastric epithelium.[21] SPEM is a regenerative lesion found in chronically inflamed mice that is believed to precede the formation of intestinal metaplasia and gastric adenocarcinoma. It is also a potential homologue of intestinal metaplasia in humans. As such, it is important to better understand SPEM (and metaplasia generally) to be able to better model human disease

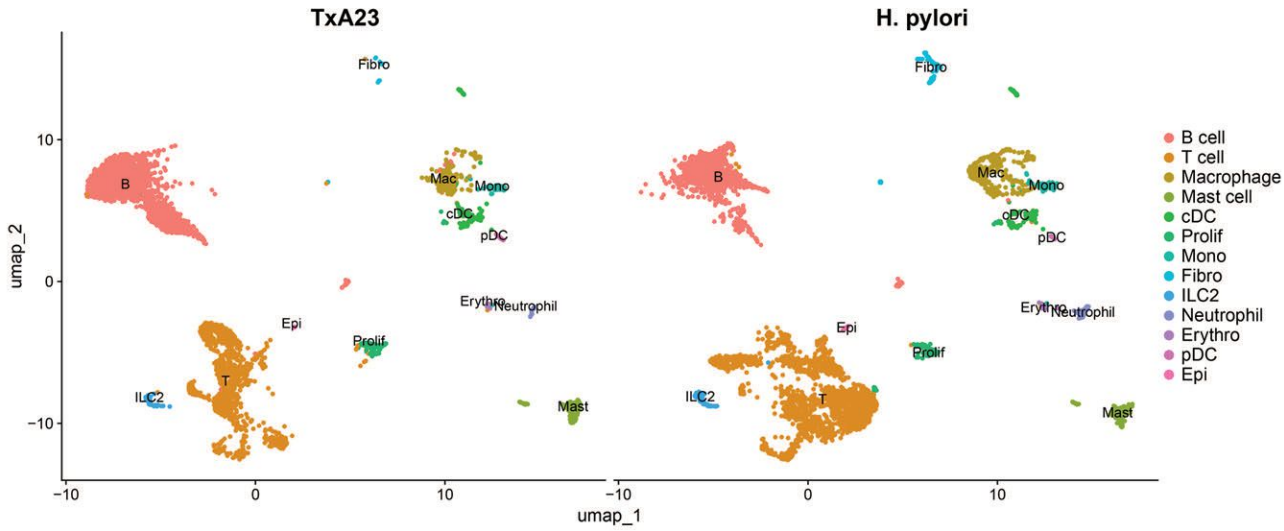


Figure 2. Similar immune infiltrates in the stomachs of TxA23 and *H. pylori infection*. UMAP plots of the immune cells (CD45+) taken from single cell RNAseq datasets generated from either TxA23 (left) or *H. pylori* infected (right) mice. Each colored cluster represents a group of cell types as indicated in the legend.

in mice. To define the transcriptional program of SPEM, scRNAseq was performed on a) healthy control mice, b) high-dose tamoxifen-induced parietal cell ablation, and c) TxA23-induced autoimmune gastric metaplasia.[22]

Several important conclusions can be made from this single cell transcriptional analysis of metaplastic epithelial cells. First, *Muc6+Gif+* epithelial cells found in healthy gastric corpus do not express SPEM transcripts such as *Tff2, Cd44, and Cftr. Muc6, Tff2*, and *Gif* expression are not sufficient to define SPEM regions in gastric epithelium, as there are Tff2+Muc6+ cells that do not express Gif. This led us to propose that the identification of SPEM should be expanded to include any cell that loses mature chief cell transcripts. Under this paradigm, we would expand the definition of head/neck metaplasia to include a broader group of transcripts and their proteins, including *Cd44, Dmbt1, Gkn3* and *Cftr*. These transcripts are absent in healthy epithelium and are expressed only in metaplastic epithelial lineages in both autoimmune and chemically induced gastritis.

These observations were validated by examining expression of *Gkn3* in mice and humans. *Gkn3* transcript and protein expression were absent in healthy mice but was detectable in SPEM regions following both AIG and *H. pylori*-induced gastritis.[22] This was shown by both scRNAseq and immunostaining of stomach tissues from normal and diseased mice. Examination of human stomachs extended this finding by showing that normal healthy human antrum expresses *Gkn3* transcript and protein and human corpus expresses *Gkn3* only during atrophic gastritis.

The identification of *Gkn3* as a SPEM/metaplasia specific transcript in epithelial cells allowed the execution of pseudo time trajectory analysis to determine the developmental origin of metaplastic cells. This approach is a machine learning tool that examines all the cells in a developing population to trace their ontogeny. This revealed that both healthy mucous neck cells and chief cells converge into a "pre-SPEM" progenitor that then becomes two lineages of metaplastic/SPEM cell lineages. Importantly, the transcripts that delimited the final transition to SPEM were not metaplasia-associated genes such as *Gkn3* and *Tff2*, rather inflammation associated genes such as those involved in antigen processing and presentation.

Since gastric metaplasia increases the risk of stomach cancer, it is important to know how metaplasia caused by different triggers affects cancer risk. To this end, metaplasia caused by infection with *H. pylori* and AIG were compared at the pathological and molecular level.[23] Anatomically, *H. pylori* and AIG induced similar gastric pathologies both containing severe inflammation, atrophy, mucous cell expansion, and mucosal hyperplasia. Immunofluorescent staining for markers of metaplasia showed aberrant GKN3 and expanded MUC6 staining in both metaplastic conditions. Similar immune infiltrates

were found in TxA23 and *H. pylori* infection (Figure 2). Pathological severity was higher in AIG than *H. pylori* infection probably reflecting the patchy nature of *H. pylori* infection in the corpus versus disseminated autoreactivity in AIG. These results show that there is a high degree of similarity between infection and autoimmune gastritis, and that both can create pathological conditions conducive to cancer development.

Future Directions

Currently, there is a lack of early predictive tools for gastric cancer, and prognosis is poor when the disease is detected at advanced stages. *H. pylori*, the largest risk factor for development of gastric cancer, infects half of the world's population, making widespread preventative treatment ineffective. While antibiotic treatment of chronic *H. pylori* infection can reduce a patient's cancer risk, efficacy of this treatment in endemic regions is limited by high cost and high reinfection rates, underscoring the need for additional preventative treatment.[24]

Preliminary data suggests that infection induces autoimmunity and a subset of patients with gastric cancer developing autoantibodies, the hallmark diagnostic test for AIG. Ongoing projects are attempting to establish the etiologic relationship between infection, autoimmunity, and cancer to identify a subset of infected individuals at most risk for developing gastric cancer. Integrating low-cost autoantibody screening into clinical practice could enable preemptive intervention and reduce gastric cancer-related deaths.

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Allogenic Memory-Like Natural Killer Cell Therapy

by Alaa M. Khalifa, PhD & Melissa M. Berrien-Elliott, PhD

We now require controlled model systems to assess pertinent questions in the field and dissect underlying mechanisms operative in adoptive cell therapy immunomodulation.



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Abstract

Adoptive cell therapy has witnessed significant progress with the success of chimeric antigen receptor (CAR) T cells for treating cancer. However, their autologous nature limits scalability, and increases production time and manufacturing costs. Additionally, CAR-T cell administration can result in severe toxicities, including cytokine release syndrome (CRS) and neurotoxicity. To address these issues, allogeneic, natural killer (NK) cells are being explored as an alternative. NK cells are cytotoxic lymphocytes that play a pivotal role in tumor surveillance and eradication. Unlike T cells, NK cells can identify and eliminate targets without MHC restriction or prior sensitization. Furthermore, NK cells exhibit enhanced responses after exposure to virus infections or cytokine activation (cytokine induced memory-like). Allogeneic NK cell therapies offer a promising alternative to autologous cell therapies, with reduced risk of graft-versus-host disease and rapid availability. This review summarizes the current landscape of allogeneic memory-like NK cell therapies, including clinical applications and challenges.

Key Words

Transplantation, natural killer cells, cancer immunotherapy, adoptive transfer.

Introduction

Natural killer (NK) cells are cytotoxic lymphocytes, critical to the innate immune system, that can attack and eliminate virally infected cells or cancerous cells without prior sensitization.[1] NK cells are the founding members to a rapidly expanding family of innate lymphoid cells (ILCs) and represent about 5-20% of all circulating lymphocytes in humans.[2,3] NK cells can respond immediately and independently of antigen presentation, making them valuable for off-the-shelf therapies.[4] While chimeric antigen receptor (CAR) T cells have revolutionized the field of adoptive cell therapy for treating cancer, especially the hematological malignancies including multiple myeloma and lymphoma, their use is associated with severe adverse events such as cytokine release syndrome (CRS), neurotoxicity, and graft versus host disease (GVHD).[5] Current CAR-T therapies are autologous (e.g. patient-derived), thus limiting scalability and increasing manufacturing time and cost. A safer and scalable allogeneic (unrelated donor-derived) alternative approach is vital for expanding the patient pool for cell therapies. Recent

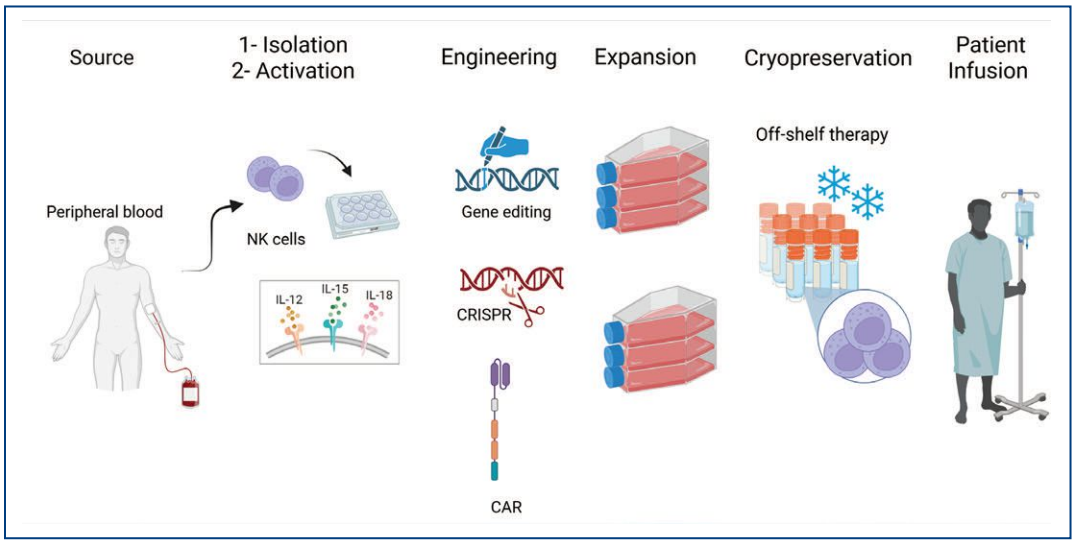


Figure 1. Schema for generating memory-like NK cell therapies. Briefly, peripheral blood NK cells are isolated from healthy donors and are activated, may be engineered, expanded, and cryopreserved for off-the-shelf administration.

advances have been made in our understanding of NK cell biology, including their memory-like (ML) properties. Additionally, improved techniques for NK cell isolation, expansion, and engineering have been developed. These discoveries have led to advances in both preclinical and clinical research into NK cell-based therapies. In this context, CAR NK and CAR ML NK cells represent a promising, safer, allogeneic, off-the-shelf alternative to CAR T cells with lower risk of CRS, broader tumor recognition, and improved activity in immunosuppressive conditions.[4]

NK Cell Biology

Development

NK cells are innate immune cells that develop from common lymphoid progenitors in the bone marrow.[6] NK cell development requires IL-15 and transcription factors like T-bet and eomesodermin.[7,8] Human NK cells are phenotypically identified by CD56 expression and the absence of CD3. They are mainly classified into two major subsets based on the expression levels of CD56: $CD56^{bright}$ and $CD56^{dim}$ NK cells.[3] $CD56^{dim}$ NK cells are mature, express the activating receptor CD16 that is critical in mediating antibody induced cellular cytotoxicity,[9] and comprise most circulating NK cells.[3] $CD56^{dim}$ NK cells mainly mediate cytotoxic responses due to their high content of cytolytic granules containing perforin and granzymes.[10] $CD56^{bright}$ NK cells are less differentiated and give rise to 56^{dim} NK cell subsets; they constitute a minority of circulating NK cells while representing the predominant NK subset in tissues.[3] $CD56^{bright}$ NK cells are highly responsive to cytokine stimulation and produce cytokines robustly in response to activation. Recent studies reveal that $CD56^{bright}$ NK cells can be primed by IL-15 allowing for increased cytotoxicity, challenging the paradigm that immature $CD56^{bright}$ NK cells do not kill.[11]

NK Receptors

Human NK cells express a variety of activating and inhibitory receptors that regulate their activity against target cells.[12] They are a complex group of receptors using opposing signaling motifs to either stimulate or inhibit activation. NK cell activating receptors (e.g. NKp46, Nkp30) detect stress-induced ligands, viral antigens and altered self-cells like infected or cancerous cells. NK cell activating receptors associate with adaptor molecules (e.g. DAP10/12), which contain immunoreceptor tyrosine-based activation motifs (ITAMs).[12] Upon binding to ligand, adaptor associated ITAMs start a cascade of intracellular signaling which leads to the release of cytotoxic granules containing perforin and granzymes, and cytokine production (e.g., IFN-γ). It is reported that stimulation of one activation receptor is not sufficient for cytokine secretion and cytotoxic function, rather stimulation of multiple receptors is required for NK cell effector

function.[13,14] The inhibitory receptors prevent NK cell cytotoxicity against normal, healthy cells.[15] Self-tolerance occurs via their recognition of the major histocompatibility complex (MHC) class I molecule, which is expressed on almost all normal nucleated cells. The key inhibitory receptors on human NK cells include inhibitory killer-cell immunoglobulin-like receptors (iKIR) and NKG2A, which contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs).[16] Upon binding of the receptor to their cognate ligands, ITIMs recruit phosphatases that inhibit activating signaling cascades. Healthy cells express MHC-I and thereby avoid NK cell attack. In contrast, virus-infected or malignantly-transformed cells may down regulate MHC-I expression to evade recognition by cytotoxic T cells, while simultaneously becoming targets for NK cell killing.[6,17,18]

Cytokine Receptors

Understanding the primary role of the cytokine receptors that NK cells express and how they tune NK responses is critical for the development of optimal memory-like NK cell therapies, particularly in off-the-shelf, allogeneic, settings.[19,20] Cytokine receptor signaling not only regulates the survival, activation, proliferation, and effector function of NK cells, but also modulates the epigenetic and metabolic reprogramming involved in memory-like NK cell development.[21–23] Key cytokines that play the main role in regulating NK cell biology include common gamma chain cytokines like interleukin (IL)-2,15,7, 21.[24] IL-12 and IL-18 are also potent NK cell activators that are secreted by dendritic cells and macrophages; they promote NK cell production of cytokines including IFN-γ, and prime NK cells for maximal effector function.[25] IL-2 and IL-15 signal via STAT5 and are indispensable for NK cell development, homeostasis and in vivo persistence.[26] Additionally, type I interferon (IFN) receptor signaling is important for NK cell maturation, activation, expansion and cytotoxicity.[27,28] Cytokine-induced ML NK cells, a distinct subset of NK cells, have emerged in the field of allogeneic cell therapies and have shown promising therapeutic results via clinical trials.[22,29–32] Brief activation of NK cells with IL-12, IL-15 and IL-18 endow them with memory-like properties including enhanced functionality upon restimulation and persistence *in vivo*, all underwritten by distinct epigenetic changes.[4,17,21,23]

Generating ML NK cells. Allogeneic ML NK cell therapy involves isolating NK cells from healthy donors, followed by brief activation with IL-12/15/18. The activated NK cells are washed of cytokines and infused into recipients. While peripheral blood is the most common source of mature, cytotoxic NK cells suitable for rapid therapeutic use, limited cell numbers may necessitate ex vivo expansion for an off the-shelf-product.

Clinical Applications

Studies in both mice and humans have shown that the enhanced functional properties of ML NK cells are maintained following cell division, indicating a heritable and epigenetically programmed phenotype.[23,33] Moreover, ML NK cells have been detected *in vivo* for prolonged periods, highlighting their potential for sustained therapeutic activity.[21,30,31] ML NK cells have also shown improved anti-tumor responses across several malignancies, including lymphoma, multiple myeloma, melanoma, colorectal cancer, and head and neck cancers.[34–37] These preclinical insights led to the clinical translation of donor-derived ML NK cells in a first-in-human trial for leukemia, where the approach was well tolerated and resulted in complete remissions (CR) in 87% (13/15) treated patients.[30] Although ML NK cell therapies have shown promise in clinical settings for hematologic cancers, their effectiveness against solid tumors requires further investigaton. To fully harness the therapeutic potential of ML NK cells, especially against solid tumors, CAR-engineered ML NK cells have become a potent next-generation cell therapy strategy.[38] In this approach, ML NK cells are genetically modified to express CARs which are synthetic receptors designed to target NK cells to tumor-specific antigens.[39]

Table 1. Overview of current active and enrolling clinical trials using cytokine-induced memory-like NK cells.

NCT Number	Study Title	Conditions	Sponsor	Collaborators
NCT05629546	Memory-Like Natural Killer Cells With Nivolumab and Relatlimab in Advanced or Metastatic Melanoma After Progression on Checkpoint Inhibitors	Melanoma	Washington University School of Medicine	Melanoma Research Alliance|Rising Tide Foundation
NCT06138587	Preemptive CIML NK Cell Therapy After Hematopoietic Stem Cell Transplantation	Acute Myeloid Leukemia, Leukemia, Myeloproliferative Neoplasm Myeloproliferative Disorders	Dana-Farber Cancer Institute	
NCT06321484	Intraperitoneal Cytokine-Induced Memory Like (CIML) Natural Killer (NK) Cells in Recurrent Ovarian Cancer	Ovarian Cancer	Dana-Farber Cancer Institute	ImmunityBio, Inc.
NCT06318871	Memory-like Natural Killer (NK) Cell Therapy in Patients With Renal Cell Carcinoma or Urothelial Carcinoma	Renal Cell Carcinoma, Urothelial Carcinoma	Dana-Farber Cancer Institute	Kidney Cancer Association
NCT06152809	CIML NK Cells With Venetoclax for AML	Acute Myeloid Leukemia	Dana-Farber Cancer Institute	
NCT07011004	A Study of Natural Killer Cells in Combination With Atezolizumab in People With Acute Myelogenous Leukemia	Acute Myeloid Leukemia	Memorial Sloan Kettering Cancer Center	Genentech, Inc.
NCT05580601	Cytokine-Induced Memory-Like Natural Killer Cells (CIML-NK) for Relapsed & Refractory Acute Myeloid Leukemia	Acute Myeloid Leukemia	Children's Hospital Medical Center, Cincinnati	
NCT06158828	Pilot Study of Memory-like Natural Killer (ML NK) Cells After TCRαβ T Cell Depleted Haploidentical Transplant in AML	Pediatric Acute Myeloid Leukemia	Washington University School of Medicine	The Leukemia and Lymphoma Society|Rising Tide Foundation|St. Louis Children's Hospital Foundation|Children's' Discovery Institute

Several tumor-associated antigens have been studied as CAR targets, including CD33, CD19, BCMA, and EphA2. Through these studies, CAR engineering has consistently been shown to enhance the responsiveness of ML NK cells against their targets.[34,37,40] Combining the specificity of CAR technology and enhanced functionality of ML NK cells, may be a promising treatment for solid tumors. Currently, significant efforts are directed towards investigating ML NK cell therapies in allogeneic settings through ongoing clinical trials (Table 1).

Challenges and Limitations

Despite the breakthrough and the promising results of allogeneic ML NK cells as off-the-shelf therapy in clinical trials, this strategy requires further optimization. Although ML NK cells showed enhanced longevity and persistence over conventional NK cells, long-term persistence may be limited by host immune rejection of donor cells due to HLA mismatching.[41] Limited *in vivo* persistence will require either repeated administration or further engineering to enhance NK cell lifespan. Moreover, the immunosuppressive tumor microenvironment (TME) may limit the infiltration and functionality of ML NK cells. Combination therapies that dampen inhibitory signaling in the TME should be investigated.

Strategies for Overcoming Barriers

Strategies for improving ML NK cell-based therapies are directed towards enhancing their targeting, decreasing TME suppression, and increasing functional persistence. Genome editing via CRISPR/Cas9 is being researched as a way to reduce alloreactivity by knocking out HLA molecules or inhibitory molecules.[22,42] Engineered ML NK cells to express CARs designed to target

specific tumor antigens and increase ML NK cell homing into solid tumors are also being explored.[39] Additionally, combining ML NK cells with immune checkpoint inhibitors such as anti-PD-1/PD-L1 and anti-NKG2A may help overcome the immunosuppressive TME.[37] Moreover, optimizing cytokine support to enhance in vivo expansion, persistence and cytotoxic potential, may be critical for improving the clinical efficacy of ML NK cell therapies. A potential method may be to engineer ML NK cells to express membrane-bound IL-15 or IL-2 to improve therapeutic durability and reduce associated toxicities.[43,44]

Conclusions and Future Directions

Substantial clinical progress has been made in demonstrating the feasibility and efficacy of cellular immunotherapies for cancer and are starting to be made for viral infections. However, studies have revealed that choices made in the clinic without pre-clinical testing, can negatively impact patient outcomes.[45] Outstanding questions include 1) How do *in vivo* dynamics like NK cell expansion correlate with patient responses? 2) Does CAR ML NK cell persistence matter for response? 3) Can factors like chemokine receptors and integrins help direct and retain CAR ML NK cells in the tumor? and 4) What is the capacity for CAR ML NK cells to modulate the endogenous immune system? One major challenge in using CAR-engineered cells in patients is identifying safe, high-density tumor antigens that have the potential for minor on-target-off tumor effects yet maximum on-target-on-tumor activity. However, even with the best scFv, delivering CAR-engineered lymphocytes to solid tumors is a major barrier in the field, underscored by the challenges that have been experienced in moving CAR T cell therapy beyond hematologic malignancies and into solid tumors.[46] Advances made in correlative immunology associated with clinical trials have revealed a new wave of questions that cannot be experimentally addressed in the clinical setting or using xenograft systems. Indeed, we now require controlled model systems to assess pertinent questions in the field and dissect underlying mechanisms operative in adoptive cell therapy immunomodulation. Basic, mechanistic studies are required to determine factors that impact CAR ML NK cell trafficking, persistence, and effector functions. Murine models will also allow us to simultaneously understand the basic cellular mechanisms that distinguish cytokine-induced ML NK and CAR versus endogenous receptor mediated signals that impact NK cell anti-tumor activity in immune-competent settings.

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Integrase Inhibitors: Redefining HIV-1 Treatment and Future Challenges

by Krishan K. Pandey, PhD

The WHO and CDC guidelines recommend INSTI-based regimens as preferred first-line therapy for most people living with HIV.



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Abstract

The discovery and development of HIV-1 integrase strand transfer inhibitors (INSTIs) represent one of the remarkable success stories in antiviral drug discovery, transforming fundamental insights of retroviral enzymology into highly effective first-line therapies for HIV-1/AIDS patients. Currently there are five INSTIs approved for clinical use. Among these, second generation drugs Dolutegravir, Bictegravir and Cabotegravir offer excellent virologic suppression, favorable tolerability and a high genetic barrier to resistance. The long-acting formulation of the most recent INSTI Cabotegravir has also been approved for pre-exposure prophylaxis in high-risk populations. Apart from inhibiting the active site of integrase, a new class of allosteric integrase inhibitors (ALLINIs), which target integrase multimerization affecting multiple steps in viral life cycle, are in the advanced phases of clinical trials. The mechanisms of HIV-1 integration, development of integrase inhibitors, structural biology breakthroughs that guided inhibitor optimization and clinical use of INSTIs, their successes and challenges for HIV/1AIDS management are reviewed here.

Key Words

HIV-1, AIDS, integrase strand transfer inhibitors, antiviral drugs.

Introduction

Since the introduction of combination antiretroviral therapy (ART) in the mid-1990s, HIV-1 infection, once almost invariably fatal, has been transformed into a chronic, manageable condition rather than a progressive, fatal disease leading to acquired immunodeficiency syndrome (AIDS). The first antiretroviral drugs targeted HIV-1 reverse transcriptase (RT), the enzyme responsible for converting the viral RNA genome into double-stranded DNA, a crucial step in viral replication cycle. Subsequently, the development of inhibitors against another key viral enzyme, the protease, led to the advent of highly active antiretroviral therapy (HAART). This combination of both class of drugs marked a major turning point in HIV treatment by dramatically reducing AIDS-related morbidity and mortality. Despite this success, the limitations such as adverse side effects, high pill burden, and emerging drug resistance underscored the continual need for developing

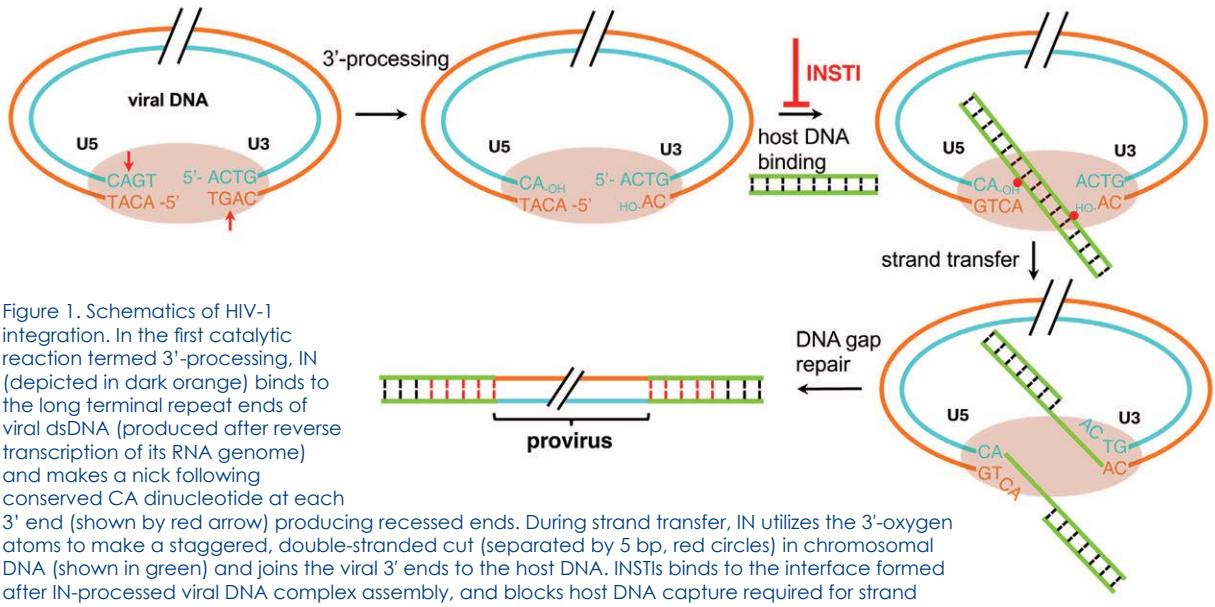


Figure 1. Schematics of HIV-1 integration. In the first catalytic reaction termed 3'-processing, IN (depicted in dark orange) binds to the long terminal repeat ends of viral dsDNA (produced after reverse transcription of its RNA genome) and makes a nick following conserved CA dinucleotide at each 3' end (shown by red arrow) producing recessed ends. During strand transfer, IN utilizes the 3'-oxygen atoms to make a staggered, double-stranded cut (separated by 5 bp, red circles) in chromosomal DNA (shown in green) and joins the viral 3' ends to the host DNA. INSTIs binds to the interface formed after IN-processed viral DNA complex assembly, and blocks host DNA capture required for strand transfer. The noncomplementary 5' dinucleotides of viral DNA are removed and gaps in host DNA repaired by cellular repair machinery resulting in 5 bp of target site duplication at each end (shown in red base pairing). The integrated viral DNA is termed "provirus".

new drug targets. HIV-1 integrase (IN), the enzyme responsible for inserting viral cDNA into the host genome, emerged as an attractive target. Decades of basic research in retroviral enzymology and medicinal chemistry ultimately culminated in the approval of Raltegravir in 2007—the first-in-class IN inhibitor. Today, IN inhibitors are recommended as the preferred first-line ART regimens for most patients worldwide. This review follows the bench-to-bedside journey of HIV-1 IN inhibitors: from early biochemical discoveries using surrogate model systems, some predating the identification of HIV-1 itself, through the structural insights that informed drug design, to clinical translation, resistance challenges, and future directions.

HIV-1 IN: Structure and Function

HIV-1 IN is a 288 amino acid protein encoded at the 3' end of the polymerase gene. It mediates the integration of the linear viral dsDNA, generated by reverse transcription, into the host genome thus making the HIV-1 infection permanent. Occasionally, the integrated provirus becomes transcriptionally silenced leading to the formation of a reservoir of latently infected cells. As these cells are no longer producing new virions, they remain nonsusceptible to treatment with ART.

Before HIV-1 was identified to be the etiological agent for AIDS, a plethora of basic research was being done in other retroviral systems. The first retroviral IN was identified in 1978 by Grandgenett et al.,[1] as a 32 kDa protein (p32), possessing DNA endonuclease activity in avian myeloblastosis virus (AMV) and Rous sarcoma virus (RSV). Later, in the mid 1980s after discovery of similar enzyme in HIV-1, the protein was named integrase. IN mediated integration proceeds through two catalytic steps (Figure 1). In the first step (3' processing), IN removes a dinucleotide adjacent to the conserved CA residues from each 3' end of the viral long terminal repeat (LTR), generating recessed 3' hydroxyl groups.

In the second step (strand transfer), IN mediates the nucleophilic attack of the processed viral DNA ends into host chromosomal DNA staggered by 5 bp which joins the viral DNA CA 3'-ends with host DNA. The resulting single stranded gaps are repaired by host DNA repair enzymes and yields integrated provirus flanked by 5 bp host DNA sequence duplication. The length of the gap between staggered cuts and the resulting host site duplication varies (4 to 6 bp)

among different retroviruses.[2] These reactions utilize Mg^{2+} and are catalyzed within a highly conserved DDE (Asp-Asp-Glu) motif in the IN catalytic core. IN comprises three domains: the N-terminal domain (NTD), possessing a zinc-binding helix bundle critical for multimerization; a catalytic core domain (CCD), containing the DDE active site motif; and a C-terminal domain (CTD), having SH3-like fold contributing to DNA binding. Oligomerization of IN on viral DNA ends forming higher-order complexes is essential for concerted integration.

Development of HIV-1 IN Inhibitors

Unlike RT or protease, HIV-1 IN has no human homolog, making it an attractive target for selective drug design. However, despite its conceptual appeal it took more than two decades for the development of the first FDA approved IN inhibitor.[3] There were two major reasons for this delay. First, RT and protease were considered more promising early targets as their functions provide higher probability of inhibiting viral DNA synthesis and polyprotein processing. In contrast, the IN performs just a single strand transfer event during infection. Second, the basic biochemical mechanisms of IN were not well understood and there was a lack of an in vitro assay to recapitulate the catalytic events carried out by IN. Since IN required both viral and host DNA ends in a particular configuration to function, early assays often failed to reproduce physiologically relevant conditions.

Development of robust *in vitro* assays in 1990s,[4,5] gave a boost to high throughput screening for IN inhibitors and identified diketo acids (DKAs) as inhibitors of strand transfer activity, hence classified as IN strand transfer inhibitor (INSTI).[6] DKAs selectively block the second step of integration without affecting 3' processing. Despite promising leads, initial IN inhibitors faced problems of poor cellular permeability, metabolic instability, and insufficient antiviral activity *in vivo*. L-870,810, a DKA derivative developed by Merck Research Laboratories was a potent inhibitor of strand transfer with an IC_{50} of 8 nM and was effective in antiviral cell-based assays. However this compound failed in experimental animal models due to liver and kidney toxicity in dogs.[7] Serendipitously, a class of drug dihydroxypyrimidine carboxamides, also being developed by Merck against Hepatitis C virus (HCV) polymerase, selectively inhibited strand transfer mediated by HIV-1 IN. Structure-activity studies demonstrated that these compounds shared a metal-chelating pharmacophore similar to DKAs but exhibited superior metabolic stability and bioavailability. Further optimization of the scaffold of this drug class led to the development of raltegravir (MK-0518) which received FDA approval in 2007.[3] Raltegravir's approval established the proof-of-concept that IN inhibition could yield potent, well-tolerated, and selective antiviral activity, even in treatment-experienced patients harboring multidrug-resistant HIV-1. Soon after, Elvitegravir[8] (Gilead Sciences) was approved in 2012. In the following years, three more INSTIs were approved for treatment of HIV-1 infection: Dolutegravir[9] (ViiV Healthcare, 2013), Bictegravir[10] (Gilead Sciences, 2018) and Cabotegravir[11] (ViiV Healthcare, 2021) (Figure 2). All of these drugs have similar mechanisms of action as they chelate the metal ion (Mg^{2+}) in the active site, effectively trapping the IN-viral DNA complex in an inactive stage and blocks the binding of host DNA for strand transfer.[12,13] Importantly, INSTIs do not bind to the IN or DNA directly, rather to the IN-viral DNA complex (intasomes) thus behaving like interfacial inhibitors.

Clinical Translation: Bench to Bedside

The clinical translation of INSTIs represents one of the most significant milestones in ART. Despite the initial challenges in developing the drugs against HIV-1 IN, now INSTIs are the preferred first-line component of ART for most people living with HIV-1. In clinical practice, INSTIs are used in combination with other classes of HIV-1 drugs, most commonly RT inhibitors. It reduces the probability of acquisition of drug-resistant mutations that can occur with monotherapy.

Raltegravir, the first-in-class INSTI, demonstrated rapid viral load reduction and excellent tolerability in both treatment-naïve and -experienced patients, including those with multidrug-resistant HIV-1.[14,15] Soon after, elvitegravir offered comparable potency but required boosting with ritonavir or cobicistat

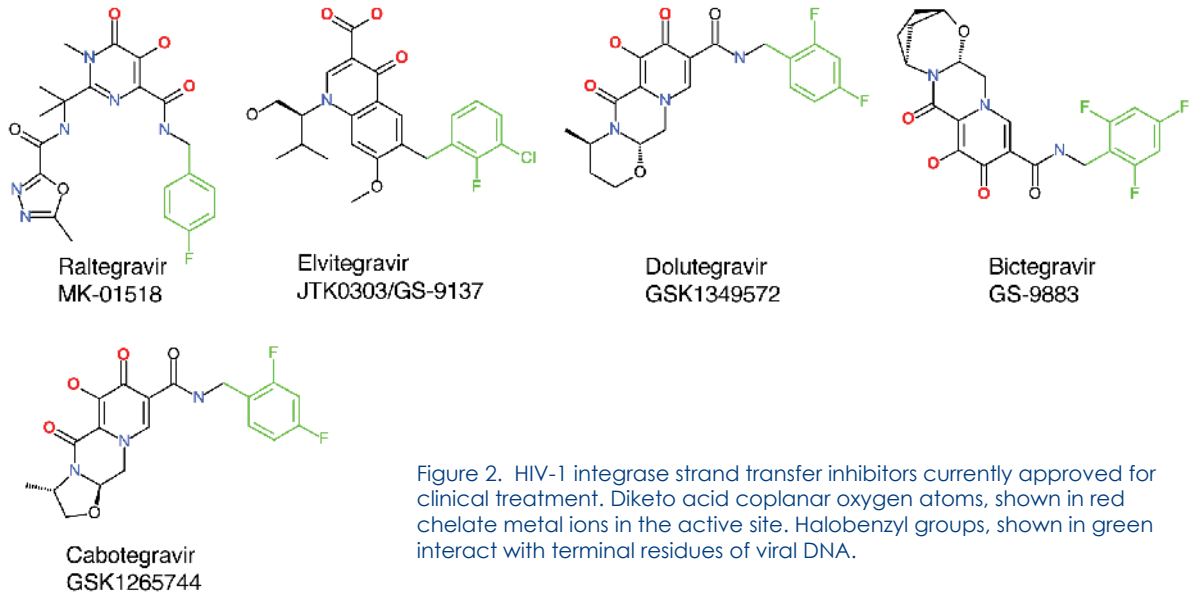


Figure 2. HIV-1 integrase strand transfer inhibitors currently approved for clinical treatment. Diketo acid coplanar oxygen atoms, shown in red chelate metal ions in the active site. Halobenzyl groups, shown in green interact with terminal residues of viral DNA.

to maintain the therapeutic concentration.[16-18] INSTIs possess a favorable pharmacologic profile compared to earlier drug classes. Most are metabolized predominantly via UGT1A1-mediated glucuronidation and to a limited extent via CYP3A4.[19-22] This confers several advantages like reduced mitochondrial toxicity, less severe hepatic effects and fewer drug-drug interactions. In contrast, elvitegravir boosted with cobicistat is primarily metabolized via the CYP3A4 pathway.[23]

Due to the high mutation rate in HIV-1 replication, development of resistance to antiviral drugs seems to be an inevitable phenomenon. This is true for INSTIs as well. Mutations such as N155H, Q148H/K/R, G140S/A and Y143C/H rapidly conferred cross-resistance to both raltegravir and elvitegravir, first generation INSTIs. These mutations reduce drug binding by altering the active-site conformation or metal ion coordination. It highlighted the need for next generation inhibitors with greater binding stability and flexibility. For years, biochemical studies suggested that functional intasomes require multiple IN subunits assembled on the viral DNA ends. The crystal structure of raltegravir bound to the active site of the prototype foamy virus (PFV) IN, a surrogate model system for retroviruses, in a complex with its cognate viral DNA was determined in 2010.[24] It revealed how IN tetramers synapse two viral DNA ends and accommodate INSTIs. This landmark finding provided the blueprint for rational design of next-generation INSTIs targeting the active site with high potency. In the following years, with advances in single particle cryo-electron microscopy (cryo-EM), a number of retroviral intasome structures including HIV-1 were solved.[25-29] Detailed knowledge of IN-DNA and INSTI interactions from these structures provided more in-depth knowledge of intasome architectures and mechanisms of development of drug resistance. It facilitated the rational design and development of tight binding INSTIs with greater tolerance of resistance mutations, longer dissociation half-lives with wild-type IN as well as resistant mutants observed with first generation INSTIs and improved pharmacokinetics.

The second generation INSTIs dolutegravir, bictegravir and cabotegravir retain activity against most single mutations, though infrequent complex resistance pathways (e.g., Q148H+G140S) can reduce susceptibility. These drugs demonstrated high efficacy, once-daily dosing, and a robust resistance barrier, becoming a cornerstone of first-line ART addressing adherence challenges and offering new models of HIV care. Cabotegravir is structurally similar to dolutegravir (Figure 2) but with an improved pharmacokinetic profile, a superior genetic barrier

to resistance, and importantly, a much longer half-life of ~40h following oral administration. It has very low water solubility and binds more efficiently to serum proteins. These properties were an impetus to co-formulate cabotegravir as long-acting (LA) injectable nanosuspension along with RT inhibitor rilpivirine which had similar physiochemical properties suitable for long lasting effectiveness.[30] Nanoparticle formulation enhances the plasma half-life of cabotegravir to ~3-7 weeks. Combination coformulation, now marketed as Cabenuva has been approved (2021) for monthly or every other month intramuscular injection. This was the first INSTI based LA treatment option and showed non-inferiority to daily oral therapy.[31]

Cabotegravir-LA alone, marketed as Apretude was approved (2021) as pre-exposure prophylaxis (PrEP) in people who are at high risk for exposure to HIV-1.[32] It is administered as an intramuscular injection once every month for the first two months, then once every two-month making it an ideal choice for a population which may have barriers to adhering to a daily medication regimen. Before starting the LA therapy, an oral lead-in period is recommended with individual tablets to assess tolerance. Recently, Lenacapavir, an HIV-1 capsid inhibitor was approved for PrEP (brand name Yeztugo) as twice-yearly injections after initial starting oral dosing schedule.[33]

While LA regimens offer new solutions for populations with adherence challenges including less adverse events, improved care consistency and avoidance of HIV related stigma, it does come with logistical challenges. LA therapy requires robust delivery systems, professional healthcare workers for administration, injection site monitoring and strict dosing schedules to avoid resistance development during the "pharmacological tail" period when subtherapeutic drug concentration persists. Improvements in delivery systems like microneedle array patches, subdermal implants, intravaginal rings, and oral regimens will make the adoption of LA regimens for treatment and PrEP easier and broaden access globally.

Today, WHO and CDC guidelines recommend INSTI-based regimens as preferred first-line therapy for most people living with HIV. Large-scale clinical trials have demonstrated superior viral suppression, fewer adverse events, and better tolerability compared to other regimens in treatment-naïve as well as treatment-experienced individuals. The most common adverse effects associated with second generation INSTIs which cause treatment discontinuation are insomnia, anxiety, or weight gain in select groups of individuals. Nevertheless, their overall safety and durability remain superior to alternative regimens, and ongoing pharmacogenomic studies aim to further personalize INSTI therapy.

Novel Drug Targets Against IN

It has been known for a long time that several mutations in HIV-1 IN affect viral replication at multiple steps like reverse transcription and viral maturation.[34] Better understandings of non-catalytic IN functions in viral replication in recent years prompted the efforts to develop drugs against these alternate IN functions rather than strand transfer, offering complementary inhibition mechanisms. Allosteric IN inhibitors (ALLINIs) engage the IN CCD dimerization interface at positions distal from the enzyme active site. ALLINIs induce hyper-multimerization of IN which in turn affect its catalytic activity, interaction with cellular protein involved in targeting integration into transcriptionally active genes, virus maturation and infectivity.[35-37] One such compound, Pirmitegravir (STP0404),[38] developed by ST Pharm is undergoing phase 2a clinical trials (NCT05869643) to evaluate its antiviral activity, safety, tolerability, and pharmacokinetics. Initial results are promising with statistically significant reduction in plasma HIV-1 RNA level, favorable pharmacokinetic profile, strong safety and tolerability. After 10 days of treatment, patients receiving 200 mg and 400 mg doses of pirmitegravir once daily saw significant viral load reductions of 1.19 to 1.55 log10 copies/ml (15.5-to-35.6-fold reduction in plasma HIV-1 RNA), respectively, compared to non-significant effect in the placebo group. Pharmacokinetics showed rapid drug absorption within one hour and a half-life of

12-14 h supporting once-daily dosing. These findings confirm that a novel mechanism of allosteric inhibition of IN is clinically viable, validating a new antiviral drug class with potential to complement current ART regimens.

Future Challenges

Despite the tremendous success of IN based therapy for HIV-1, numerous challenges remain. Second generation INSTIs are relatively new and HIV-1 treatment needs to be life-long. Continual monitoring of patients on these drugs is essential to study long-term adverse effects and arising resistance mutations. How we ensure the equitable global access to advanced therapy regimens is one of foremost challenge for worldwide health policy. Despite guideline recommendations, access to second-generation INSTIs remains limited in many low and middle-income countries due to cost and supply constraints. Emerging data show association of second generation INSTIs (especially dolutegravir and bictegravir) with weight gain, insulin resistance, and dyslipidemia.[39] Mild but notable neuropsychiatric (sleep disturbance, anxiety) symptoms are reported, requiring monitoring in long-term therapy.

The IN-resistance mutations in treatment naïve or INSTI naïve patient are rare; however, due to a large number of patients on INSTI-based therapy globally, resistance can develop due to poor adherence or treatment failure. Cabotegravir-based LA treatment poses new challenges with adherence. Prolonged sub-therapeutic drug levels after missed or delayed injections may select for drug resistance. Contingency plans should include oral cover during discontinuation and robust delivery systems. Maintaining cold-chain storage and injection delivery infrastructure for extended-release cabotegravir is challenging in many regions.

Co-morbidities associated with HIV-1 complicate the treatment options as the probability of drug-drug interactions rises. Tuberculosis patients on Rifampin treatment necessitate INSTI dose adjustment or an altogether different INSTI-based regimen. Like other HIV-1 treatment options, INSTIs do not cure the infection. INSTIs block new cells from getting infected but are ineffective against the latent reservoir of HIV-1 infected cells. Clonal expansion of HIV-1 infected cells maintain the proviral DNA despite full viral suppression, and remains a major barrier to eradication.

Conclusion

IN inhibitors have redefined HIV-1 therapy by offering potent, durable, and well-tolerated regimens that anchor first-line ART globally. Their success stands as a paradigm of how fundamental enzymology and structural biology, availability of suitable surrogate model systems can drive translational bench-to-bedside breakthroughs. Yet, challenges remain: the threat of resistance, global access disparities, and the imperative to manage long-term safety. As the INSTI era continues to unfold, continued innovation in long-acting formulations, allosteric inhibitors, resistance management, and novel mechanisms promises to further enhance the role of IN inhibitors in HIV treatment and prevention.

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In Silico Studies of the Hepatitis B Virus Polymerase: Efforts to Identify Novel Drug Binding Sites to Inhibit HBV Replication

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Approximately 250-300 million people are chronically infected with HBV and 1.1 million deaths are estimated to have occurred in 2022 from chronic HBV infection, mainly due to liver failure and hepatocellular carcinoma.



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Abstract

Hepatitis B virus (HBV) replicates via reverse transcription, a reaction catalyzed by the HBV polymerase (P) protein. P is a multidomain protein consists of terminal protein, spacer, reverse transcriptase, and ribonuclease H domains. We used a predicted structural model of HBV P and computationally identified potential non-nucleos(t)ide reverse transcriptase inhibitor (NNRTI) sites. Out of the three potential NNRTI sites, we selected Site 3 for virtual high throughput screening of 837,000 virtual compounds. We selected 53 compounds considering their docking scores, ligand uniqueness, stability and rigidity for screening in HBV replication inhibition assay, but all of them failed to suppress the HBV reverse transcription. Molecular dynamic simulations showed that Site 3 binding pocket is not conformationally stable even after binding of the ligand. This study indicates that additional potential NNRTI sites should be explored, and that rigorous molecular dynamic simulations studies should be performed before virtual compound screening.

Key Words

Hepatitis B, HBV polymerase protein, non-nucleos(t)ide reverse transcriptase inhibitor sites.

Introduction

Hepatitis B virus (HBV) is a partially double stranded DNA virus which replicates via reverse transcription.[1] HBV causes both acute and chronic infections. Acute infections resolve in >90% of adults but <10% in children, and chronic infections can lead to cirrhosis, liver failure, and hepatocellular carcinoma. Approximately 250-300 million people are chronically infected with HBV and 1.1 million deaths are estimated to have occurred in 2022 from chronic HBV infection, mainly due to liver failure and hepatocellular carcinoma.[2] There is an effective vaccine available which can prevent HBV infection, and nucleos(t)ide analogs (NAs) and pegylated interferon α therapies are available to treat the infection.[3] Interferons can have serious side effects and rarely clear the infection, while NAs are well tolerated but also rarely clear the infection, in part because NAs fail to block HBV reverse transcription completely.[4]

HBV reverse transcription is catalyzed by the viral polymerase (P) protein within viral nucleocapsid particles. P has four domains, the terminal protein (TP), spacer (SP), reverse transcriptase (RT) and ribonuclease H (RH) domains (Figure 1).[5] We recently predicted and validated the structure of P for the first time.[6] Newly synthesized HBV genomes can either be secreted within infectious virions, or they can be trafficked to the nucleus, where they are repaired to form the viral covalently closed circular DNA (cccDNA), which is the template for all viral mRNAs.[1] The goal of this study was to identify targets for non-nucleoside reverse transcriptase inhibitors (NNRTI) on the HBV P protein that could contribute to more completely blocking formation of the viral cccDNA. In this study, we identified NNRTI sites on the predicted structure of HBV P and conducted virtual screening of small molecule libraries against a predicted NNRTI site.

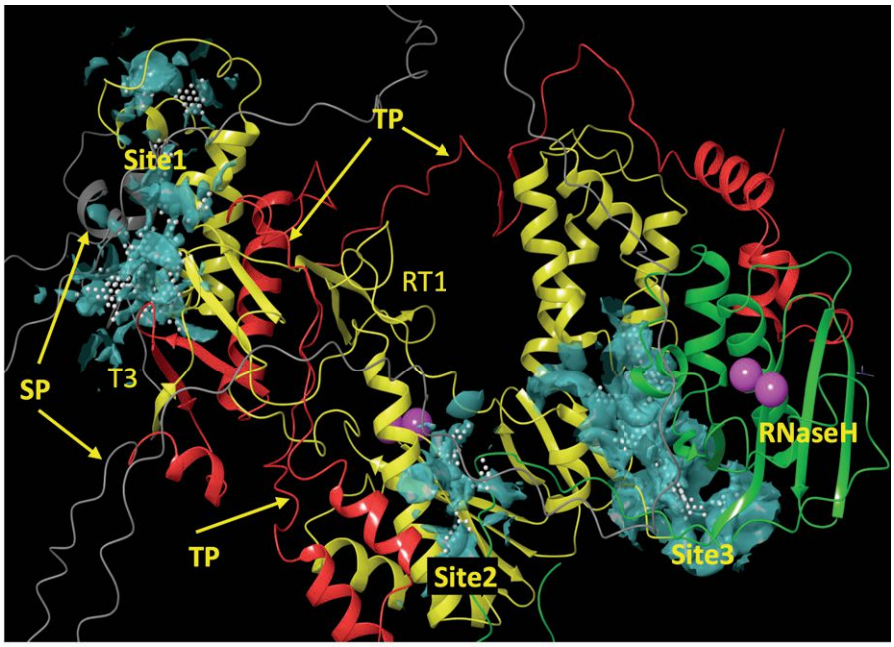


Figure 1. Predicted HBV P structure. HBV P (genotype B) structure showing three potential NNRTI sites (cyan). Terminal protein domain (TP, red); spacer domain (SP, grey); reverse transcriptase domain (RT, yellow); ribonuclease H domain (RH, green); and Mg2+ ions (pink); T3 (a motif essential for RNA binding); RT1; (a motif essential for RNA binding).

Materials and Methods

Preparation of the Protein Structure for Docking Studies

The structure of P protein from HBV genotype B (accession number AB246341) predicted via AlphaFold2[6,7] was prepared using the protein preparation wizard program in the Schrödinger suite (Schrödinger LLC). Different protonation states of P protein generated at pH 7.5 ± 2, H-bond networks and correct protonation states were assigned using the PROPKA within the Schrödinger molecular analysis suite. Energy minimization of the P structure was done using the OPSL4 force field.

Identification of Potential Binding Sites on P and Receptor Grid Generation

The Site Map program within the Schrödinger suite was used to identify 10 potential NNRTI binding sites using the default settings. After visual inspection, three binding sites were selected for detailed consideration, and 10 Å receptor grid was established around the sites that were selected for testing in replication inhibition studies (Figure1).

Virtual High Throughput Screening

The ChemBridge CORE virtual compound library that consists of 837,000 of lead-like and drug-like molecules was downloaded from the ChemBridge website (https://chembridge.com/screening-compounds/lead-like-drug-like-compounds/#core-library-stock). The virtual high throughput (VHTS) screening workflow within the Maestro interface of Schrödinger suite was used for screening of compounds (Figure 2). The compound library was filtered by running QikProp and prefiltered against Linpinski's Rule of Five.[8] Compounds were prepared by energy minimization using OPSL4 force field

and different protonation states were assigned at pH 7.5 ± 2 using the Epik utility. Metal binding states were added when necessary and high energy ionization and tautomer states were removed while retaining the specified chiralities of the compounds. Epik state penalties were used during docking, and scaling of ligand van der Waals radii for nonpolar atoms was done using scaling factor 0.8 and a partial charge cutoff 0.15. Virtual screening was done by flexible docking of the compound with one binding pose being generated by high throughput virtual screening followed by refinement employing the Glide SP and XP docking utilities, with the 10% of compounds with the best docking scores being retained after each docking step.

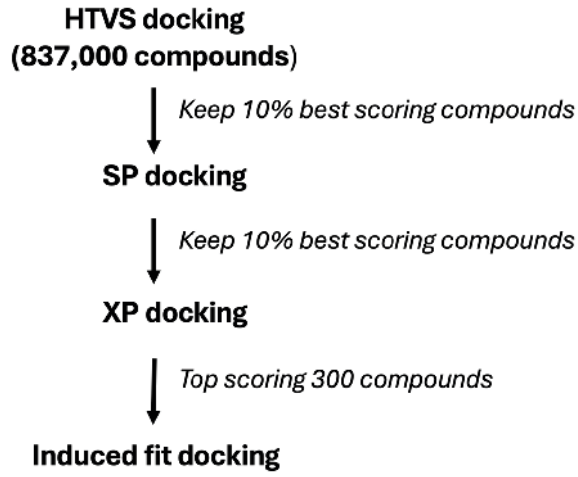


Figure 2. Virtual high throughput screening workflow. HTVS; high throughput virtual screen.

Induced Fit Docking

The top 300 compounds from the XP screening step of the VHTS were selected for further screening through induced-fit docking (IFD) in which both the compounds and protein were flexible. Receptor grids of 12Å were generated around each binding site, with a ligand ring conformation sampling energy window of 2.5 kcal/mol. Glide docking was then done by keeping receptor and ligand van der Waals scaling at value of 0.5, and 10 binding poses were generated per compound. The Prime program within the Schrödinger suite was then used to refine residues within 5 Å of the docked ligand and redocking was done into the structures with a 30 kcal/mol energy barrier for the 10 best structures per compound generated by XP docking.

Compound Clustering and Selection

To diversify the chemotypes, clustering of compounds passing the induced fit docking step was done using the Tanimonto coefficient to calculate the similarity of the compounds based on their fingerprints using the Canvas similarity and clustering program within Maestro interface of Schrödinger suite, and 23 clusters were created consisting of 121 compounds. Final selection of 53 compounds for wet-bench analysis was made from 17 clusters after considering their numbers of rotatable bonds, docking scores, the range of docking score among different binding poses of a compound, and predicted hydrophobicity (cLogP).

Viral Replication Inhibition Studies

HBV replication inhibition screen was done using HepDES19 cells[9] as previously described.[10] In brief, HepDES19 cells were seeded in 96-well plates at 4 × 10^4 cells per well. Two days later, compounds were added to cells in the presence of 1% DMSO at 5 µM and 20 µM in the qualitative screens or as a serial dilution of compounds from 100 to 0.39 µM in the quantitative screens. Lamivudine at 5 µM and 20 µM was used as a positive control. HepDES19 cells were lysed as described previously.[10] The lysate was used in strand-preferential quantitative polymerase chain reaction (qPCR) assays for detection of HBV DNAs. qPCR was performed with 40 cycles of 95 °C for 15 sec. and 60 °C for 1 min. employing the Kappa Probe Force universal PCR master mix (Roche 07959354001). Two sets of primers and probe (IDT Inc.) were used for the plus-polarity and minus-polarity DNA strands.[10] In the qualitative screens, inhibition was defined as 50% suppression of the (+) and/or (-) polarity DNA strand accumulation levels compared to the DMSO vehicle-treated controls. In quantitative assay, effective concentration 50% (EC_{50}) values were calculated with GraphPad Prism using

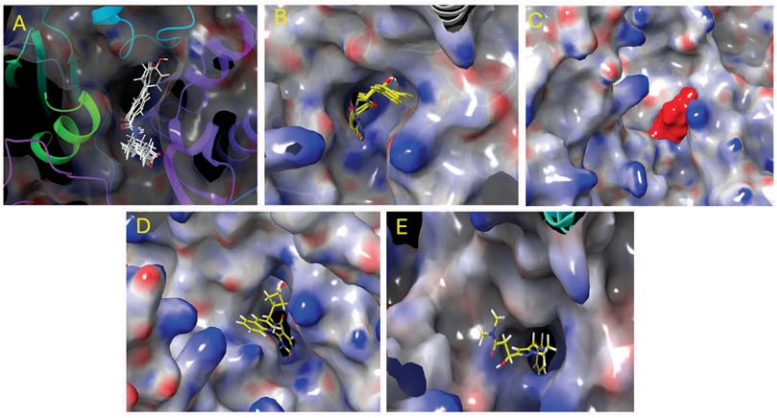


Figure 3. Docking poses from induced-fit docking to Site 3. (A) Four binding poses for a compound with docking scores ranging from −10.1 to −8.6 kcal/mol. (B) Docking poses for a linear hit extending through the Site 3 pocket (scores −11.8 to −9.7 kcal/mol). (C) Binding pose for a hit filling the binding pocket (−10.4 kcal/mol). (D) Binding pose for a 3-armed hit (−8.99 kCal/mol). (E) Docking pose for a hit extending from the Site 3 pocket into the binding nucleic acid binding groove (−10.5 kcal/mol).

the four-parameter log(inhibitor)-versus-response algorithm

Molecular Dynamic Simulations

Molecular dynamic simulations (MDs) were done under physiological temperature (300 K), pressure (1.01325 bar) and salt concentration (150 mM) on the P protein and on P-ligand (PL) complexes with a ligand docked inside Site 3 for analysis of flexibility and stability of binding pocket and receptor-ligand interactions. MDs were done using the Desmond module within the Maestro interface of the Schrödinger suite. Before performing the MDs, the receptor and receptor-ligand systems were prepared using System Builder program in Maestro. The receptor and receptor-ligand systems were solvated within a orthorhombic box with a 10 Å distance between the protein surface and the box edges using solvent model simple point charge (SPC). The systems were neutralized by adding Na^+ or Cl^- counterions and energy minimized using the OPSL4 system. MDs were performed for 100 ns with recording trajectory intervals of 100 ps, resulted in 1000 frames. Temperature and pressure were controlled during the simulation through the Nose Hoover chain and Martyna-Tobias-Klein algorithms, respectively. Ligand interaction maps were used to analyze ligand-binding site stability, and structural variations were assessed by root mean square deviations (RMSD) and root mean square fluctuations (RMSF) of P protein and PL complexes.

Results and Discussion

Selection of Binding Sites for Compound Screening

We used a predicted and validated structure of P protein[6] for the identification of the NNRTI sites. The sitemap program within the Schrödinger molecular analysis suite was used to identify 10 possible sites. Out of the 10 sites, three sites looked promising for virtual screening based on visual inspection and importance of the location in the substrate binding and interactions with other domains (Figure 1). Binding a compound to Site 1 should block RNA binding by obscuring RNA interactions with the T3 motif needed for interaction between P and the HBV RNA[11] and/or DNA elongation by altering the fingers subdomain grip on the template. We did not move forward with Site 1 for screening because the pocket is shallow and the very dynamic spacer domain which folds in a different conformation in every variant structure produced using Alphafold2 is part of the site (Figure 1). A compound binding to Site 2 should block template binding by steric occlusion, priming by mis-aligning the RNA template, and/or DNA elongation by displacing the template. Site 2 is in the palm subdomain of P and is analogous to the HIV NNRTI site, where NNRTI compound binding blocks reverse transcription.[12] The last 42 residues of the C-terminus of the RH domain form a flexible loop, and in the predicted structure it is folded over the palm subdomain and also forms a few H-bonds with residues in the palm subdomain, thus blocking part of Site 2 (Figure 1). We performed VHTS using the ChemBridge virtual library for the Site 2, but very few compounds docked with promising docking

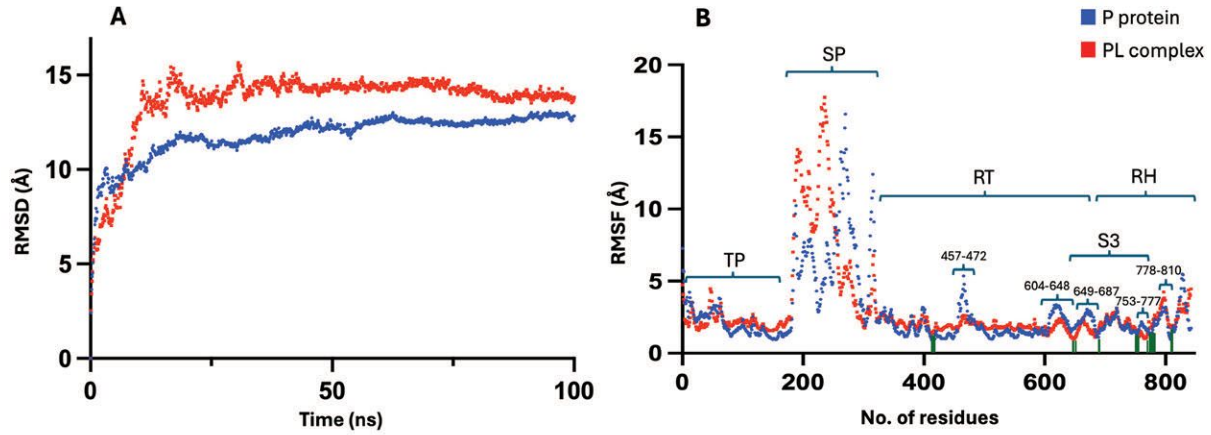


Figure 4. Structural variations during molecular dynamics simulations of P and PL complexes. (A) RMSD values of the P protein backbone in the absence (blue) and the presence of ligand (PL, red). (B) RMSF values of P (blue) and the PL complex (red). TP; terminal protein, SP; spacer domain, RT; reverse transcriptase, RH; ribonuclease H, S3; Site 3. Residue positions interacting with the ligand are shown as green lines.

scores, so wet-bench testing was not pursued. Ligand binding to Site 3 should block DNA elongation by suppressing lateral flexibility of the RT domain as it flexes during its catalytic cycle. Additionally, Site 3 is located close to the substrate binding channel and forms a deep pocket which would likely be able to form a high-affinity binding site. Therefore, we selected Site 3 for the screening efforts as it appeared to be the most promising of the potential sites and as inhibitors binding to it would work by a novel mechanism.

Virtual Hit Identification

The ChemBridge virtual small molecule compound library consisting of 837,000 compounds was prepared as described in the methods to ensure protonation and conformation states were appropriately represented. Computational screening was done involving sequential use of the high throughput virtual screening, SP, and XP docking utilities within the Schrödinger molecular analysis suite, with the best 10% of the compounds being retained at each step. This resulted in 837 virtual hits after the final XP screening step (0.1% of the library) with docking scores ranging from -6.50 to -10.1 kcal/mol, corresponding to estimated K_ds of 0.025 - 9.9 μM calculated using the relation $\Delta G=InRT(K_d)$. For comparison, the approved nucleos(t)ide analogs dock to P:nucleic acid complexes with scores of -9.3 to -13.3 kcal/mol. Several compounds returned multiple binding poses in the binding sites with promising docking scores.

The top 300 hit compounds by XP docking score were further screened with induced-fit docking (IFD) where both ligand and receptor were flexible. Rescoring with IFD improved the docking scores for 49 compounds, 41 had negligible changes, 81 got worse, and 129 failed to dock. The 30 kcal/mol energy barrier employed in IFD binding presumably eliminated compounds that had to traverse a high energy barrier to bind. The range of changes in the docking scores following IFD was -4.40 to +3.36 kcal/mol. The best change in predicted K_d for any compound following IFD was 47.3 nM (XP docking) to 0.348 nM (IFD docking).

Selection of Virtual Hit Compounds for Replication Inhibition Studies

Selection of compounds for HBV replication inhibition studies employed a docking score cutoff of -7.0 kcal/mol, ligand uniqueness, and compound conformational stability and rigidity. Compound clustering was used to diversify the chemotypes in our final selection. Final selection of 53 compounds was made from 17 clusters considering their rotatable bonds, docking scores and range of docking scores

among different binding poses of a compound. Rotatable bonds in an inhibitor determine its rigidity i.e., compounds with fewer rotatable bonds are more rigid; this was evaluated because effective inhibitors typically have ≤10 rotatable bonds.[13] We also selected a few more flexible compounds having 10 or more rotatable bonds to test the hypothesis that more flexible compounds may fit better as the flexibility of the binding site on P was unknown and because we were screening against a predicted model rather than an experimentally determined structure. The degree of change in docking scores of different bindings poses of a docked compound was considered to reduce entropic penalties during binding, e.g., a compound having four binding poses with docking scores range from -10.1 to -8.6 kcal/mol was given priority over a compound with drastic difference in its docking scores in multiple binding poses (Figure 3). We also considered compounds with unique shapes and binding poses, e.g., including some that projected into the nucleic acid binding groove (Figure 3). Finally, only compounds with cLogP values from 1.0 – 5.0 were selected to improve chances that they had favorable solubility and cellular permeability profiles.

Screening of In Silico *Hits for HBV Replication Inhibition*

We performed quantitative and qualitative screens using our standard HBV inhibition replication assay.[10] HepDES19 cells carrying a tetracycline-repressible HBV genomic expression cassette[9] were plated in a 96 well plate and grown for 48 h in the absence of tetracycline to induce HBV replication. HBV nucleic acids within cytoplasmic nucleocapsids were harvested from lysates of cells expressing HBV after incubation with compound for three days. Strand-preferential qPCR was done to independently assess accumulation of the (+) and (-) polarity HBV DNA strands of the viral genome. None of the compounds inhibited HBV DNA accumulation to below 50% at either 5 or 20 μM in the initial qualitative analysis. Two compounds with detectable inhibition in the qualitative screen, 1779 and 1785, were moved forward to quantitative analysis. EC_{50} values were determined in replication inhibition studies where serially diluted compounds were added to HepDES19 cells replicating HBV for three days. Both compounds had EC_{50}s >100 μM, indicating failure to inhibit HBV replication.

MD Simulation Studies

To assess reasons for failure of the virtual hit compounds to inhibit HBV replication, molecular dynamics simulations (MDs) were done for 100 ns to study the protein-ligand (PL) interactions plus the stability and compactness of Site 3. We compared the MDs results of P protein in its apo form with results from P bound to a ligand having the highest docking score bound to Site 3. The Site 3 binding pocket consists of residues N411, L412, Q413, S414, N417, P645, T647, Y651, V687, A688, and Q690 from the RT domain plus residues V752, L753, S754, R755, K756, Y757, N769, R773, G774, T775, S776, F777, Y779, Y806, R807 and T809 from the RH domain. Conformational and structural changes and stability were analyzed using root mean square deviation (RMSD) and root mean square fluctuation (RMSF). RMSD is the average distance between atoms of a protein's conformation compared to a reference conformation over a period of time during an MDs and quantifies global changes in the protein structure. This was analyzed because the binding of a compound can alter the conformational stability of a protein by providing stabilizing molecular contacts.[14,15] The P protein RMSD changed by 11.8 Å from its initial conformation in 19 ns before stabilizing with slight changes for the rest of simulation. In the case of PL complex, the conformation changed by an RMSD of 13.8 Å in 12.2 ns, after which the structure stabilized over the next 87.2 ns of the simulation (Figure 4A). Overall, the PL complex structure stabilized slightly earlier than the apo structure, and the mean conformational change of the PL complex after stabilization was 2 Å more than the P protein alone.

The RMSF values were analyzed to observe the local conformational fluctuations in protein structure near Site 3 during MDs. Site 3 is located between 644-778 aa and also contains a few residues from the RT palm subdomain (411-417 aa) and the RNase H C-terminus (806-809 aa). Structural fluctuations in the apo form of P were primarily observed in residues 457-472, with an average RMSF value of 3.73 Å, but this region lies in the fingers subdomain of RT and is

not close to Site 3. In the case of the PL simulations, the average fluctuation in residues 457-472 was reduced to 2.26 Å, and the ligand interacted with residues N411 and Q413 for >30% of simulation time. We observed three fluctuating peaks in the region comprised of residues 600-810 in the apo enzyme simulation. The average RMSF values of residues 604-648, 649-687 and 753-777 were 2.53, 2.25 and 1.67 Å, respectively, which was reduced slightly in the PL complex structure to 1.79, 1.6 and 1.39 Å. The ligand had interactions (>30% of simulation time) with residues Q690, L753, S754, R755 and F777 in these regions in the PL complex. The fluctuation peak in the P protein region was comprised of residues 776-810. This region had an RMSF value of 2.14 Å in the apo enzyme, which was slightly increased in the PL complex to 2.64 Å. This enhanced flexibility was unexpected because the ligand interacted with residues F777, Y806 and T809 in 776-810 for >50% of the MDs time in the PL complex (Figure 4B). Overall, the MD simulation results showed that binding of ligand did not markedly stabilize the Site 3, and that large conformational fluctuations in the region containing Site 3 may result in unstable binding of ligand.

Conclusion

We identified potential NNRTI sites using a predicted and validated structure of the HBV.[6] Site 3 was selected for detailed analysis based on its location near the substrate binding channel and the potential for compound binding to the site to constrain lateral motion of the polymerase during its catalytic cycle. However, none of the compounds suppressed HBV reverse transcription by more than 50% during viral replication inhibition studies. MDs studies revealed that the conformation of Site 3 fluctuates constantly and ligand binding did not significantly stabilize the Site 3. These findings led us to conclude that new binding sites should be identified, and that selection of sites should include rigorous MDs experiments before performing high throughput screening experiments and replication inhibition studies. They also imply that using polymerase structures derived from MD simulations after the molecule has stabilized its conformation may improve results from VHTS.

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No financial disclosure reported. AlphaFold2 was used to generate the structure of the HBV P protein used in this study. AlphaFold2 is a machine-learning tool, but its use was consistent with the JAMA guidelines because it was used to generate the starting point for these studies but played no role in the execution of the virtual screening study or interpretation of the results. **MM**

Electric Scooters: A Modern Method of Trauma

by Ryan Hoffman, DO, James O'Brien, MD & Matthew Cook, MD

E-scooters have established themselves as economical modes of transportation in our modern society, and thus, safe use should be encouraged based on the early data.



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Abstract

Electric scooters (e-scooters) have become a popular means of short-distance transportation for major metropolitan areas in several countries over the past decade. Their eco-friendliness, affordability, and ease of operation are selling points to people of all ages, particularly youth and young adults. Societal normalization has unfortunately overshadowed the risks entailed with their operation including severe injury and death. Alcohol, lack of protective equipment, and inadequate infrastructure appear to be common preventable risk factors. Emergency clinicians and trauma teams experience the aftermath of e-scooter injuries, which promise a rising trajectory. Common injury patterns have been identified by researchers around the world, most notably orthopedic, head, and maxillofacial in nature. Becoming familiar with these injury patterns and risk factors contributing to e-scooter accidents can be advantageous to the emergency physician's preparedness and public officials' approach to injury prevention.

Key Words

Electric scooter injury, orthopedic head, and maxillofacial trauma.

Introduction

A ten-year-old boy was riding his electric scooter (e-scooter) home from a friend's house during the early evening hours on October 13, 2025, when he collided with a vehicle at an intersection in the Kansas City metropolitan area. Local news reports that he later died at Children's Mercy Hospital from his injuries including severe head trauma.[1] Though few fatalities have been reported among the early literature discussing e-scooter injuries, this tragedy draws attention to a rising mode of transportation spreading like wildfire nearly one century since the device's conception as the "Autoped" in 1915 (Figure 1).[2]

In addition to privately owned devices zipping through neighborhoods and college campuses, many cities have implemented e-scooter rideshare programs into their infrastructure. The first dockless scooter program was launched by Bird in Santa Monica, California in 2017. Since then, "micro-mobility" transportation has made its mark on several major metropolitan areas with programs appearing in over 100 cities and 20-plus states.[3] Companies, including Bird and Lime, promote scooters as an environmentally friendly mode of transportation that reduces



Figure 1. The "Autoped" was the first e-scooter developed by Arthur Hugo Cecil Gibson in the 1910s.[2]
Source: Smithsonian Magazine

traffic congestion and offers a cost-effective method of travel (Figure 2). Rentals require only a smartphone application, which operates on a pay-by-minute basis following a nominal fee. While no protective equipment is necessary, age verification is required for rental, usually once through the associated application. Nevertheless, it is not uncommon to spot children and teenagers riding privately-owned devices to school or recreationally.

The privately-owned e-scooter has a broad price range from approximately $300 to $2000. The customer expense of a rideshare unit varies by brand and distance traveled. Bird reported in 2018 the cost of a scooter with a GPS tracker installation totaled approximately $400.[4] E-scooters are intended for short-distance travel in urban areas, powered by a small electric motor and a standing platform designed for a single rider. They typically move at speeds comparable to bicycles, but specifications can range in maximum velocity, acceleration, and suspension. Their basic and nearly universal configuration allows ease of operation. Figure 3 depicts the dashboard of a simple rideshare scooter with throttle, brake, bell, and unlocking mechanism. Rideshare programs have continued to update their fleets with new features such as turn signals and smartphone mounts (Figure 4).



Figure 2. Basic rideshare e-scooter model manufactured by Bird. Photo taken at Hospital Hill, Kansas City, Missouri, USA.



Figure 4. Newer rideshare e-scooter model by Bird featuring smartphone mount and turn signaling capabilities. Photo taken at Hospital Hill, Kansas City, Missouri, USA.



Figure 3. Basic dashboard of e-scooter depicted in Figure 2 are as follows: A - handlebars; B - QR code for smartphone application connection (unlock mechanism); C - throttle lever; D - brake; E - bell.

Missouri law dictates that the maximum speed for an e-scooter on a public road is 20 mph when in a bike lane or 30 mph on the road. According to the Missouri State Highway Patrol (MSHP), the operator "must abide by the same laws governing a motor vehicle (i.e., stopping at posted STOP signs, driving on the right-side of the roadway, yielding to approaching traffic when turning left, and not exceeding the posted speed limit)."[5] As e-scooters are technically motor vehicles, intoxicated operators are subject to DWI penalties.[6] The minimum age requirement varies by state in the US with the most common age set at 16, coinciding with the legal driving age.

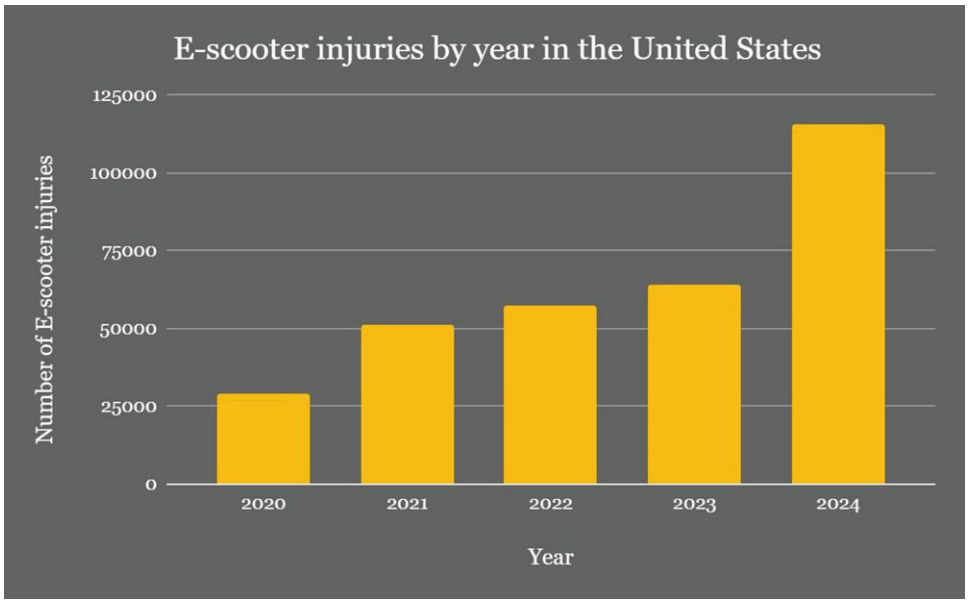


Figure 5. Data reported by the United States Consumer Product Safety Commission via the NEISS database. Values are based on a weighted sample from 100 emergency departments.[7]

Accidents are common, and rules are not always followed. The surge in e-scooter use has corresponded to a spike in scooter-related trauma presenting to emergency departments. Figure 5 provides a visual representation of injury trends in the US according to data from the US Consumer Product Safety Commission.[7] These e-scooters have the potential to create new trends in trauma incidence and alter trauma patterns seen in a range of emergency department settings. Here we present an investigation into the demographics, risk factors, and injury patterns entailed with e-scooter use. We close with a discussion of primary prevention strategies and what scooter injuries entail for emergency medical providers.

Demographics

The most common patient who presents to the emergency department (ED) following an e-scooter event is a male in his early thirties. Accidents occur most often during the late afternoon and evening hours. The patient is usually the rider who lost control and fell, although it is not uncommon to be struck by a vehicle while riding in densely populated areas such as southern California.[8] Riders sustain injuries from colliding with stationary objects, pedestrians, and other riders at a much lower rate.

Most e-scooter operators, namely those renting through a mobile application requiring age verification, are older than 16. However, younger riders board private e-scooters without sufficient knowledge of rules of the road, increasing risk to themselves and people they encounter. Cruz et al. reports orthopedic injuries in patients as young as 11-years-old with 14 of 83 patients (17%) under the age of 16.[9] It should be noted that privately-owned scooters were utilized during this study and, thus, no form of ID was required to unlock the scooter. On the other end of the age spectrum, some studies report elderly falls by means of tripping over scooters, though the reported incidence is low.[9,10] Injuries sustained by older individuals tend to be more severe as observed by researchers in Vienna.[11]

Risk Factors

E-scooters are equipped with few safety features. Arguably the only safety feature accompanying standard brakes in rideshare scooters is a programmed speed cap of about 15 mph. This speed may easily be exceeded by private, recreational scooters. Despite this limit, Cruz et al. reports that more than half of patients who sustained orthopedic injuries admitted to traveling at a speed less than 15 mph.[9]

Personal protective equipment is merely a recommendation in most regions and most patients who presented to the ED in the literature were not wearing a helmet. Data from southern California and the UK show helmet use in about one-fifth to one-third of individuals who sustained injuries while only one of 268 patients reported helmet use in a retrospective German cohort.[8,9,10] Authors in Helsinki report a 2-6% helmet compliance rate among those injured.[12]

People are generally familiar with the consequences of driving an automobile under the influence of alcohol, but consequences of drunk e-scooter operation may be less clear. Operating an e-scooter under the influence may deceptively pose as a safer alternative to an automobile with fewer legal repercussions through the lens of alcohol. Kleinhertz et al. reports alcohol use in 33% of patients with a startling rate of head and facial injury in 84% of those who were intoxicated.[10] Their data do not specify whether injuries were more severe among those who consumed alcohol, but it is noted that intoxicated patients were more likely to be brought to the ED by ambulance. The few patients who were intoxicated in the Vienna study all were noted to have severe head injuries.[11] Snäll et. al. reports that the intoxication rate among patients with facial injuries was over twice that of patients without facial injuries.[13]

A rider's geography may play a role in injury severity based on data out of southern California. Dhillon et al. report an ICU admission rate of 20.7% with one fatality.[8] These findings differ from other studies in which falls were by far the most common mechanism of injury. We cannot conclude that the population density of southern California is directly related to the rate of vehicular strikes or that injury severity was worse in riders struck by a vehicle. These observations are worth considering as southern California has one of the highest population densities in the US.

Lack of daylight shows association with injury occurrence, though it is unclear whether it is poor visibility itself or nighttime activities that drive these numbers. The data consistently show that most injuries occur in the late afternoon through overnight hours. Urban terrain presents multiple hazards including curbs, light posts, cars, pedestrians, and animals. Nevertheless, such obstacles become more difficult to dodge when coordination has been compromised by alcohol or other recreational substances.

Injury Patterns and Mechanisms

A fall from a moving e-scooter is the most common mechanism of injury. There is a higher incidence of upper extremity injuries than lower according to the orthopedic literature reviewed and this may be attributed to the reflexive fall on an outstretched hand or elbow. The most fractured bone of the upper extremity is consistently the radius, specifically the radial head. Shichman et al. reported that more than half of fractures involving the radius were intra-articular and the distal aspect had a higher rate of operative management than proximal.[14] This finding is supported by Ishmael and colleagues who analyzed operative orthopedic cases over a two-year span, starting around the time of Bird's debut in Santa Monica.[15]

Tibial plateau and ankle fractures appear to be the most injured regions of the lower extremity. Accompanying tibial plateau fractures are ACL injuries and other capsular components of the knee. Although fractures of the lower limb may be less common than upper, they are more likely to require operative intervention and subsequently longer hospital admission.[14] Ishmael et al. found that five of seven patients (N=73) who sustained hip fractures with e-scooter involvement were noted to be under the age of 46.[15] It is clear that e-scooter injuries may entail extensive rehabilitation and elicit long-term effects on mobility when the lower extremity is involved.

The upper extremities are the first line of defense, however at speeds sometimes exceeding 15 mph, they do not reliably serve as a failsafe against head and facial trauma, especially in the setting of alcohol use and delayed reaction times. Fortunately, the reported incidence of intracranial hemorrhage was very low among studies. Most head injuries were non-operative and coinciding cervical spine fractures were low. Soft tissue injuries including lacerations predominated and there was a lower incidence of traumatic brain injury (TBI).[8,10] As TBI is a clinical diagnosis, injury severity and resulting long-term impacts on cerebral function were not detailed in the trauma literature, but certainly are a valid consideration.

Discussion

E-scooters have become a common form of recreation and transportation among adults and children, leading to a rise in ED visits for a range of traumatic injuries. With modern technology creating a seamless way to rent scooters, their accessibility has only increased in urban and suburban settings. Their attractive features overshadow associated operational risks, which may subject the rider to devastating injury. The early data present opportunities for both public officials and ED providers to respectively promote safe transit and be mindful of common injury patterns for optimal care. Since it has been less than a decade from

their introduction on a large scale, further research is needed to form trends and optimally recommend safety procedures.

It is clear from the literature that use of protective equipment is minimal and, intuitively, that is no surprise. Most people do not routinely lug around a helmet (much less elbow, wrist, and knee pads) as they are bulky and not particularly fashionable. Unfortunately, helmet enforcement presents a tall order in some states. Missouri, for example, does not require licensed motorcyclists over the age of 26 with adequate health insurance to wear them.[16] Loose legislation should not discourage micro-mobility companies or public officials from increasing awareness of safety precautions. Accentuating existing guidelines listed in the fine print and encouraging additional joint padding may be a start.

We cannot rely on riders to don protective gear, so we must seek alternative means of prevention. Protecting the public from e-scooter injuries may yet be accomplished by introducing city ordinances that restrict their use to daylight hours given many e-scooter-related ED visits tend to occur in the evening. Helsinki introduced legislation in September 2021 that reduced the speed cap of its rideshare scooters from 25 km/h to 20 km/h (15.5 to 12.4 mi/h) between the hours of 5:00 a.m. to midnight and further to 15 km/h (9.3 mi/h) between midnight and 5:00 a.m. Monday through Friday. Their use was banned altogether between midnight and 5:00 a.m. on Saturday and Sunday. Pakarinen and colleagues found a nearly 40% decrease in e-scooter injuries from 2021 to 2022 during the time frame of January-August.[12] Snäll et al. reported a decrease in maxillofacial injuries of 88% during the summer months following the legislation.[13] Trialing ordinances of this nature in other cities may cut down on injuries related to poor visibility and alcohol intoxication.

Injuries to pedestrians on foot may be minimized by expansion of infrastructure to keep riders in bike lanes instead of sidewalks. By the same token, expansion of bike lanes would endow a safer commute for riders at risk of vehicular collision. Increased distribution of designated scooter racks at drop-off zones may prevent the rare elderly trip-and-fall as many scooters are deposited in the middle of sidewalks or street corners.

The ED physician is faced with the challenge of expediently identifying the rider's injuries, consulting appropriate services, and determining ultimate disposition. Asking about injury mechanisms like fall or vehicle strike along with predicted speed become helpful in determining injury location and severity. For example, a fall on an outstretched hand may prompt the provider to obtain radiographs of both the proximal and distal radius. Discerning whether the e-scooter was part of a rideshare program or privately owned can be helpful in predicting approximate speed as rideshare e-scooters are often max out at 15 mph. Higher speeds should raise suspicion of injuries that are not clinically apparent. Suspected alcohol or drug use should support the decision to obtain a non-contrasted CT scan of the head and face, especially if the patient is altered as intoxication may easily mask intracranial injury. Fortunately, the rate of reported intracranial hemorrhage and cervical spine fracture was low despite poor helmet compliance.

E-scooters have established themselves as economical modes of transportation in our modern society, and thus, safe use should be encouraged based on the early data. Continued data collection would be helpful for ED and trauma providers to remain familiar with injury trends. As physicians, we must remain mindful of our duty to educate our patients and the community beyond the hospital and clinic walls. Healthcare providers should maintain a healthy respect for serious injury when scooter related trauma presents. As always, perform a thorough head-to-toe trauma evaluation to promptly identify and treat injury when primary prevention measures fall short.

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Case In Point

I Almost Killed That Kid on the Electric Scooter

by John C. Hagan III, MD

One minute I'm enjoying a beautiful fall afternoon drive with my wife in Riverside Park in Parkville, Missouri. Next minute I'm having an epic adrenaline dump, shaking and ghostly pale.

"I almost killed that kid on the e-scooter," I tell my equally shaken wife. In the rearview mirror, I see the male scooter driver, apparently unfazed, continue on as he gives me a rude gesture. He can give a finger but he apparently can't give a left-hand turn signal prior to suddenly turning left.



Five hours later, I'm still thinking of what might have happened had I hit the boy. Stopping, calling 911, delivering the best first aid a retired ophthalmologist can, watching the ambulance take him away, enduring insults, perhaps threats, from the rider's friends and family.

Inevitably I would be served by a deputy. I'm being sued for many millions of dollars for careless and reckless driving causing great bodily harm or death. I wonder how the jury would rule. The road was a narrow, asphalt road, barely wide enough for two cars to pass. No center line, no sidewalk. The e-scooter driver, who had on no helmet and was below legal driving age, made no turn signal when I attempted to pass him, giving the widest clearance possible on the relatively narrow road. "Ladies and gentlemen of the jury, he turned into me without any hand signal." Given the erratic path of 'jury justice', who knows whether I would be found innocent or guilty?

Please read the e-scooter article carefully. Every time you see one of these ubiquitous e-contrivances while driving, please treat them like a injury-death-lawsuit waiting to happen. Two weeks after this incident, my blood pressure and pulse returned to normal.

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Ulnar Collateral Ligament Injuries in Youth Throwing Athletes

by Matthew J. Steffes, MD

The recent rediscovery of UCL repair has shown great promise, demonstrating the inherent advantage of repairing native tissues as evidenced by faster recovery time.



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Abstract

The medial ulnar collateral ligament (UCL) of the elbow is a vital structure for the overhead throwing motion. Injuries to this ligament are becoming increasingly common and specifically in youth baseball players. Significant work has been done to provide pitch count guidelines to monitor workload and prevent UCL injuries. Yet despite these efforts, injuries to the ulnar collateral ligament continue to occur at an alarming rate, leading to lost playing time and impacting player development. Complete tears of the UCL have traditionally been addressed surgically by reconstructing the ligament with a tendon graft, known as Tommy John Surgery. Newer techniques have allowed for repair of the native UCL tissue in many cases, drastically changing the treatment paradigm and subsequent rehabilitation.

Key Words

Ulnar collateral ligament, elbow, overhead throwing, tendon graft.

Introduction

The ulnar collateral ligament (UCL) originates on the anterior face of the humeral medial epicondyle, attaching broadly to the sublime tubercle of the ulna, and acting as the primary stabilizer to valgus force at the elbow. Most daily activities do not require full stability of the UCL, but it is essential for the overhead throwing motion. While the first UCL injury was described in a Scandinavian javelin thrower, these injuries are now synonymous with baseball pitchers.[1] During the overhead throwing motion, the upper extremity is placed into maximal abduction and external rotation, described in baseball terms as the "lay-back" position. The body is then propelled towards the target, the lead leg contacts the ground which allows the torso to rotate and create supra-physiologic rotational force on the arm to generate ball velocity. This motion has shown to create 64-120 N m of valgus force at the elbow, which can lead to both acute traumatic tears of the UCL and chronic attritional damage with repetitive throwing.[2]

Given this, it is no surprise that the volume and velocity of pitches thrown have been implicated in the well-publicized rise of UCL injuries at the youth level.[3,4] In a review of the New York State Health department database, Mahure et al. found the incidence of UCL reconstructions being performed increased 343% from 2003 to 2014, with pitchers aged 15-19 years old showing the largest proportion of surgeries.[3] Based on this trend they projected continued increases by

year 2025, from 6.3 procedures per 100,000, to 14.6 per 100,000. This trend was the nidus for the MLB Pitch Smart Guidelines for youth baseball pitchers as means of workload management, which have been adopted by most states and governing bodies.[5] Despite these guidelines, youth pitchers commonly violate pitch counts, with a recent study showing 84% of high school pitchers self-reporting one or more violations.[6] Other causative factors shown to be highly correlated with youth UCL injury is the use of weighted balls to gain velocity, playing for multiple baseball teams, and pitching more than eight months in a year.[7,8] With such emphasis on injury prevention with continued increases in UCL injuries, a more diligent evaluation of pitch count guidance and athlete/coach education may be necessary to effect injury rates.

Treatment

Treatment decisions for UCL tears are made based on tear location, degree of injury, and chronicity. Treatment for partial thickness tears of the UCL typically employs a period shutdown from throwing and avoiding any valgus load to the elbow. During this time physical therapy is employed to identify any pathologic movement patterns that may have contributed to UCL injury, as deficits anywhere along the kinetic chain have been shown to contribute to UCL injury.[9,10] It has been shown proximal partial thickness tears respond best to this type of treatment as opposed to mid-substance and distal tears.[11] Once pain-free motion is obtained, activity is advanced to plyometric activities of the upper extremity followed by a graduated return to throwing program and then to competition.

The role of platelet-rich plasma (PRP) has been evaluated as an adjuvant to augment ligament healing. The first reported series of this in 34 overhead athletes of various ages and levels of competition, showed 88% were able to return to sport without complaint by mean 70 weeks follow-up.[12] Dines et al. showed excellent results with PRP for proximal partial tears but less encouraging results for distal partial tears.[13] A larger study using the MLB HITS injury surveillance system compared groups of position players and pitchers with partial UCL tears who either received PRP injection or did not, and found no benefit.[14] This paucity of evidence delineating superiority of PRP, specific PRP preparations, or certain injection protocols prevent advocating for routine use for partial UCL tears.

Initially, full thickness UCL injuries were considered career-ending as returning to baseball amongst professional pitchers with either surgical or non-surgical treatment was uncommon and performance upon return highly variable. With the development of the UCL reconstruction in 1974 by Frank Jobe, MD, on Dodgers pitcher Tommy John, professional pitchers were now able to return to professional pitching and at a high level. This pivotal development forever changed the paradigm of UCL treatment, establishing UCL reconstruction as the gold standard for all UCL tears.

Dr. Jobe's initial technique involved removing the flexor musculature to expose the epicondyle and making full length tunnels for a palmaris longus tendon graft.[15] In the decades since, refinement and improvements in surgical exposure, fixation techniques, and rehabilitation have consistently shown return to play rates among professional pitchers of 80-92%, and performance upon return at or surpassing pre-injury levels.[16–18] Similar results have been shown in several studies of adolescent throwers, with a large series by Saper et al. showing 98% return to sport at a mean of 11.6 months and 90% returning to the same level of performance.[19]

Despite these exceptional results UCL reconstruction is not without its drawbacks. Chief among them is the prolonged rehabilitation timeline, driven mostly by the time required for the tendon graft to fully incorporate and mature, causing the typical timeline to return to competitive pitching anywhere from 12-18 months. Also, the size of the tunnels required for graft passage causes bone loss and can lead to issues should subsequent UCL tear occur and a revision procedure necessary.

Given these cited shortcomings to UCL reconstruction, many surgeons began revisiting the role of UCL repair, particularly in avulsion tear patterns from either the humerus or ulna in an otherwise healthy ligament. First among these was Argo et al., who followed a group of female throwing and non-throwing athletes who underwent UCL repair by various surgical techniques. Ages ranged from 15-37 years and they demonstrated a return to sport rate of 93% by six months from the initial procedure.[20] This same study group then detailed outcomes of teenaged throwing athletes following UCL repair by various repair techniques. Of the 51 throwing athletes included, only one was unable to return to competitive



Figure 1. MRI Coronal fluid-sensitive sequence showing a full thickness proximal UCL tear with the healthy residual UCL denoted by the arrow.



Figure 2. Intra-operative view of a left elbow. The proximal torn end of the UCL is within the forceps. A longitudinal split of the ligament shows remaining healthy tissue but gapping and instability of the ulnohumeral joint.

baseball.[21] These results were redemonstrated by Dugas et al. who followed a cohort of adolescent throwers who underwent UCL repair using a suture anchor construct and augmented with a suture tape. Of the 111 athletes followed, 92% returned to either the same or higher level of competition by 6.7 months.[22] This same group also evaluated the outcomes of 461 teenaged throwers who underwent either UCL reconstruction or UCL repair with suture augmentation. They showed no difference in various patient-reported outcome scores or return to sport rate (98% UCL repair vs. 99% UCL reconstruction cohorts). The most notable finding was the UCL repair cohort returned to competition at mean of 9.2 months, compared to 13.4 months for the UCL reconstructions.[23] This body of work overwhelmingly shows the role of UCL repair in the treatment algorithm for UCL injuries, and contemporary indications for this procedure are for partial or full thickness avulsion tears in otherwise healthy ligament tissue.. Considering avulsion UCL tear patterns are frequently seen amongst youth throwers, this procedure will likely find wider adoption, ideally with continued excellent and durable outcomes (Figures 1, 2, 3, 4).

Conclusion

Injuries to the UCL are common in youth athletics and are occurring with increasing frequency. This rise is multifactorial but there is an established link between volume of pitches thrown and risk of UCL injury. Prevention of these injuries and adherence to age-based pitch count guidelines should be a point of emphasis at all levels as this has shown to lessen risk of injury. While results following UCL repair and reconstruction continue to allow players to return to competition and perform well, the time loss and impact on athlete development is significant and should not be discounted. The recent rediscovery of UCL repair has shown great promise, demonstrating the inherent advantage of repairing native tissues as evidenced by faster recovery time. Future work should delineate the longevity of this procedure and risk of re-injury, but at this time midterm results are promising and UCL repair will likely see an expanded role in treatment of youth UCL injuries.

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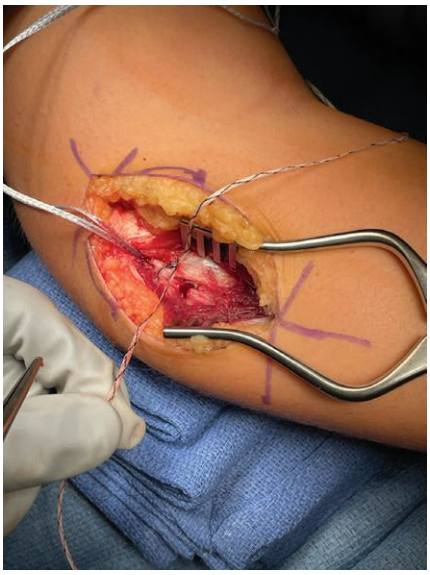


Figure 3. Intra-operative view of a left elbow. The torn proximal end of the UCL has been sutured with the white and black sutures. The blue suturetapes remain for later suture augmentation.



Figure 4. Intra-operative view a left elbow. The blue suturetapes sutures are secured into a suture anchor distally on the ulna to augment the repair and reinforce ligament stability.

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Disclosure

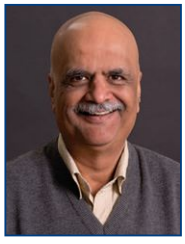
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Sleep Disturbances in PTSD: Insight from Clinical Studies

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Continued research targeting sleep as a therapeutic entry point may significantly advance PTSD management and improve overall patient outcomes.



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Abstract

The review article on posttraumatic stress disorder (PTSD) and sleep provides a comprehensive overview of circadian genes in PTSD-related sleep disturbances, drawing insights from patient studies. PTSD is often accompanied by significant sleep-related disturbances, which can manifest as nightmares, disrupted sleep continuity, and alterations in sleep architecture, particularly changes in Rapid Eye Movement (REM) sleep-regulation. Despite the well-documented symptomatic complaints of poor sleep among PTSD patients, the underlying mechanism remains poorly understood. This review underscores the need for a deeper understanding of how PTSD affects sleep patterns, including the potential role of specific sleep stages like REM sleep. By synthesizing findings from various studies, the review aims to clarify these relationships and explore potential mechanisms, ultimately informing better diagnostic and therapeutic strategies for managing sleep disturbances in PTSD patients.

Key Words

PTSD, sleep, clinical, circadian.

Introduction

PTSD is a neuro-psychiatric condition in which an individual often suffers from the nightmares/sleep disturbances caused by recalling the life-threatening events happened in the past.[1] However, PTSD patients are always diagnosed with sleep problems. Most of the PTSD patients suffer from nightmares and hyperarousal.[2,3] The first-ever clinical trial specifically targeting PTSD patients was conducted in the late 1980s. The trial focused on the use of fluoxetine (Prozac), a selective serotonin reuptake inhibitor (SSRI), to treat PTSD symptoms.[4] This marked the beginning of evidence-based pharmacological treatment for PTSD, setting the stage for further research and the development of standardized therapeutic approaches for the disorder. However, the condition known as "shell shock" during World War I, experienced by soldiers exposed to the horrors of trench warfare, is earliest recognition of PTSD symptoms during 16th century.[5]

PTSD is a debilitating psychiatric condition that often develops following exposure to traumatic events such as combat, assault, or natural disasters.[6] Among its core symptoms, sleep disturbances—particularly insomnia, nightmares, and fragmented sleep—are some of the most persistent and

distressing, often exacerbating daytime symptoms like hyperarousal, mood instability, and impaired cognition.[7] These sleep-related symptoms not only contribute to reduced quality of life but are also predictive of PTSD severity and treatment resistance. Despite their clinical significance, sleep disturbances in PTSD have historically been underrecognized and undertreated. In recent years, growing evidence has underscored the importance of addressing sleep symptoms as a central component of PTSD treatment.[8] This review aims to summarize current clinical approaches and emerging therapies targeting sleep disturbances in PTSD.

History

The first patient commonly recognized as having PTSD-like symptoms, Sprenger exhibited symptoms like flashbacks, anxiety, and insomnia following his experiences in battle, resembling what we now understand as PTSD.[9] Ralph Harrington argues that "railway spine," traditionally seen as an organic disorder, played a crucial role in shaping modern conceptions of traumatic disorders.[10] With early descriptions of PTSD-like symptoms is Samuel Pepys, a 17th-century English naval administrator documented in his diary the distressing nightmares and flashbacks he experienced after witnessing the Great Fire of London in 1666.[11] The first research group to systematically study PTSD symptoms was led by Dr. Ann Wolbert Burgess conducted pioneering research on rape trauma syndrome, which contributed significantly to the understanding of PTSD.[12] With the formal recognition of PTSD as a diagnosis in the DSM-III in 1980, sleep problems such as nightmares, insomnia, and disrupted sleep patterns were acknowledged as core symptoms of the disorder.[13]

Evolution in the definitions of PTSD Over Time

PTSD was first officially defined in the DSM-III as a psychiatric disorder resulting from exposure to a traumatic event involving actual or threatened death, serious injury, or a threat to physical integrity.[14] The definition of PTSD was revised in the DSM-IV to include additional symptoms and a more detailed description of trauma exposure[15]. DSM-V introduced significant changes to the PTSD diagnosis. PTSD was reclassified under a new category, "Trauma- and Stressor-Related Disorders," separating it from anxiety disorders. This edition also recognized PTSD in children and included dissociative symptoms as part of the diagnostic criteria.[16] The International Classification of Diseases, 11th Revision (ICD-11) provided the presence of three core symptoms: re-experiencing the trauma in the present (e.g., flashbacks), avoidance of reminders of the trauma, and a persistent sense of heightened current threat.[17]

Sleep disturbances in PTSD

Sleep disturbances have been recognized as a core feature of PTSD since the 1970s, when Dr. Robert Jay Lifton and Dr. Chaim Shatan first described severe nightmares and insomnia in war veterans.[18] Later, researchers like Dr. Philip Ross emphasized the persistent nature of sleep issues, while Dr. Anne Germain explored how trauma specifically alters sleep patterns (Figure 1).[19]

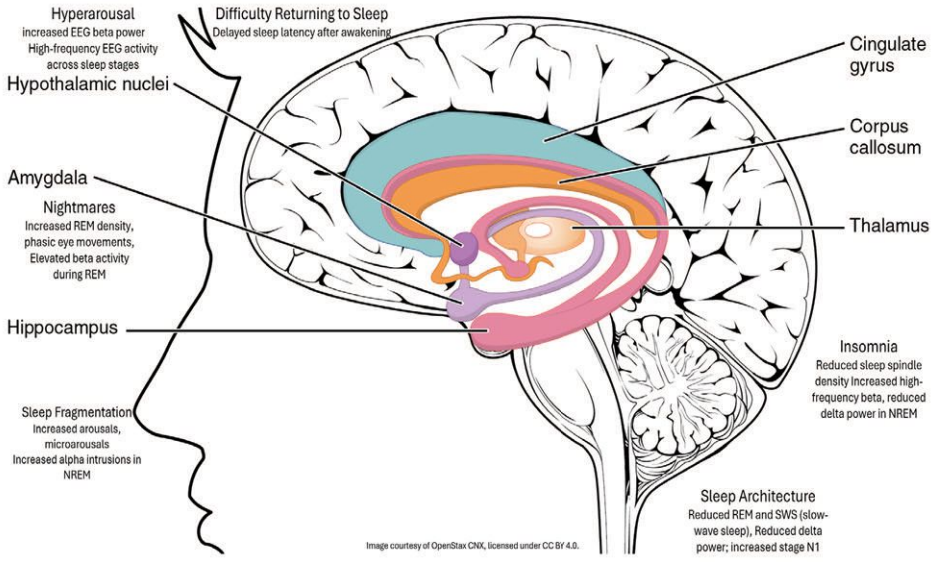


Figure 1. Brain regions implicated in PTSD-related sleep disturbances, highlighting areas associated with elevated electrophysiological markers. Regions include the amygdala, hippocampus, thalamus, prefrontal cortex, brainstem structures (including the pontine- tegmentum and reticular activating system), anterior cingulate cortex, hypothalamus.)

PTSD closely interacts with circadian dysregulation and sleep pathology. Studies show that PTSD patients experience fragmented REM sleep, reduced REM theta activity, and heightened sympathetic tone, which drive insomnia, nightmares, and hyperarousal.[20] Early post-trauma sleep problems such as insomnia and REM fragmentation predict later PTSD by disrupting extinction memory consolidation.[21] Pre-deployment short sleep and nightmares significantly raise the risk of PTSD in service members.[22] PTSD symptoms related to sleep disturbances are typically classified into the following categories:

Nightmares

Recurrent distressing dreams where the content or emotions are related to the traumatic event. These nightmares often cause significant distress and can disrupt sleep patterns.[23] Mysliwiec and colleagues studied four young US Army Soldiers with trauma-related sleep disturbances. They found disruptive nocturnal behaviors, nightmares, and REM without atonia. Polysomnography confirmed REM without atonia in all patients, and one showed both a nightmare and disruptive behavior during REM sleep.[24] According to the Melynda D. Casement, a meta-analysis study found that this therapy significantly reduces nightmare frequency, enhances sleep quality, and alleviates PTSD symptoms, with effects lasting up to 12 months.[25] Previous study shows that cognitive-behavioral treatment (CBT) effectively reduces chronic nightmares in trauma-exposed adults. After treatment, 84% of participants reported no nightmares within the past week and experienced significant improvements in depression, posttraumatic stress, sleep quality, and quantity.[26] Phelps and colleagues found that nightmares occurred in both REM and non-REM sleep, were linked to increased heart rate and were frequently associated with respiratory or leg movement disturbances.[27] Anne Germain compared sleep disturbances between posttraumatic nightmare (P-NM) sufferers, idiopathic nightmare (I-NM) sufferers, and healthy controls. They found that P-NM sufferers experienced significantly more nocturnal awakenings compared to both I-NM sufferers and controls, supporting the idea of increased sleep hyperarousal in PTSD. Both P-NM and I-NM sufferers exhibited elevated periodic leg movements (PLMs) during REM and non-REM sleep. This suggests that while PLMs are common in both groups, they might not be specific to PTSD but rather related to the intensity of negative dreaming.[28] Thordardottir et al. examined avalanche survivors 16 years post-trauma and found lasting sleep disturbances. Childhood exposure predicted dream-enactment behaviors, while adulthood exposure predicted trauma-related nightmares. The study shows PTSD-related sleep problems persist long-term and vary with age at trauma exposure.[29] In PTSD, heightened activation in the temporo-parieto-occipital junction and ventrolateral prefrontal cortex during REM sleep—regions crucial for mental imagery—combined with limbic system hyperactivation, may underlie the vivid and intrusive nature of trauma-related nightmares.[30-32]

Insomnia

Although no single quantitative marker defines insomnia disorder, Lichstein et al. (2003) outlined key thresholds—such as sleep latency or wake after sleep onset >30 minutes, sleep efficiency <85%, and total sleep time <6.5 hours—as common features when paired with persistent sleep complaints. Their work provided foundational quantitative criteria still used to support the clinical diagnosis of chronic insomnia.[33] Laura D. Straus's study highlights that veterans with PTSD experience notably worse sleep efficiency and greater variability in sleep patterns compared to individuals with primary insomnia. This variability is evident both within individuals across nights and between individuals, emphasizing the need for personalized treatment strategies for PTSD-related sleep disturbances.[34] The study by Virginia Lewis investigates whether sleep disturbances are specific to veterans with PTSD or if they are a broader issue among veterans. This pilot study involved 152 Vietnam War veterans, 87 of whom did not meet PTSD criteria. Findings reveal that both PTSD and non-PTSD veterans reported clinically significant sleep disturbances, suggesting that poor sleep is a common issue across the veteran population.[35] Raymond C. Rosen's study investigated the relationship between PTSD severity and insomnia-related sleep disturbances in a large, gender-balanced cohort of combat-exposed veterans. The study found that a significant proportion of veterans with PTSD experienced insomnia-related issues and that sleep problems at the beginning of the study predicted worsening PTSD severity over time. The study highlighted the high prevalence of sleep issues among veterans, emphasizing the need for targeted clinical attention and effective management of sleep disturbances in this population.[36] Riemann and colleagues proposed

a hyperarousal model which describe insomnia as a "final common pathway" resulting from an imbalance between arousing and sleep-promoting brain activity, shaped by stress, maladaptive behaviors, and cognitive tendencies such as rumination and worry.[37] Despite controlling for depression, individuals with insomnia in PTSD exhibit intact amygdala reactivity to arousal but show blunted responses to emotional valence and heightened sensitivity to insomnia-related stimuli, highlighting a specific affective processing bias.[38-41]

Sleep Fragmentation

Frequent awakenings during the night, leading to non-restorative sleep. This is often associated with hypervigilance, or intrusive thoughts related to trauma. The study by Anne Germain found that early-life traumatic experiences were linked to increased fragmentation of REM sleep. This REM sleep fragmentation, in turn, was associated with more disruptive nocturnal behaviors in later life.[42] Oton's study found that military veterans with PTSD spend less time in REM and deep sleep (<1 Hz) and more time in higher-frequency deep sleep compared to controls. The study also revealed that PTSD patients experience greater sleep fragmentation. These findings underscore significant alterations in sleep architecture related to PTSD and highlight the effects of medications and psychological factors on sleep quality.[42,43] Calhoun and colleagues studied sleep disturbances in women with PTSD using actigraphy over three nights in their home environment. They found that PTSD patients had significantly poorer sleep efficiency, longer sleep latency, and more restless sleep compared to controls.[44] Initiation insomnia, sleep fragmentation, and nightmares, common in PTSD, are all potential barriers to Obstructive sleep apnea (OSA). Data from patients with OSA in the absence of PTSD have shown that respiratory indices and sleep fragmentation.[45,46] Poor sleep quality is significantly associated with reduced cortical thickness in frontal and cingulate regions among veterans with mTBI with PTSD, suggesting sleep disruption may be a key neurobiological target in addressing brain structure changes linked to trauma-related injuries.

Difficulty Returning to Sleep

Difficulty falling asleep, staying asleep, or waking too early is common in PTSD, often driven by hyperarousal and trauma-related anxiety. Nightmares and anxiety can prolong wakefulness after awakenings, further disrupting sleep. In a double-blind, randomized, placebo-controlled trial, Thomas A. Mellman and colleagues showed that trauma-exposed individuals with early PTSD symptoms had more wakefulness after sleep onset and fragmented REM sleep, marked by shorter and more frequent REM periods. These findings suggest that early REM sleep disruption may contribute to PTSD developments.[47] Anne Germain highlights the critical role of sleep in PTSD, building on Ross and colleagues' hypothesis that sleep disturbances, particularly REM sleep, are central to PTSD. While evidence from human studies supports the link between sleep and PTSD-related emotion and memory processing, definitive biological mechanisms remain unclear. Germain points out that individual differences in REM sleep may influence stress responses, but the impact is likely broader, encompassing general sleep disturbances. This underscores the importance of sleep in both the manifestation and treatment of PTSD, suggesting a need for further research to clarify these mechanisms and improve clinical approaches.[8,48] Hananyah Glaubman and colleagues studied the sleep patterns of seven chronic PTSD patients compared to seven matched controls. They found that PTSD patients experienced poorer sleep, characterized by reduced sleep efficiency, increased awakenings, decreased slow-wave sleep (SWS), and longer REM latency. These sleep disturbances correlated with the patients' self-reported sleep complaints, supporting the inclusion of sleep difficulties in the PTSD diagnosis.[49] Delahanty conducted a landmark meta-analysis revealing objective sleep abnormalities in PTSD, including increased Stage 1 sleep and decreased slow-wave sleep, firmly establishing that PTSD involves measurable disruptions in sleep architecture beyond subjective reports.[50]

Altered Sleep Architecture

Changes in the structure of sleep, such as reduced REM sleep or disruptions in sleep stages, are often observed in PTSD patients. These alterations can contribute to the persistence of symptoms like nightmares and poor sleep quality.[51] Ihori Kobayashi and colleagues performed a meta-analysis of 20 polysomnographic studies to address inconsistencies in sleep findings among PTSD patients. Their review found that PTSD patients exhibit increased stage 1 sleep, reduced slow-wave sleep, and higher rapid-

eye-movement (REM) density compared to controls. Exploratory analyses indicated that factors such as a higher proportion of male participants and lower rates of comorbid depression were associated with more pronounced sleep disturbances in PTSD. The study highlights persistent sleep abnormalities in PTSD and suggests that variability in previous findings may be influenced by moderating variables like age, sex, and comorbid conditions.[2] In a community-based polysomnographic study, Naomi Breslau and colleagues found no significant overall sleep disturbances in individuals with lifetime PTSD compared to controls. However, PTSD patients exhibited increased brief arousals from REM sleep and more frequent transitions to lighter sleep or wakefulness during REM. This suggests that sleep complaints in PTSD may reflect heightened sensitivity to brief REM arousals rather than widespread sleep issues.[52] In a study by Mitsunari Habukawa and colleagues, young adult PTSD patients without substance dependence or personality disorders displayed significantly poorer sleep compared to controls. They experienced reduced sleep efficiency due to increased wake time after sleep onset and more frequent awakenings from REM sleep. The severity of trauma-related nightmares positively correlated with the percentage of REM interruptions and wake time after sleep onset, highlighting the impact of nightmares on sleep disturbances in PTSD.[53] Thomas A. Mellman and colleagues investigated the relationship between REM sleep and PTSD symptoms shortly after trauma. Their study found that shorter REM segment duration correlated negatively with initial PTSD and insomnia severity, and total sleep time was also affected. Additionally, increased beta frequency during REM sleep was linked to greater PTSD and nightmare severity. These results suggest a connection between subjective PTSD symptoms and REM sleep disturbances shortly after traumatic events.[54] Brian E. Engdahl and colleagues examined sleep patterns in elderly war veterans with and without PTSD. They found that veterans with PTSD had significantly higher percentages of REM sleep and fewer arousals from non-REM sleep compared to those without PTSD. Despite these objective findings, PTSD veterans reported poorer sleep quality than their non-PTSD counterparts. The study suggests that PTSD-related sleep disturbances may lessen over time, especially when comorbid sleep disorders are accounted for.[55] Sanford et al. (2014) conducted a landmark meta-analysis of 34 studies, revealing that PTSD is consistently associated with polysomnographic disruptions—especially reduced total sleep time, slow-wave sleep, and sleep efficiency—highlighting age, sex, and PTSD severity as key modulators of sleep architecture.[56]

Hyperarousal

Persistent heightened state of alertness that can interfere with the ability to relax and initiate sleep. This can manifest as an exaggerated startle response or feeling constantly "on edge," further complicating sleep. Steven H. Woodward and colleagues studied hyperarousal in PTSD by examining heart rate and EEG spectral power during sleep in 56 unmedicated Vietnam veterans with PTSD and 14 controls. They found no group differences in heart rate, but PTSD patients showed reduced slow-wave EEG power during NREM sleep and elevated beta power during REM. PTSD patients also displayed altered REM–NREM EEG power relationships and an inverse correlation between REM–NREM heart rate difference and REM percentage. These findings suggest distinct central arousal mechanisms in PTSD and highlight the need for further investigation into sleep-related neurophysiology.[2] Peretz Lavie and colleagues studied 12 chronic war-related PTSD patients and 12 controls to explore sleep disturbances. They found that PTSD patients had significantly higher awaking thresholds from REM sleep compared to controls. Despite similar overall sleep data between groups, higher awaking thresholds were positively correlated with increased depression and anxiety. Patients' dreams were notably more aggressive and hostile, with some including explicit war-related content. The severity of PTSD symptoms was associated with higher aggression in dreams and poorer sleep quality. This suggests that elevated awaking thresholds are a distinct feature of chronic PTSD.[57] Thomas A. Mellman and colleagues investigated sleep disturbances in combat veterans with PTSD. They found that recurrent awakenings, threatening dreams, and thrashing movements were common symptoms. Objective data revealed longer periods of wakefulness, frequent micro-awakenings, and increased body and limb movements during sleep. Symptomatic awakenings, often associated with startle or panic, were disproportionately preceded by REM sleep. These findings highlight that PTSD is characterized by heightened arousal during sleep,

Table 1. Sleep Disturbances and Corresponding Drug Therapies for PTSD-Related Sleep Disturbances

Sleep Disturbance	Drug Treatment	Mechanism of Action	References
Nightmares	Prazosin	a_1-Adrenergic receptor antagonist; reduces noradrenergic activity	62,73-77
	Clonidine	a_2-Adrenergic agonist; reduces sympathetic outflow	78-81
	Doxazosin	a_1-Adrenergic blocker	82
	Trazodone	Serotonin antagonist and reuptake inhibitor	83,84
	Risperidone, Olanzapine	Atypical antipsychotics; dopamine (D2) and serotonin (5-HT2) antagonists	85-87
Insomnia	Eszopiclone	Non-benzodiazepine hypnotic; GABA-A receptor agonist	65,88
	Zolpidem	Non-benzodiazepine hypnotic; GABA-A receptor agonist	89,90
	Trazodone	Serotonin antagonist and reuptake inhibitor	91,92
	Suvorexant (dual orexin receptor antagonist)	Orexin receptor antagonist	71
Sleep Fragmentation	Prazosin	a_1-Adrenergic blocker	93
	Lorazepam, Midazolam	Benzodiazepines; GABA-A receptor agonists	94
Difficulty Returning to Sleep	Prazosin, Clonidine	a_1/a_2-Adrenergic agents	79,95,96
	Trazodone	Serotonin antagonist and reuptake inhibitor	92
Sleep Architecture	Phenobarbital (historical)	Barbiturates; CNS depressants	97,98
	Lorazepam	Benzodiazepines	94
	Midazolam	Short-acting benzodiazepine	94
	Eszopiclone, Zolpidem	Non-benzodiazepine hypnotics	
	Suvorexant	Orexin receptor antagonists	71
Hyperarousal	Olanzapine, Risperidone	Atypical antipsychotics; D2 and 5-HT2 antagonists	99,100
	Prazosin, Clonidine, Doxazosin	a_1/a_2-Adrenergic agents	78,101,102
	Ketamine	NMDA receptor antagonist	103,104
	Venlafaxine	Serotonin-norepinephrine reuptake inhibitor	105,106
	Sertraline	Selective serotonin reuptake inhibitor	107

contributing to disrupted sleep patterns and increased physiological reactivity.[58] Hyperarousal in PTSD sleep disturbances is driven by overactivation of the amygdala, locus coeruleus, and hypothalamus, leading to heightened noradrenergic tone, elevated EEG beta activity, and disrupted sleep regulation.[30,59-61]

Clinical Treatments

Among available treatments, prazosin has shown the most consistent efficacy in reducing trauma-related nightmares and improving sleep quality by attenuating noradrenergic hyperactivity.[62] Barbiturates, while historically used for managing intracranial pressure, have been shown to suppress REM sleep and worsen sleep architecture upon withdrawal, limiting their clinical use for PTSD-related sleep disorders.[63] Benzodiazepines, including lorazepam, are commonly prescribed but pose risks such as REM suppression, memory impairment, and increased suicidal ideation; midazolam appears to have a more favorable safety profile.[63,64] Adjunctive agents like eszopiclone showing superior effects on sleep maintenance.[65] Trazodone has shown utility in treating insomnia and nightmares, though side effects such as priapism can limit tolerability.[66] Clonidine and doxazosin, both alpha-adrenergic blockers, have also shown effectiveness

in reducing nightmares in case reports and open-label studies.[67-69] More recently, ketamine has shown rapid antidepressant and anti-PTSD effects, with improvements observed within 24 hours post-infusion and sustained over several weeks.[70] Emerging therapies like dual orexin receptor antagonists (DORAs) offer potential benefits for sleep and mood regulation without worsening psychiatric symptoms. Currently, the U.S. Department of Defense is sponsoring a multi-year clinical trial evaluating daridorexant (marketed as QUVIVIQ) for PTSD treatment. This study aims to assess daridorexant's impact on PTSD symptom severity and insomnia over a 12-week period, with results anticipated in the coming years. These investigations highlight the potential of DORAs to address sleep and PTSD symptoms, though further research is necessary to determine their efficacy and optimal application in PTSD therapy.[71,72]

Future Directions

Current evidence highlights the complex relationship between PTSD and sleep disturbances, with insomnia and nightmares being among the most prevalent and debilitating symptoms. While traditional pharmacological and behavioral treatments provide partial relief, emerging therapies offer promising alternatives. Preliminary studies suggest that improving sleep quality in PTSD may also alleviate core psychiatric symptoms, supporting an integrated treatment approach. However, limitations in sample size, study design, and long-term data necessitate further large-scale, well-controlled trials. Continued research targeting sleep as a therapeutic entry point may significantly advance PTSD management and improve overall patient outcomes. Thus, this review provides the basic clinical studies underlying the PTSD-related sleep disturbances.

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Sexual Dream Frequency and Self-Reported Cardiovascular Morbidity: Findings From the SLEEP Study

by Emmanuel Eroume A Egom MD, PhD

This study identifies a significant association between sexual-dream frequency and self-reported cardiovascular morbidity.



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Abstract

Background

Dreams often evoke autonomic arousal, yet the relationship between dream patterns and cardiovascular health has rarely been examined.

Methods

In this cross-sectional SLEEP Study, 301 adults recruited via the Prolific research platform completed an online questionnaire assessing sexual-dream frequency, post-dream symptoms, and self-reported cardiovascular history. Associations between sexual-dream frequency and self-reported cardiovascular outcomes were examined using chi-square tests and multivariable logistic regression. The primary multivariable model evaluated self-reported heart issues after adjustment for age, smoking, depression, anxiety, and medication use.

Results

The most commonly reported post-dream symptoms were increased heart rate (57%), sweating (35%), and anxiety (34%). Compared with rare sexual dreams (≤ once per year), sexual dreams occurring a few times per month were associated with higher odds of self-reported heart issues (OR 3.03, 95% CI 1.38–6.67; p = 0.005), and dreams occurring a few times per week were associated with still higher odds (OR 6.62, 95% CI 1.64–26.7; p = 0.004).

Conclusions

In this sample, sexual-dream frequency was associated with self-reported heart issues. Prospective studies using objective cardiovascular outcomes are needed before clinical application.

Keywords

Sleep, dreams, cardiovascular disease, psychosomatic cardiology, screening.

Introduction

Sleep is a fundamental aspect of human health, influencing both physical, mental and spiritual well-being.[1,2] While much is known about the restorative functions of sleep, the content and implications of our dreams, particularly sexual dreams, remain underexplored.[2-8] Sexual dreams, which occur across cultures and demographics, are often dismissed as mere nocturnal phenomena.[2,9-13] However, recent evidence suggests that the frequency and intensity of these dreams may be linked to underlying health conditions, particularly those affecting the cardiovascular system.

The connection between sleep and heart health has long been

established, with sleep disturbances like obstructive sleep apnea and insomnia being recognized risk factors for cardiovascular disease.[14-18] Dreams, as a byproduct of sleep, are also influenced by physiological states, yet the potential relationship between specific types of dreams—such as sexual dreams—and heart health has not been fully investigated.[2] This study seeks to bridge that gap by examining whether the frequency of sexual dreams could serve as an early indicator of cardiovascular issues, particularly heart disease.

Sexual dreams are nearly universal, but their physiological and emotional aftermaths can vary.[2-4,6-17,19] For some, these dreams are accompanied by physical symptoms like increased heart rate, sweating, and anxiety—symptoms that may mirror those seen in individuals with heart-related conditions. These common physical responses to sexual dreams raise a compelling question: could frequent sexual dreams be reflective of underlying cardiovascular health problems?

The aim of this study is to explore the potential link between sexual dream frequency and heart health, focusing on two key cardiovascular outcomes: general heart issues and coronary artery disease. By leveraging a large and diverse dataset, this research seeks to determine whether individuals who frequently experience sexual dreams are more likely to report heart-related symptoms or conditions. In doing so, we hope to contribute to the growing body of knowledge on the intersections between sleep, dream content, and cardiovascular health.[2-4,6-17,19]

This study is one of the first to investigate the potential physiological implications of sexual dreams, offering a novel perspective on how our nocturnal experiences might reflect broader health patterns.[2-4,6-17,19] Given the increasing prevalence of cardiovascular disease globally, identifying unique and overlooked predictors of heart health could have significant implications for early diagnosis and intervention.

Methods

Study Design, Recruitment, and Participants

This investigation was part of the SLEEP Study (Sexual Dreams, Longitudinal Effects, and Emotional and Physical Impact), a cross-sectional survey designed to examine associations between sexual-dream frequency and self-reported cardiovascular morbidity.[20] Companion analyses from the same SLEEP Study cohort have previously described the prevalence and patterns of sexual dreams and their associations with mental health; the present report focuses specifically on self-reported cardiovascular morbidity.[20,21] Participants were recruited through Prolific, an online research platform widely used in behavioral research and recognized for data quality and transparent participant compensation.[22-24] Eligibility criteria included age 18 years or older, English fluency, and US residence.

A total of 301 participants completed the questionnaire. Recruitment followed a convenience-sampling approach typical of online psychosomatic and behavioral research. All participants provided electronic informed consent before participation. The study was anonymous, minimal risk, and collected no directly identifying information.

Participants were compensated according to Prolific's ethical payment framework, equivalent to approximately USD 15 per hour. The final dataset included demographic characteristics, smoking status, relationship and mental-health variables, and self-reported cardiovascular history.

Procedure and Measures

Participants completed a structured online questionnaire assessing four domains:

1. sexual-dream experience and frequency;
2. post-dream physical and emotional symptoms;
3. perceived effects on mood, behavior, and relationships; and
4. health and lifestyle factors, including cardiovascular history, smoking, and mental-health treatment.

The primary predictor was sexual-dream frequency, categorized with "Rarely (≤ once per year)" as the reference group. The primary outcomes were self-reported "heart issues" and coronary artery disease.

Statistical Analysis

Analyses were performed in Python. Descriptive statistics summarized participant characteristics and symptom frequencies. Associations between sexual-dream frequency and cardiovascular outcomes were first examined using chi-square tests.

Binary logistic regression was then used to evaluate the relationship between sexual-dream frequency and self-reported heart issues. The unadjusted model assessed the crude association, and the adjusted model included age, smoking status, self-reported depression, anxiety, and medication use as covariates selected a priori. Odds ratios with 95% confidence intervals were derived from model coefficients. Statistical significance was defined as $p < 0.05$.

Participants were recruited through a platform that enforces research ethics standards. Given the anonymous,

Table 1. Demographic, lifestyle, and cardiovascular characteristics of participants (N = 301) in the SLEEP Study. Percentages are calculated within valid responses. Continuous variables are expressed as mean ± standard deviation. "Heart issues" represent a composite outcome encompassing any self-reported cardiovascular symptom, diagnosis, or procedure.

Table 1. Sample Characteristics of the Study Population (N = 301)

Characteristic	n (%) or Mean ± SD
Age (years)	39.5 ± 12.3 (19–104)
Gender identity	
- Woman (incl. Trans Woman)	271 (90.0)
- Non-binary / Gender diverse	30 (10.0)
Ethnicity	
- White	203 (67.4)
- Black / African American	41 (13.6)
- Hispanic / Latino	26 (8.6)
- Asian	19 (6.3)
- Mixed / Other	12 (4.1)
Highest education completed	
- High school or equivalent	41 (13.6)
- Undergraduate degree	136 (45.2)
- Graduate / Doctoral degree	108 (35.9)
- Other / not stated	16 (5.3)
Smoking status	
- Never smoker	202 (67.1)
- Former smoker	61 (20.3)
- Current smoker	38 (12.6)
Self-reported anxiety disorder	94 (31.2)
Self-reported depression	88 (29.2)
Reported "heart issues"*	108 (35.9)
Reported coronary artery disease (CAD)	23 (7.7)

* "Heart issues" includes participants who self-identified having chest pain on exertion, treatment for heart disease, or previous cardiovascular procedures.

minimal-risk design and absence of identifiable data, additional institutional ethics approval was not required under local policy. The questionnaire is provided in Supplementary Appendix A.

Results

The final dataset included 301 participants. Mean age was 39.5 ± 12.3 years. The sample was predominantly female-identifying (90%), with most participants identifying as White (67%); nearly half had an undergraduate degree. Anxiety and depression were each reported by approximately one-third of respondents, and 7.6% reported a prior diagnosis or procedure consistent with coronary artery disease.

Almost all respondents reported having experienced sexual dreams, indicating that such dreams were nearly universal in this sample. This is consistent with the companion sexual-medicine report from the same cohort, which found a lifetime prevalence of 99.67% and further characterized effects on mood, thoughts, behavior, and relationships.[21] The predominance of female participants likely reflects self-selection rather than recruitment restriction, as prior dream research suggests that women are more likely to volunteer for studies involving emotional or introspective content.[11,12]

The most commonly reported post-dream symptom was increased heart rate (57.38%), followed by sweating (34.97%), anxiety (33.88%), and muscle tension (22.95%). Approximately one-fifth reported no symptoms.

With respect to cardiovascular outcomes, 108 participants (35.9%) met the composite definition of self-reported heart issues and 23 (7.6%) reported coronary artery disease (Table 1). Sexual-dream frequency was significantly associated with self-reported heart issues (Pearson $\chi^2 = 23.68$, $p < 0.001$) and with coronary artery disease (Pearson $\chi^2 = 13.98$, $p < 0.001$) (Table 2).

In multivariable logistic regression predicting self-reported heart issues, more frequent sexual dreams remained independently associated with the outcome after adjustment for age, smoking, depression, anxiety, and medication use (Table 3). Compared with participants reporting sexual dreams rarely (≤ once per year), those reporting dreams a few times per month had higher odds of self-reported heart issues ($\beta = 1.11$; OR 3.03, 95% CI 1.38–6.67; $p = 0.005$), and those reporting dreams a few times per week had still higher odds ($\beta = 1.89$; OR 6.62, 95% CI 1.64–26.7; $p = 0.004$). Figure 1 presents the adjusted model graphically.

Discussion

This study found a significant association between sexual-dream frequency and self-reported heart issues, the primary modeled cardiovascular outcome. In the adjusted model, compared with rare sexual dreams, dreams occurring a few times per month, and a few times per week were each associated with higher odds of the heart-issues composite. These findings suggest that sexual-dream frequency may reflect a psychophysiological correlate of cardiovascular vulnerability. A companion analysis of the same cohort also found that frequent sexual dreams were associated with self-reported mental health diagnoses, supporting the possibility that these dream experiences reflect broader psychophysiological stress rather than an isolated sleep phenomenon.[20]

Table 2. Cross-tabulations of sexual-dream frequency with self-reported cardiovascular outcomes in the SLEEP Study (N = 301). Values are n (row %). Pearson χ^2 and p values are reported separately for self-reported heart issues and coronary artery disease.

Table 2. Crosstabulation of sexual-dream frequency with self-reported cardiovascular outcomes

Sexual-dream frequency	Heart issues n (%)	No heart issues n (%)	CAD n (%)	No CAD n (%)
Rarely (≤ once per year)*	18 (17.8)	83 (82.2)	4 (4.0)	97 (96.0)
Frequently (a few times / month)	77 (43.3)	101 (56.7)	13 (7.3)	165 (92.7)
Very frequently (a few times / week)	13 (59.1)	9 (40.9)	6 (27.3)	16 (72.7)
Total (N = 301)	**108 (35.9)**	**193 (64.1)**	**23 (7.6)**	**278 (92.4)**

Note. Percentages are within rows. CAD = coronary artery disease. Pearson's χ^2 tests: heart issues, $\chi^2 = 23.68$, $p < 0.001$; CAD, $\chi^2 = 13.98$, $p < 0.001$. * One participant reporting "Never" was folded into the rare reference category, so the table aligns with the manuscript's three-level frequency coding.

Table 3. Binary logistic-regression models predicting self-reported heart issues (N = 301). Reference category = "Rarely (≤ once per year)." OR = e^β. Adjusted model includes age, smoking, depression, anxiety, and medication use.

Table 3. Binary logistic-regression models predicting self-reported heart issues

Predictor	β	SE	OR (95 % CI)	p
Unadjusted model				
Frequently (a few times / month)	1.23	0.36	3.41 (1.67–6.95)	0.001
Very frequently (a few times / week)	1.96	0.66	7.10 (2.00–25.1)	0.003
Adjusted model*				
Frequently (a few times / month)	1.11	0.40	3.03 (1.38–6.67)	0.005
Very frequently (a few times / week)	1.89	0.72	6.62 (1.64–26.7)	0.004
Age (years)	0.02	0.01	1.02 (1.00–1.04)	0.046
Current smoker (vs non-current smoker)	0.58	0.34	1.79 (0.91–3.54)	0.094
Depression (yes vs no)	0.74	0.30	2.09 (1.15–3.81)	0.016
Anxiety (yes vs no)	0.42	0.28	1.52 (0.88–2.62)	0.130
Medication use (yes vs no)	0.67	0.33	1.95 (1.02–3.73)	0.043

Reference category = "Rarely (≤ once per year)." OR = e^β. Adjusted model includes covariates age, smoking, depression, anxiety, and medication use.

To our knowledge, few studies have explored the relationship between sexual dreams and cardiovascular outcomes. Our findings are nonetheless consistent with adjacent literature showing that emotionally intense dream experiences, particularly nightmares, are associated with cardiovascular symptoms, hypertension, and adverse cardiac outcomes.[25-29] Together, these studies suggest that dream-related phenomena may reflect underlying autonomic, emotional, or sleep-related processes relevant to cardiovascular health.

Several mechanisms may explain the observed association. First, sexual dreams can evoke autonomic arousal, including increased heart rate, sweating, and anxiety, all of which were frequently reported in this cohort. This interpretation is reinforced by a focused symptom analysis from the same dataset, in which increased heart rate, sweating, anxiety, and muscle tension were the dominant post-dream autonomic-like experiences.[30] Repeated nocturnal arousal may reflect or amplify sympathetic activation in vulnerable individuals.[31-36] Second, frequent sexual dreams may be a marker of disturbed or fragmented REM sleep. REM dysregulation has been linked to elevated nocturnal sympathetic tone, blood-pressure variability, and adverse cardiovascular physiology.[2,37-43] Third, hormonal factors may contribute. Sexual dream patterns may be influenced by stress-related or sex-related hormonal fluctuations, which themselves may intersect with cardiovascular risk.[2,44-55] Finally, medication effects may be relevant, as some cardiovascular and neuropsychiatric medications can alter dream vividness and REM sleep.[2,56-60]

An additional clinically relevant finding was the large proportion of participants who were either uncertain of their cardiovascular status or symptomatic without a diagnosis. More than one-third had never been assessed or were unsure of their heart health, and 16.2% reported exertional chest pain without formal diagnosis. This raises the possibility that dream-related questions might serve as low-cost adjunctive prompts during broader cardiovascular or psychosomatic screening, especially in preventive settings.

At the same time, these findings should be interpreted with caution. The study was cross-sectional and relied on self-reported dream frequency and self-reported cardiovascular outcomes. Established cardiovascular determinants such as hypertension, diabetes, dyslipidemia, obesity, physical activity, diet, alcohol use, family history, and sleep disorders were not comprehensively assessed. Sexual-dream frequency is therefore unlikely to function as a stand-alone diagnostic tool; rather, it may represent one component of a broader biopsychophysiological profile. Future studies should determine whether this signal adds information beyond conventional risk factors in independent cohorts.

This study has several limitations. First, the cross-sectional design precludes causal inference. It is not possible to determine whether frequent sexual dreams

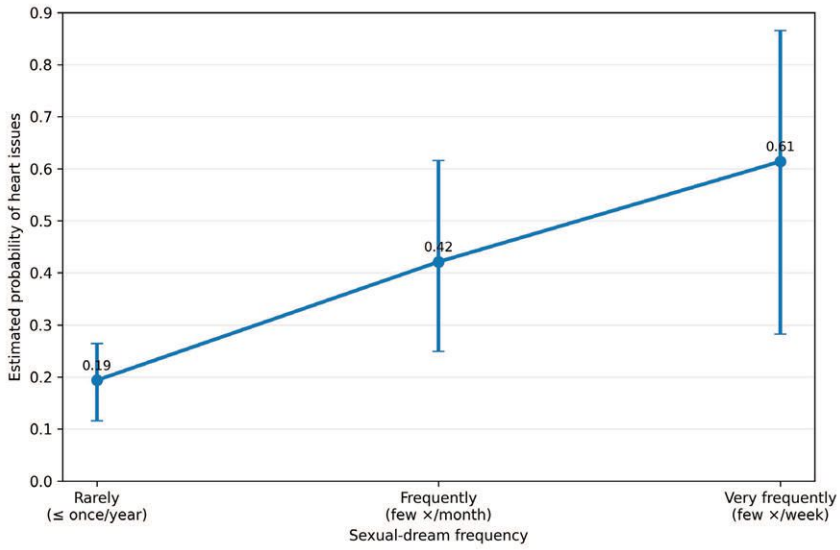


Figure 1. Adjusted estimated probability of self-reported heart issues by sexual-dream frequency. Error bars indicate approximate 95% confidence intervals.

precede cardiovascular symptoms, result from them, or simply co-occur due to shared mechanisms. Second, the study relied on self-reported dream frequency and self-reported cardiovascular morbidity, which introduces recall bias and possible misclassification. Third, the sample was predominantly female-identifying, limiting generalizability across genders. Fourth, the online convenience-sampling design may have introduced selection bias. Finally, relevant confounders such as diet, alcohol use, exercise, sleep duration, sleep apnea, and family history were not fully captured.

Despite these limitations, the study contributes a novel perspective to psychosomatic and sleep-related cardiovascular research. It suggests that dream frequency, particularly when associated with autonomic symptoms, may reflect broader physiological states relevant to cardiovascular health. Future studies should test this relationship in larger and more diverse cohorts, incorporate objective cardiovascular and sleep measurements, and determine whether dream-related variables add incremental value to established screening frameworks.

Conclusion

This study identified a significant association between sexual-dream frequency and self-reported cardiovascular morbidity. Individuals reporting more frequent sexual dreams were more likely to report heart-related symptoms or diagnoses, including coronary artery disease. Although causality cannot be inferred, the findings suggest that dream frequency may function as a behavioral or psychophysiological marker of cardiovascular vulnerability.

The association may reflect shared contributions from autonomic arousal, sleep disturbance, hormonal factors, medication effects, or broader psychosomatic stress processes. Because many participants were either unaware of their cardiovascular status or symptomatic without formal diagnosis, questions about dream patterns may have value as part of wider preventive or psychosomatic assessment.

Further research is needed to confirm these findings in prospective studies with objective cardiovascular endpoints and more comprehensive measurement of confounding factors. If validated, dream-related markers may enrich our understanding of the connections between sleep, emotional physiology, and cardiovascular risk.

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Supplementary Appendix A. SLEEP Study Questionnaire

Section 1. Demographics

1. Age (years): ____
2. Gender identity (choose one): Man (including Trans Man), Woman (including Trans Woman), Non-binary , Rather not say.
3. Ethnicity (select one): White, Black/African American, Asian, Hispanic/Latino, Mixed, Other, Prefer not to say.
4. Highest education level: No formal qualification, High school, Undergraduate degree, Graduate/Doctorate degree.
5. Employment status: Full-time, Part-time, Unemployed, Student, Other.

Section 2. Dream Experience

6. Have you ever had a sexual dream? Yes / No.
7. How often do you have sexual dreams? Never / Rarely (≤ once per year) / Frequently (a few times per month) / Very frequently (a few times per week).
8. What is the gender of individuals in your sexual dreams? Same sex / Different sex / Both / Prefer not to say.
9. Do you experience any physical or emotional symptoms after a sexual dream? Select all that apply: Increased heart rate, Sweating, Muscle tension, Anxiety, None, Other (specify ____).
10. Do sexual dreams affect your daily life? No / Yes—affect mood / Yes—thoughts or behavior / Yes—relationships / Other (specify ____).

Section 3. Health and Lifestyle

11. Do you currently smoke cigarettes? Never / Former / Current.
12. Have you been diagnosed with any mental health conditions (e.g., depression, anxiety, PTSD)? Yes / No / Prefer not to say.
13. Are you currently receiving mental-health treatment? None / Psychological therapy / Medication / Both / Other.
14. Do you have any chronic medical conditions (e.g., diabetes, hypertension, autoimmune disease)? Yes / No / Prefer not to say.

Section 4. Cardiovascular Health

15. Have you ever been diagnosed with a heart condition (e.g., heart failure, arrhythmia, CAD)? Yes / No / Unsure.
16. If Yes, please indicate which applies: Heart attack / Stent or bypass / Medication (e.g., statin, aspirin) / Implantable device / Other (specify ____).
17. Do you experience chest pain on exertion? Yes / No / Sometimes.
18. Have you had a cardiology check-up or stress test in the past year? Yes / No / Not sure.

Section 5. Spiritual and Lifestyle Factors (optional)

19. Do you participate in religious or spiritual activities? Public / Private / Both / None.
20. Do you believe your dreams carry personal or spiritual meaning? Yes / No / Unsure.

Ethics and Data Protection

Participation was voluntary and anonymous. No personal identifiers were collected. Participants could withdraw at any time without penalty. The study complied with Prolific's research ethics standards and data protection guidelines.

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Disclosure

No financial disclosure reported. Artificial intelligence, language models, machine learning or similar technologies were not used in the conceptualization, study, research, preparation or writing of this manuscript.

Ethics/Privacy

Anonymized, minimal-risk online survey; no identifiable data collected; formal institutional ethics approval not required per local policy. **MM**



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