SEPTEMBER / OCTOBER 2022 # 86

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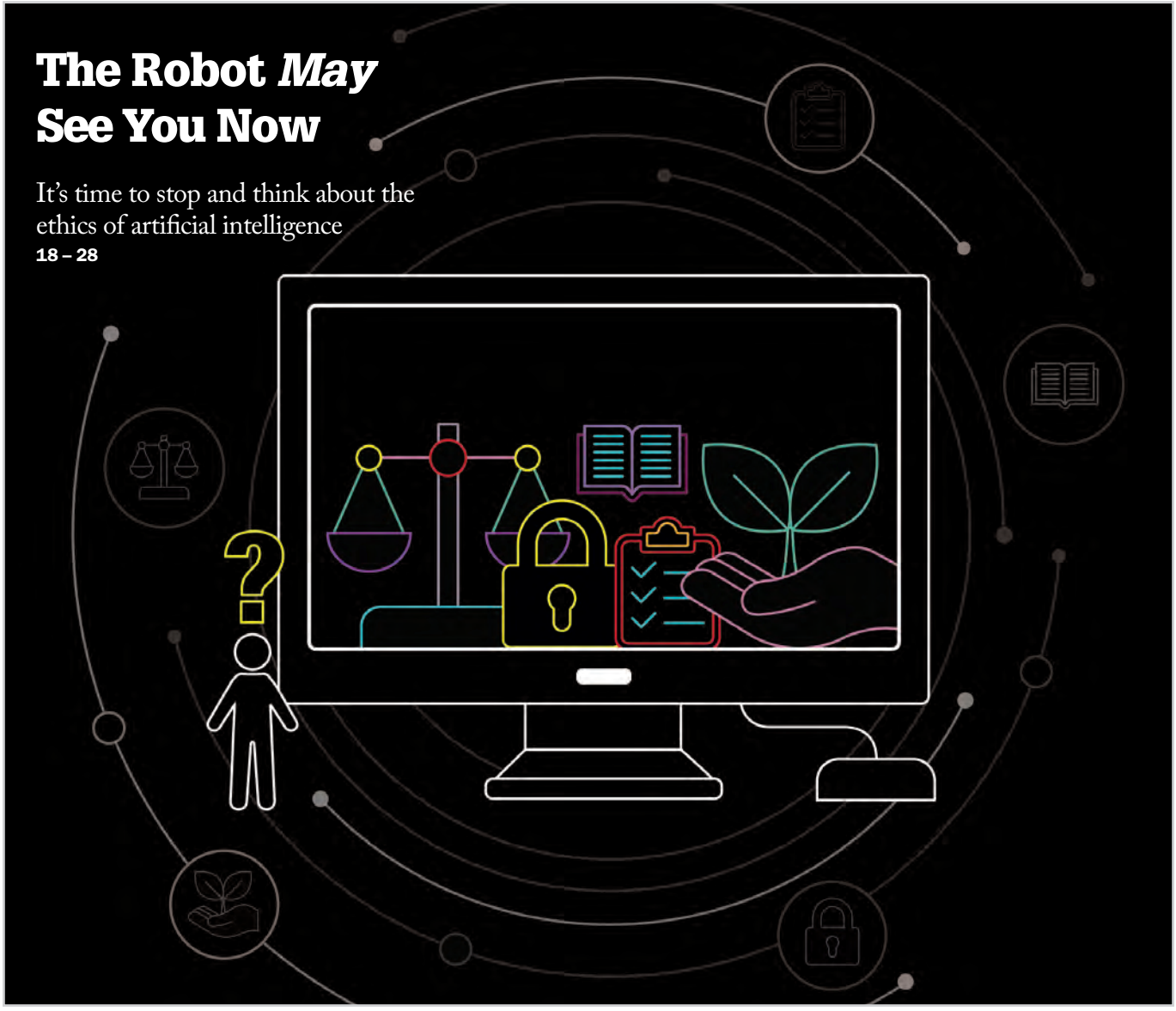
The Robot *May* See You Now

It's time to stop and think about the ethics of artificial intelligence
18 – 28



Confidently Drive the Future of Precision Cancer Care.

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AMP | Booth 1300

Corporate Workshop

November 2 | 1:00 pm | Room 229AB

Comprehensive Genomic Profiling for Tumor Micro-environment Assessment and Liquid Biopsy Analysis

Prashant K. Singh, Ph.D., Roswell Park Comprehensive Cancer Center, Buffalo, NY

Kyle Manning, Exosome Diagnostics, a Bio-Techne brand, Waltham, MA

Michael Ruvolo Ph.D., Agilent Technologies

Innovation Spotlight

November 3 | 1:20 pm | Innovation Spotlight Stage

Who Says You Can't Have It All: Rapid Comprehensive Molecular Profiling of Hematological Malignancies

Sean T. Glenn, Ph.D., Roswell Park Comprehensive Cancer Center, Buffalo, NY

Poster Presentation

Poster Number: ST033 | Abstract Number: 1303675

Development of a Modular Set of Hybridization-Capture Panels and Analysis Workflows for Tumor Molecular Characterization

Michael Ruvolo, Ph.D., Agilent Technologies

Live Demos

November 3 - 5 | Booth 1300

Visit the Agilent Booth for a Live Alissa Software Demonstration

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D73288/01

CAP | Booth 302

Quick PD-L1 IHC 22C3 pharmDx CPS Training Cases

October 9 | 10:30 am | 12:30 pm | 2:30 pm

October 10 | 10:30 am | 12:30 pm | 2:30 pm

Booth 302 – No registration needed.

Dr. Ronald Paler, CND Life Sciences

Virtual PD-L1Trainings

PD-L1 IHC 22C3 pharmDx CPS Scoring Session (3 hour)

October 20 | 10:00 am PT / 1:00 pm ET

Dr. Corrado D'Arrigo, Poundbury Cancer Institute for Personalized Medicine

November 17 | 10:00 am PT / 1:00 pm ET

Dr. Sunil Badve, Emory University School of Medicine

Scan here to register.

PD-L1 CPS Training Utilizing the Atlas of Stains (1 hour)

November 9 | 10:00 am PT / 1:00 pm ET

Dr. Allen Gown, University of British Columbia

Scan here to register.

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Trusted Answers

Choose Your Words Wisely

Using the power of good science communication to combat the rise of fake news and misinformation

A bold question: Being a scientist and a science communicator aren't mutually exclusive, but should they be?

Earlier this year, researchers in Australia identified a biomarker that could one day help identify newborns at risk of sudden infant death syndrome (SIDS) (1). Important? Absolutely. Groundbreaking to the point where SIDS "may soon be a thing of the past" as suggested in an accompanying press release (2)? Not quite. But that didn't stop many media outlets and social media users running with the "miracle finding" narrative. Don't get me wrong, I love a good catchy headline and gripping opening paragraph as much as the next writer, but that shouldn't come at the cost of sacrificing scientific or journalistic integrity.

As scientists, we're taught to critically appraise papers, avoid sensationalist messaging, and question everything we read. Media professionals with no science background may not be afforded the same training but, then again, media and communication training aren't typically covered in-depth (or at all) in most science degrees.

Those working in science or media have clear complementary skills that, if combined correctly, can deliver the most effective messaging. Scientists who struggle with science communication and simplifying complex topics should work with the media to create engaging messaging; likewise, journalists with no scientific background should be fact- (or sanity-) checking with experts to avoid sensationalist language and the risk of misleading readers.

The SIDS biomarker story appears to be a relatively harmless case of accidental misinformation, rather than a deliberate, malicious attempt to spread fake news. But what happens when the latter is the aim?

The process of scientific understanding unfolded in real time – and on a global stage – during the COVID-19 pandemic, which inevitably led to conflicting information from governments and health officials. Trust in experts, researchers, and healthcare providers unraveled in some camps, and allowed misinformation – and its nasty cousin, disinformation – to run rampant. If we have learned anything from the COVID-19 pandemic, it's that current anti-misinformation laws and regulations struggle to combat committed science-deniers and die-hard anti-vaxxers who go to great lengths to peddle their falsehoods.

Until more widespread disinformation laws (that actually work) are in force, using strong science–media collaborations to craft compelling but accurate communications could help avoid unintentional misinformation and dampen the impact of toxic fake news.

Liv Gaskill
Deputy Editor

See the full article online at: tp.txp.to/choose-wisely

References

1. *CT Harrington et al., EBioMedicine, 80, 104041 (2022). PMID: 35533499.*
2. *The Sydney Children's Hospital Network (2022). Available at: https://bit.ly/3NKtFKh.*

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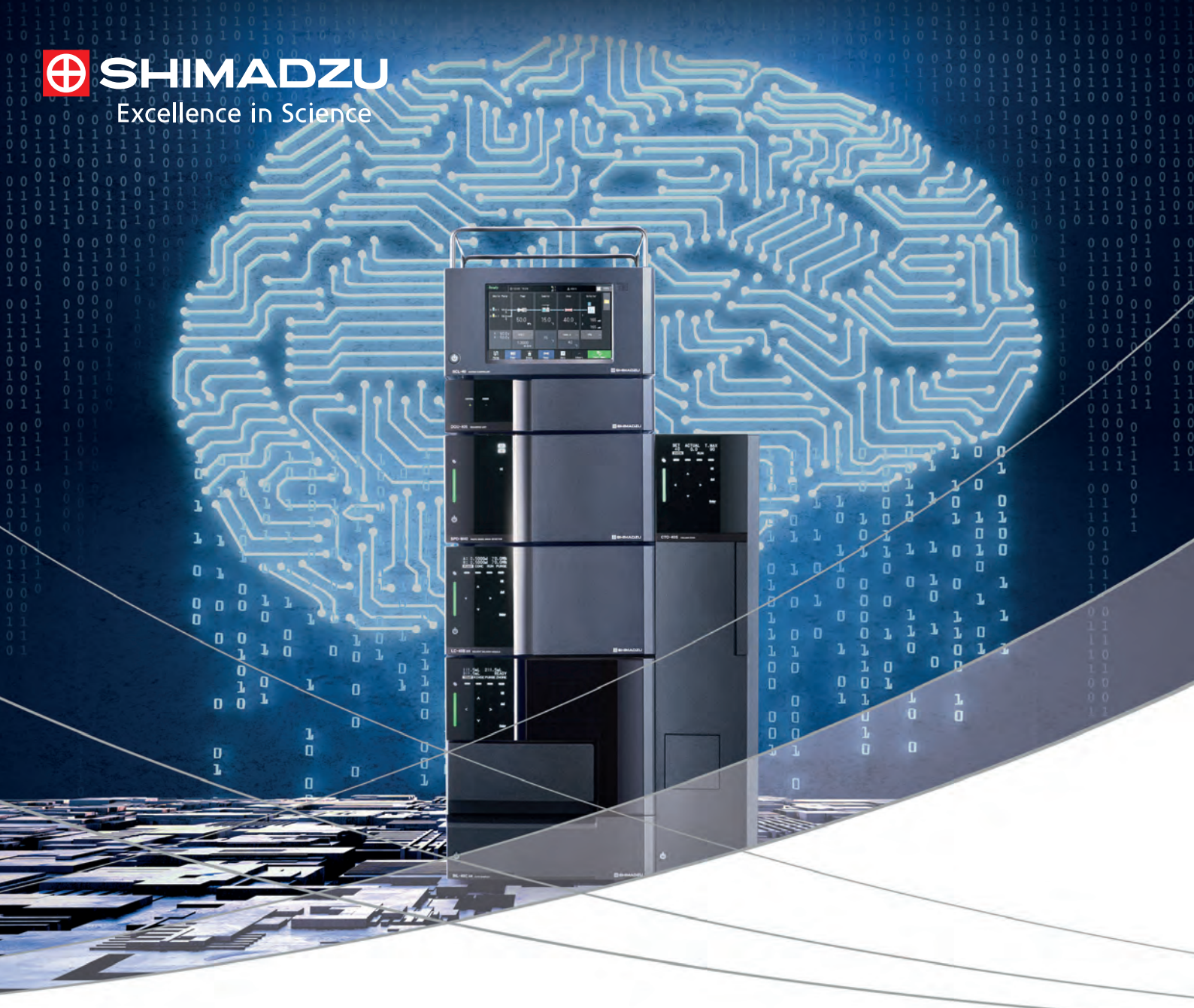

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ANALYTICAL INTELLIGENCE

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the Pathologist

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Getting to the Genetic Core of Autism

Investigating the genotype-phenotype relationship in autism to improve our understanding of the condition's heterogeneity

The core diagnostic criteria for autism rely on overtly measurable characteristics that impact daily functioning. But therein lies a problem – the criteria can lead to significant differences in phenotypic features, support needs, or co-occurring conditions in individuals with autism diagnoses. This heterogeneity may come from multiple sources, but research into genotype-phenotype associations has been limited to small sample sizes with core autism features, as opposed to underlying latent features that could further inform our understanding of these associations. Additionally, the impact of common genetic variants on co-occurring developmental disabilities is poorly understood relative to those of rare de novo mutations. Intellectual disability can affect sex differences in autism, but the impact of common variants in autistic individuals of either sex, with or without intellectual disability, remains unknown.

Recognizing these knowledge gaps, an international collaboration of researchers combined phenotypic data from autistic people to investigate genetic differences in features of autism, co-occuring developmental disabilities, and sex (1). They found an association between core factors and common, but not rare, genetic variants; they also identified a negative correlation between autism polygenic scores (PGS) and the likelihood of developmental disabilities. In those without co-occurring disability, PGS were inherited more in autistic females than in males, but the authors suggest this could be due to reduced single nucleotide polymorphism heritability of autism in females, meaning that higher PGS are needed to reach equivalent likelihood levels.

A second study highlights the negative correlation between genetic loads of rare and polygenic risk (with a greater impact in females) and the association between de novo mutations and symptom severity (2). Together, the studies demonstrate that characterizing the genotype-phenotype relationship can inform our understanding of heterogeneity in cognition, behavior, and co-occurring conditions in autistic individuals; however, deeper phenotyping at scale is needed along with an understanding of the evolving core diagnostic criteria.

References

1. *V Warrier et al., Nat Genet, [Online ahead of print] (2022). PMID: 35654973.*
2. *D Antaki et al., Nat Genet, [Online ahead of print] (2022). PMID: 35654974.*

Of Mice and MA10

Mice infected with the SARS-CoV-2 MA10 strain provide insight into long COVID-19

SARS-CoV-2 infection can lead to long-term consequences, but research into the mechanisms behind lung abnormalities associated with post-acute sequelae of SARS-CoV-2 (PASC) lack longitudinal tissue samples. Mice infected with the mouse-adapted MA10 strain suffer from acute respiratory distress syndrome similar to humans – providing researchers with a prime reference for studying PASC pathogenesis.

Researchers from the University of North Carolina at Chapel Hill studied MA10-infected mice and found that many chronic phenotypes seen at 15 days post-infection were also observed after a 120-day period (1). Furthermore, fibrotic pulmonary disease peaked at 15 days post-infection in young BALB/c and aged C57BL/6J mice, but waned by 30 days post-infection compared with aged BALB/c mice. Surviving mice also exhibited higher levels of pro-inflammatory and pro-fibrotic cytokines and, though most returned to normal after 30 days, there was prolonged up-regulation of TGF-β signaling in subpleural fibrotic regions.

See references online at: tp.txp.to/mice-and-ma10

Credit: DNA Image sourced from Pixabay.com

RESEARCH ROUNDUP

The hottest recent news in pathology – summarized!

Magnificent Yet Minute

The largest known bacterium – 50 times larger than previous record-holders – has been discovered in a marine sulfidic environment. The organism, *Candidatus Thiomargarita magnifica*, is a full centimeter in length. Its size is attributed to features of its cellular division and elongation mechanisms that allow it to surpass limits on growth (1).

A Tale of Africa

Genetic risk scores derived from data of African American individuals and multi-ancestry data perform better in sub-Saharan Africa than European-derived genetic risk scores (2). These African American-derived data enhance polygenic prediction of lipid traits, but accuracy varies between cohorts – specifically Ugandan and South African Zulu populations.

It's Alive!

A novel, nontoxic method for comprehensive longitudinal profiling can be applied to both dispersed cells and living tissue. Scission-accelerated fluorophore exchange (SAFE) removes immunofluorescent signals from the surfaces of labeled cells, enabling multiple rounds of staining of the same samples (3).

The House of Mouse

Researchers have assembled a high-quality map of the mouse proteome. The map is built from 17,883 proteins and 41 tissues using quantitative mass spectrometry-based methodology. The work substantially builds on systematic studies of genes and proteins in mouse tissues in years since 2002 (4).

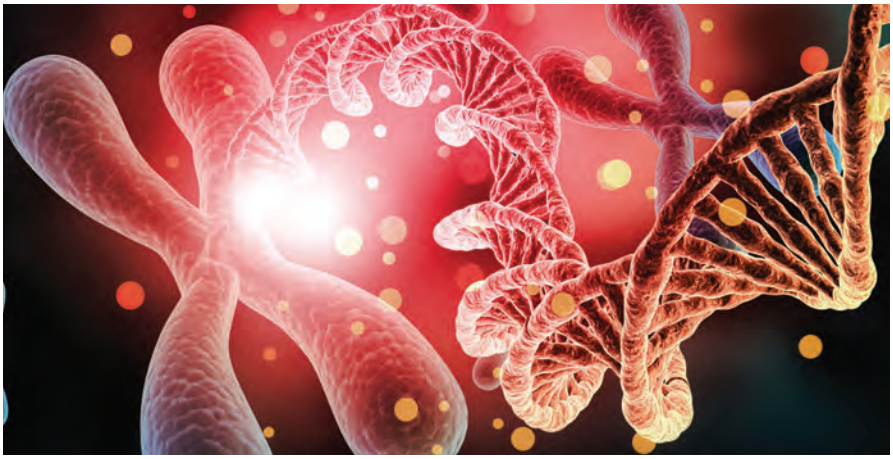
Don't Stop the Beat

Examining the genetics behind musical beat synchronicity in 606,825 individuals has shown that moving to a beat has a highly polygenic architecture, with 69 loci reaching genome-wide significance (5).

See references online at: tp.txp.to/gist-news

A Pan-Cancer Panopticon

Studies into pan-cancer omics are constantly pointing a spotlight on every potential avenue for better patient outcomes



Credit: Image sourced from Shutterstock.com

A key goal in oncology is to curtail mutations that improve cancer's ability to spread. So far, because modeling such variable mutation rates is no mean feat, most attempts to identify these types of mutations have focused on protein-coding sequences and specific noncoding elements. However, researchers have devised a novel method that uses deep neural networks to search for mutations across the cancer genome. Succinctly known as "Dig," the technique maps estimates of cancer mutations which are then refined through comparison with predicted mutation counts (1). So far, Dig has mapped mutation rates in 37 cancer types – and the data are even available for web-based exploration.

RNA studies are an equally fruitful avenue for pan-cancer research; previous research has suggested that mRNA content is linked to tumor phenotypes, but technical complexity has prevented further exploration. Recently, a team from Texas developed a technique to measure the amount of total mRNA expression (TmS) from a cancerous tumor – factoring in transcript proportion, purity, and ploidy – and compare it with the amount produced by regular cells to help predict disease progression and tumor phenotypes (2). The method was tested on 6,590 tumors across 15 cancer types, revealing a link between high TmS and risk of cancer progression and death. TmS was also seen to have a relationship with "cancer-specific patterns of gene alteration and intra-tumor genetic heterogeneity (2)," alongside cross-cancer metabolic dysregulation.

And what of chromosomal instability (CIN)? This DNA-affecting process has a long-established association with cancer, yet there is no systematic method to measure CIN types and their effects on cross-cancer phenotypes. New research has led to a comprehensive anthology of CIN origin and diversity – representing over 7,800 tumor specimens from 33 types of cancer. The researchers codified 17 copy number signatures – each exemplifying a different CIN type – that help to forecast drug response and inform potential new treatment options. The finalized compendium highlights the structure un

See references online at: tp.txp.to/cancer-panopticon

Diagnosis: Uncert(AI)n

The old phrase "garbage in, garbage out" still rings true

A notable 2021 study by Seyyed-Kalantari and colleagues identified that widespread models trained on chest X-ray datasets showed a disparity between ethnic groups when spotting disease. Specifically, Black, Hispanic, and other underserved groups received significantly more false "healthy" classifications than their White counterparts (1).

A number of papers have responded to the team's work, including one comment that raised the study's potential limitations and the original researchers' inability to classify a cause of bias, noting that disparities are likely to arise when using a single prediction threshold (2).

Like an academic tennis game, the original authors replied to agree with points such as prevalence shift, difficulties in training with biased data, and use of a natural language processing tool (3). However, they reiterated the study's main finding – that biases exist and must be addressed before AI can be considered a reliable tool in the clinic.

See references online at: tp.txp.to/diag-uncertain

THE FUTURE OF THE LABORATORY IS

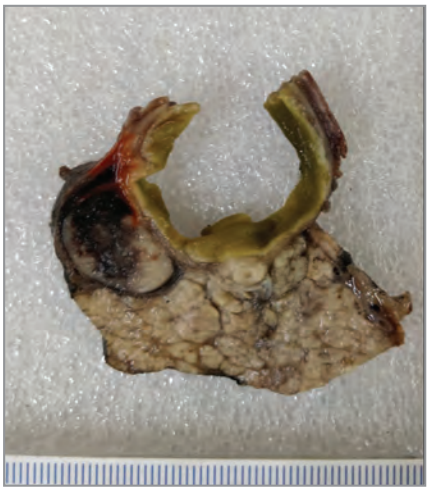
STRONGER TOGETHER

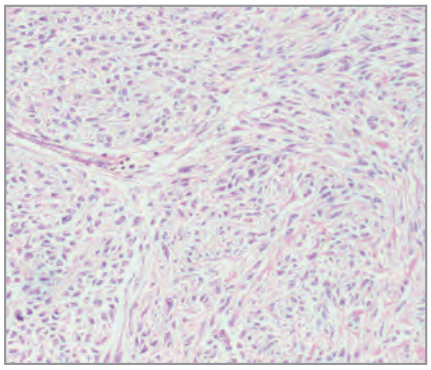
2022 marks the 100th anniversary of ASCP, and we are celebrating and honoring our members, without whom these 100 years of growth, innovation, and advancing pathology and laboratory medicine would not have been possible.

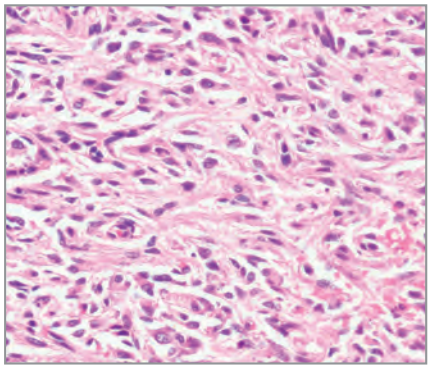
Renew your ASCP membership and be part of the evolution of pathology and laboratory medicine. Together we will create change that makes the laboratory accessible to all and recognized by healthcare executives and patients alike as the foundation of high-quality care.

Renew today at www.ascp.org/renew

CASE OF THE MONTH







A 55-year-old woman presented with intermittent, non-radiating upper abdominal pain for five months. Abdominal CT revealed a 24x14 mm, well-defined, arterial phase-enhancing lesion in the head of the pancreas, diagnosed as gastrointestinal stromal tumor (GIST).

Which of the following is true of GISTs?

a) GISTs with KIT *exon 9 mutations respond poorly to imatinib*
b) 85 percent of GISTs are associated with KIT *mutations*
c) Small intestinal GIST has a more favorable prognosis than gastric GIST
d) GIST is not associated with NF1 *mutations*

Answer to last issue's Case of the Month…

a) *Well-differentiated neuroendocrine tumor*

Low-power view of this ileal specimen exhibits both glandular areas in a cribriform pattern and solidifications. High-power view demonstrates cytologic findings of nuclei with fine "salt and pepper" chromatin and prominent nucleoli. According to the 2019 WHO classification criteria, well-differentiated tumors are classified as neuroendocrine tumors and defined as low, intermediate, or high-grade depending on the mitoses per 2 mm^2 or Ki-67 percentage index (whichever is greater). Poorly differentiated lesions are termed neuroendocrine carcinoma (4).

Immunohistochemical staining for this biopsy with pan-endocrine markers showed tumor cells strongly and diffusely positive for synaptophysin and chromogranin A. However, there was focal dim staining and negativity for neuron specific enolase and CD56, respectively. Antibody reactivity was negative for cytokeratin 7 and cytokeratin 20, decreasing the likelihood of adenocarcinoma of midgut or colorectal origin, respectively (6). The Ki-67 percentage index was less than 1 percent, supporting the morphologic diagnosis of a grade I well-differentiated neuroendocrine tumor.

Submitted by Erina McKinney, University of Kansas School of Medicine, Kansas City, Kansas; Gang He, American Diagnostic Consultation & Services, New York; and Ting Zhao, Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA.

See reference online at: tp.txp.to/0822/case-of-the-month

To register your guess, please go to http://tp.txp.to/1022/case-of-the-month
We will reveal the answer in next month's issue!

Case of the Month is curated by Anamarija M. Perry, University of Michigan, USA.

The Next Generation of Laboratory Automation

PerkinElmer's BioQule™ NGS system represents the next step in entry-level automation

An interview with Adam Snider

What makes the BioQule™ NGS system stand out from other next-generation sequencing (NGS) systems?

The BioQule™ NGS system is an easy-to-use benchtop lab automation device designed to prepare and quantitate up to eight NGS libraries in parallel. Compared with typical NGS prep automation, the BioQule™ NGS system is intended for lower throughput needs, preparing up to eight samples at a time as opposed to 96- or 384-well systems. It represents true entry-level automation for both manual users and larger labs who want to run eight or fewer samples without tying up their expensive systems.

Within this space, the BioQule™ NGS system distinguishes itself by being an open system, meaning that PerkinElmer supports third-party reagents and kits to give customers more options. Additionally, the system integrates fluorescence-based quantification into the automated workflow for library normalization and quality control. Typically, quantification is done manually offline, but the BioQule™ system automates the process.

What inspired the development of this system?

When my colleagues and I were research associates, most of our days were spent tracking sample preparation protocols because the existing automation platforms were well over our lab's budget. Additionally, new lab techs struggled with many of our library prep workflows, so I wanted to make a robust, easy-to-use, and cost-effective automation platform that frees up people's time to work on valuable research while ensuring libraries are properly prepared.

What challenges do labs face when implementing automated systems – and how does the BioQule™ NGS system's design solve them?

Many larger automation systems have a steep learning curve, even with prewritten automation scripts. Users need to be mindful of deck-setup details and attend to errors that may occur mid-run, such as pipette tip problems or low reagents. The BioQule™ technology employs a single-use cartridge system that includes the tips and an assay plate that comes pre-plated with reagents, making the few pipetting steps easier and providing a simpler solution for end users. This simplicity is especially important for smaller labs who may be new to automation; however, for larger labs that already work with large automation systems, the most important factor of the BioQule™ is its dependability, because they may run dropout samples from a larger assay and need to know that the library is prepared properly every time.

To maximize a researcher's time, an automated system should require as little manual intervention as possible. A normal NGS library prep, including quality control, has about 13 manual intervention points; the BioQule™ NGS system reduces that to two – a front-loaded setup step and library recovery at the end of the protocol. For staff facing high workflows and limited resources, this means far less time pipetting and monitoring and more time running libraries and working on research.

What assays can the system offer?

Currently, the product is focused on NGS library preparation. We've started with DNA whole-genome sequencing, and we are working on a number of other library preparation kits. Additionally, the BioQule™ technology enables some further applications that we aim to develop in future.

How does the system support expansion on the range of offered assays?

The BioQule™ NGS system contains a magnet for bead-based procedures, an integrated thermocycler, an optics module, and a liquid handling system – all features typically found in larger automation systems. With support from our application development team, you can design and run customized NGS library preparation workflows.

How do the data obtained using the BioQule™ NGS system compare with those obtained using alternative methods?

Our benchmark for sequencing metrics has always been to ensure automation is equivalent to manually prepared samples by a skilled user. From there, we add reproducibility, throughput, and walk-away time to enhance the capabilities of laboratory technicians.

Adam Snider is Product Manager for the BioQule™ system at PerkinElmer, Hopkinton, Massachusetts, USA.

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In My View

Experts from across the world share a single strongly held opinion or key idea.

Liquid Biopsies: Reach for the Stars

By overcoming the limitations of first-generation liquid biopsies, we can leap light-years ahead in clinical practice

By Dan Norton, Associate Director of Product Management at Personalis, Menlo Park, California, USA

Stargazing and medical research aren't so different. Both involve peering out into empty space and looking in awe at the breadth and scale of the things going on – things that happen without any of your input. At the same time, it's incredibly overwhelming. With so much choice, where do you look? Imagine for a moment that every star in the sky is an avenue for medical care being worked on – a method or technique with its own set of pros and cons, successes and failures, advocates and naysayers. Again comes that same question: where do you point your telescope? You can appreciate, and even take joy in, the fact that there's so much potential for improving patient outcomes – but, at the end of the day, you can only point your telescope at one spot in the "research sky" at a time.

For me, one star burns extra bright in the constellation of cancer diagnostics. When I look across the vast galaxy of projects, developing treatments, and emerging technologies, it's the shining prospect of liquid biopsies that continues to catch my eye through my telescope.

Tumor-informed liquid biopsies, for those not in the know, can be used throughout the cancer lifecycle. Their use can enable clinicians to manage a patient's course – from initial diagnosis and biopsy all the way through surgery, therapy, remission, and relapse. These tumor-informed liquid biopsies (TILBs) can show changes in cancer development – both progression and regression – earlier and more accurately than previously possible. TILBs involve first analyzing a patient's tumor tissue sample using whole genome sequencing, then designing a truly personalized liquid biopsy panel. This "informed" biopsy gives a more comprehensive view of the tumor's mutational landscape and optimizes sensitivity. After a patient undergoes surgery to remove a tumor, TILBs allow robust calculation of post-surgical survival risk. Specifically, the technique accurately detects molecular residual disease (MRD) at earlier points following surgical resection, meaning that it's much faster to ascertain whether the tumor was removed entirely or requires further treatment.

Today, most patients who undergo surgical resection of stage II or III cancer are given chemotherapy as a precaution. However, only a small portion of that population benefits from it – and the harsh side effects of chemotherapy on the body are well documented, so eliminating unnecessary treatment would improve patients' health and quality of life. TILBs can help identify MRD-negative patients who may not require chemotherapy and monitor treatment responses in MRD-positive patients who undergo further treatment by noting changes in tumor load and mutations over time. By leveraging such insights into tumor changes, care teams can adjust therapies accordingly. TILBs can also help determine the efficacy of therapy at a given time point by measuring the level of tumor burden and identifying individual tumor mutations, allowing care teams to tailor treatment on a case-by-case basis.

Beyond treatment, TILBs also have applications for recurrence monitoring. Once a patient is in remission, TILBs can detect tumor signals much earlier than tumor-agnostic liquid biopsies or the current standards of care (including imaging). Such optimized sensitivity enables physicians to provide treatment before the cancer metastasizes, potentially saving and extending lives.

It's my hope that you are now excited by the TILB-led future that lies ahead of us – and that I've inspired you to look beyond your usual patch of the "research sky." Glance elsewhere and who knows what exciting research could be waiting at the other end of your telescope?

IVDR Compliance: A Marathon, Not a Sprint

Reaching the IVDR compliance finishing line requires significant time and resources – but it's an investment that will pay off in spades

By Brice Ezzouaouy, Senior Product Manager at Beckman Coulter Life Sciences, Marseille, France

Do you use laboratory-developed tests (LDTs)? If so, new regulations in the European Union may change the way you operate. In the EU, in vitro diagnostic devices (IVDs) must now begin to comply with new regulations that considerably raise the bar for compliance, both for IVD manufacturers and for laboratories relying on LDTs. Many laboratories face an additional challenge in that their own compliance track overlaps that of their manufacturers – labs need their LDT manufacturers to cross the finish line in good time to win their own compliance race. So, when it was introduced in 2017, the In Vitro Diagnostic Regulation (IVDR) challenge – with a compliance deadline of 2022 – always looked like it would be a difficult course to run. But worse was to come; for many EU laboratories and manufacturers, crossing the finish line by 2022 was made impossible by the unprecedented impact of the COVID-19 pandemic (1) – and some did not realize early enough that their LDTs fell within the scope of the IVDR (2, 3). That's why, for a significant part of the industry, racing to meet the 2022 deadline was just not possible – and this risked supply continuity for critical IVDs and LDTs.

Accordingly, in January 2022 the EU extended transitional provisions for IVDR compliance; laboratories now have an additional two years to bring their in-house assays or LDTs up to speed, whereas IVD manufacturers may benefit from up to five additional years (1, 4). Though this helps many players catch their breath, it is not a signal to relax – or to slow down. The new requirements have not gone away, nor have their time and resource implications – such as the need to upgrade and maintain quality management systems and product design history files. Therefore, we must use the extended transition period efficiently, intensify our efforts, and commit the necessary resources to this endeavor.

In particular, it is critical for IVD end-users to know as soon as possible which products are intended to be IVDR-compliant. Note that it is each lab's responsibility to ensure its assays comply with IVDR, a task that varies significantly in difficulty depending on whether the lab uses an IVD assay according to its intended purpose or seeks to validate an LDT. For IVD manufacturers, the marathon will be longer still; manufacturers have many more regulations to implement, not least due to additional clinical evidence and post-market surveillance requirements.

Clearly, there are costs associated with running the IVDR compliance race. Equally, though, participants can expect significant long-term benefits on the other side of the finish line. Consider, for example, the pre-IVDR situation for LDTs with quality requirements that vary significantly. With more stringent demands on IVD manufacturers, it makes sense to also demand enhanced oversight of LDTs – which, after all, carry the same kinds of risk and benefits for patients as commercial IVDs.

At the same time, IVD manufacturers should appreciate that IVDR compliance will improve product quality and thereby contribute to growing product demand as clinicians opt for approved (and improved) IVDs to support their treatment decisions. Finally, IVDR is likely to improve the overall quality and transparency of healthcare by raising the standards of all diagnostic tests and medical devices – a win-win end to the compliance race.

"The race to meet new compliance standards is underway and those who take immediate action to reach the finish line will enjoy the rewards of an improved and more streamlined testing environment."

In brief, although IVDR is undoubtedly challenging to implement, we should remember that these new regulatory standards -- by contributing to a robust, consistent regulatory framework that applies to all IVD assays – will help improve both diagnostic efficacy and patient safety. Furthermore, as IVDR benefits become more broadly recognized over the coming years, this improved environment for patient diagnostics and safety is likely to spread globally (5). In other words, the race to meet new compliance standards is underway, and those who take immediate action to reach the finish line will enjoy the rewards of an improved and more streamlined testing environment.

See references at: tp.txp.to/ivdr-compliance

The Learning Never Stops

Evolution of the profession relies on education – for professionals and patients alike

By E. Blair Holladay

A century ago, patients were often treated in their homes and much of the practice of medicine went unregulated. Today, cutting-edge research and technology have helped establish guidelines and benchmarks that ensure patient safety across healthcare organizations, hospitals, and clinics.

A century ago, blood banks didn't exist, the discovery of widely used antibiotics was still years away, and the common Pap smear that changed how we test for cervical cancer had yet to be discovered.

A century ago, pathology wasn't recognized as a medical specialty. It wasn't until a group of physicians made it so at the 1922 American Medical Association meeting in St. Louis that our discipline became recognized. One hundred years later, as we gather this September in Chicago at ASCP 2022, we celebrate a century of progress and continued advancement in pathology and laboratory medicine. Innumerable technologies and practices that exist today were unheard of mere decades ago – and, as leaders in healthcare, we share our excitement over what we have accomplished in the past 100 years and acknowledge the deep pride that comes from knowing that we have changed the face of medicine for the better. We know that our contributions to medicine stem from our refusal to simply coast on what is expected from the laboratory. Rather, we push ourselves to continually improve our skills and increase our knowledge. We know that educating ourselves, our colleagues, and our patients is critical to the success of the laboratory and providing extraordinary patient care.

It is part of the American Society for Clinical Pathology's mission to provide pathologists and medical laboratory scientists – both in training and in the field – with relevant, up-to-date education that will help them at the beginning, middle, and conclusion of their careers. Our education doesn't end when we leave our academic institutions, diplomas and credentials in hand. Instead, if we are to ensure that the patient is at the center of our practice, education must be a lifelong endeavor. If we are not continually gathering knowledge and educating ourselves on the latest technologies, treatments, procedures, information, and skills, then we are doing our patients a disservice. Our commitment to pathology and medical laboratory science moves in tandem with our dedication to educating ourselves and others.

The way we learn has also changed drastically over the past century, particularly in the last two years. Classroom learning has evolved to a hybrid model and online platforms such as YouTube can help students and professionals alike hone their skills. This increase in online education has enabled pathology and laboratory medicine to reach people around the world who would never otherwise have access to the field. As such, it broadens our practice and emphasizes the importance of the laboratory in patient care.

As we move into the next 100 years, we are excited to see how the profession – and our understanding of new skills, techniques, and technologies – unfolds. We can only build on the century of progress we've already established – and the next century is already proving to be an exciting time for pathologists, medical laboratory scientists, and patients everywhere.

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Creating Made-to-Measure NGS Testing with Multiple Panels

An interview with Yvonne Wallis and James Beasley on their experience using a multi-panel approach to oncology biomarker sequencing

Tell us about your laboratory and the biomarker testing you do…

Yvonne Wallis: We both work at the West Midlands Regional Genetics Laboratory, which is the Genomics Laboratory Hub (GLH) for the Central and South GLH Consortium – a part of the NHS Genomics Medicine Service in England. The Central and South GLH serves a population of almost 13 million people across the West Midlands, Oxfordshire and South of England.

James Beasley: When it comes to volume, we currently perform just under 400 cancer panels a month. However, I'm excited to say that we now have funding to double capacity and are ramping up to perform 10,000 panel tests a year.

What is your approach to selecting next-generation sequencing (NGS) panels? Has it developed over time?

YW: We've been running NGS panels for somatic cancer testing for over a decade, starting with a nine-gene panel, progressively increasing in size – from nine to 28, 172, and now 523 genes in a single panel. However, we now appreciate that having a single capture-based technology does not fit the requirements for all cancer referrals. So we've introduced a mid-sized targeted NGS panel using amplicon-based technology for rapid testing, with plans to introduce additional panel tests using this technology to support capacity and contingency. Of course, because DNA-based panels are not always adequate to detect fusions, we also have a 103-gene RNA sequencing panel. It's remarkable that we started off with a single nine-gene panel and have now moved up to routinely delivering in excess of 500 genes, alongside a large RNA sequencing panel and two medium-sized DNA panels. It's a really broad church and meets all the criteria for delivery of somatic cancer testing.

Can you please share your results? What do they mean for patients?

JB: We initially used a single NGS platform to provide testing for all cancer clinical indications. Diversifying the cancer panel portfolio has resulted in a significant drop in failure rates. Using different chemistries – including both capture-based and amplicon-based approaches – means we are better adapted to processing poorer-quality samples. Rather than reporting failures, we're able to detect a lot of variants in tissues that may have previously been a struggle to sequence (see Table 1). Overall, our results have changed pretty dramatically just by increasing the variety of available NGS panels.

YW: Turnaround times have also changed. Over the last six months, we've been able to reduce the average turnaround time by more than seven days. When we first started the transition to include both mid-sized and large-scale panels, we were running at an average of about 24 days. Now our average is approximately 16 days and we have plans to reduce it to 10–14 days very soon. We can only do that because we have different types of platforms, which lets us work efficiently – well within the timescales professionals need to ensure the best possible outcomes for their patients.

What are your thoughts about the future of biomarker testing in precision oncology?

YW: The National Cancer Genomic Test Directory is a large list of tests available to cancer patients across England. Panel tests are becoming an important component of the technology required to facilitate delivery of the directory, because they are able to cope with the increasing number of tests required per patient on limited amounts of tissue. To me, the future will require even more in terms of the number and scope of biomarkers per patient using panel tests. A good example of this would be routine use of panel tests to identify potential clinical trials for all cancer patients. The Test Directory is quite prescriptive as to which essential genetic targets should be tested for particular cancers, which may not be useful for all patients at the end of standard-of-care treatment. I hope that, in the future, the directory will include a pan-cancer clinical trial test entry to address this. Another thing to consider is speed. This field is huge and growing, but turnaround times for tests will still need to be short to offer patients maximum benefit.

JB: My answer would be circulating tumor DNA (ctDNA). Most biomarker testing for solid tumor samples uses

> *"We're able to detect a lot of variants in tissues that may have previously been a struggle to sequence."*

1st August 2021 - 31st October 2021 (Large DNA paniel & RNA panel)

	Pre-test (both DNA and RNA)	Post-test (DNA and RNA)	Incomplete (DNA or RNA targets failed or isolated DNA/RNA pre-test QC	Detection rate (DNA or RNA finding, including partial fails
Lung	24.6%	8.2%	36.0%	35.2%
Colorectal	14.3%	28.6%	42.8%	57.1%
Melanoma	0%	25%	0%	50%

1st Nov 2021 - 31st Jan 2022 (Small DNA paniel & RNA panel)

	Pre-test (both DNA and RNA)	Post-test (DNA and RNA)	Incomplete (DNA or RNA targets failed or isolated DNA/RNA pre-test QC	Detection rate (DNA or RNA finding, including partial fails
Lung	0%	0.56%	7.9%	61.4%
Colorectal	1.1%	0%	13.5%	60.7%
Melanoma	13%	0%	8.7%	52.2%

Table 1. An example of the impact of panel choice on patient pathways. Pre-test QC fail: the DNA/RNA extracts were of insufficient quantity for testing. Post-test QC fail: the DNA/RNA extracts were of insufficient quality for testing.

biopsy tissue, and not every patient can have their tumor biopsied. As a result, there is a big drive for more regular ctDNA testing within the NHS. It has been an option in the past, but only on a small scale. I think the future will see NGS panels using ctDNA that can step in when a tissue sample is limited or unavailable (or even, for speed, as the first-line test). In light of that, liquid biopsy panel tests can really help with tissue preservation requirements as well as alleviating issues around tumor heterogeneity.

Do you have any words of wisdom for people who believe that "bigger is better" when it comes to panel size?

YW: Large panels underpin the potential to deliver highly flexible high-throughput somatic cancer testing. Once it is fully automated (end-to-end) with reporting integration, it will deliver results for cancer patients at any stage along their clinical pathway. However, it's important to remember that NGS panel testing is rarely plug-and-play. Successful implementation to deliver accurate results requires appropriate infrastructure and expertise to ensure that everything is accurate and interpreted correctly. This is especially important when using large panels that can throw up unknown variants with the potential to be germline. Quality metrics must be carefully considered; it's crucial to know when you might be looking at a false positive or false negative result. Bioinformatics is critical to the safe analysis of panel testing data. Some panels and platforms come with their own solutions, whereas others require an in-house team of bioinformatics experts to create the pipeline. Even "out of the box" solutions require expertise to fully understand the limitations of the assay and to provide the appropriate limits of detection. To ensure every cancer patient receives the genetic testing they deserve, it's important to be flexible, keep your options open, and choose the tools that are best for a variety of clinical scenarios. There are circumstances in which large panels are best – and, equally, there are situations that necessitate smaller panels.

"It's important to be flexible, keep your options open, and choose the tools that are best for a variety of clinical scenarios."

THE ROBOT *MAY* SEE YOU NOW

It's time to stop and think about the ethics of artificial intelligence

By George Lee

Every journal, website, and conference is hailing artificial intelligence (AI) as the latest, greatest laboratory tool. AI advocates say no corner of laboratory medicine will go untouched by its influence. Upon its arrival, lab professionals will have unprecedented hours to spend on difficult cases, interact with patients, or even enjoy overdue downtime baking bread or simply taking a break. The impact of this technology will be immense and its effects everlasting. Much like the microscope, AI may leave pathologists incapable of imagining a world without it. That's right; the AI train is coming – so jump on board or get off the tracks!

Despite my sarcastic techno-optimism, I should make it clear that I'm not anti-AI. The power of AI and related technologies is well-documented, and it's already in widespread use in our everyday lives – from the personal assistant on your smartphone to the way the bank assesses your credit score – and quite likely in many other aspects you've not even considered. But potential and promises do not absolve technologies of skepticism – especially when they are going to be highly integrated into healthcare settings. Though it might sound like a win-win situation for everyone involved, implementation of AI is a concept of extremes – at best, it has the potential to empower patients and professionals and increase healthcare equity; at worst, it could exacerbate the most miserable parts of healthcare in late-stage capitalism (1).

"Though it might sound like a win-win situation for everyone involved, implementation of AI is a concept of extremes."

The ethical side of AI is often less-reported compared to its ability to transform healthcare, and when it does occasionally step into the limelight, some ethical dimensions take precedence over others, even in bioethic spheres. According to one study, an analysis of 85 ethical guidelines from across the globe showed that sustainability, dignity, and solidarity were significantly underrepresented compared to other ethical considerations (2).

But what's so bad about AI? Or, more accurately, what's not-so-great about it? Certainly, it can be difficult for non-experts to dig out the answers from the PR packages and the slick speeches of wily copywriters. But, drowned out by the fanfare from Silicon Valley, more nuanced conversations are taking place – discussions that are less focused on what AI could do and more on what it shouldn't.

Welcome to the world of AI ethics!

INTERROGATING THE MACHINE

"We realized that was a massive mistake."

These are the words of Eric Brown, the IBM research scientist responsible for the creation of Watson (a supercomputer that beat Jeopardy's best two players). Out of context, Brown's bold statement might seem like some doomsayer declaration of the coming AI apocalypse, but he was actually referencing the fact that some of Watson's dataset had been pulled from Urban Dictionary – the crowdsourced and sometimes dubious definition site (3).

Like many other question-answering systems, Watson had struggled with the fluid and often instinctive way people use slang and other non-standard word forms. Perhaps not fully aware that the site is known for ironic user submissions and a lax approach to profanity, the team sought to address Watson's fluency issue by importing hundreds of thousands of entries from Urban Dictionary. They quickly noticed Watson's new proclivity for inappropriate language after it rather snarkily answered a researcher's question with the word "bullsh*t." Unsurprisingly, IBM engineers washed Watson clean of the colorful data well before its television debut.

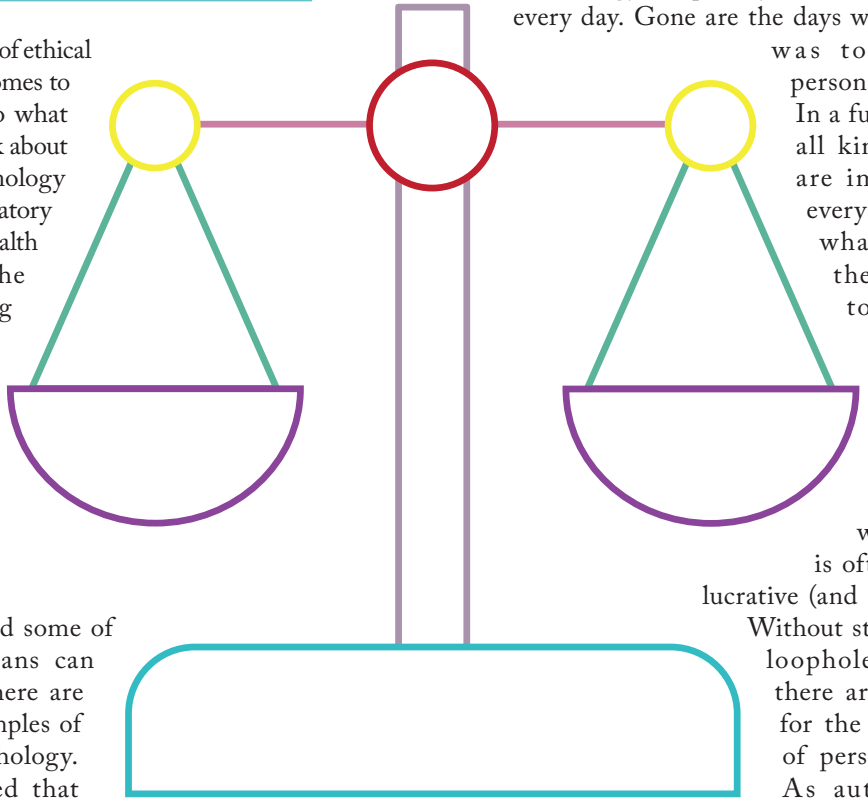
Watson's short-lived potty mouth is a good example of the effect that human input has on AI. Supercomputers with attitude is one thing – algorithm bias is quite another. The risk of humans developing skewed systems is a big topic in the AI ethics discussion. And the risk isn't just hypothetical. It's no secret that algorithms used to decide which US inmates deserve parole have been shown to replicate known human biases (4). In this case, it means that Black defendants are

deemed more likely to reoffend than the data indicate – and significantly more than their White counterparts. Can we afford to let such biases stand in healthcare?

The topic gets even more nuanced with "black box" AI, in which the mathematical models are incredibly difficult to understand, even for experts (5). What happens if the researchers asking the questions don't understand how the AI has reached a particular conclusion? Surely, if one of the supposed benefits of human-free systems is the lack of bias and other human baggage, any AI tech that purports to be entirely objective must be placed under the greatest levels of scrutiny.

FIVE PILLARS OF AI ETHICS

Bias is just one (big) area of ethical consideration when it comes to AI implementation. So what else do we need to think about when using the technology in pathology and laboratory medicine? The World Health Organization and the European Union, among others, have their own frameworks for ethical AI use but here we present five key aspects – starting with objectivity.



Objectivity

We've already outlined some of the biases that humans can impose on AI, but there are plenty of specific examples of AI bias affecting pathology. A 2021 paper showed that algorithms trained on public US datasets for chest X-rays had underdiagnosis biases consistent with underserved demographics, such as female, Black, and Hispanic patients, as well as those of low socioeconomic status (6). Another case saw Black patients missed for vital kidney transplants due to a race-based algorithm (7). In these cases and others like them, social factors influence the dataset, causing the AI to reinforce existing biases.

These weaknesses of an AI approach have historically been overshadowed by their otherwise exciting potential. Perhaps, like a technological honeymoon period, these biases that "[affect] the data and shape the design of the algorithm [are] now hidden by the promise of neutrality and [have] the power to unjustly discriminate at a much larger scale than biased individuals" (8).

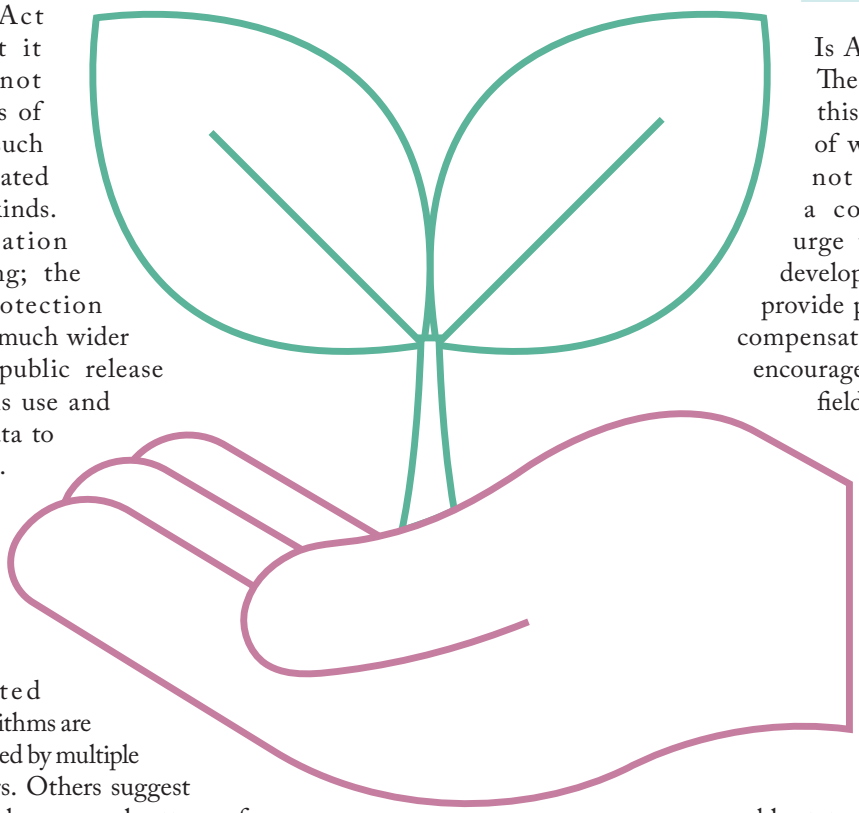
Experts recognize this issue and recommend addressing it by involving pathologists in AI development – from the start of the project. And the role of the pathologist shouldn't end at development; regular monitoring and quality control will be required if AI accuracy is to be truly reliable.

Privacy

Technology and privacy are more and more entangled every day. Gone are the days when common advice was to keep anything personal off the Internet. In a futuristic world where all kinds of technologies are implemented across every level of healthcare, what protections will there be with respect to data collection, surveillance, and consent? Who has the general public's best interests in mind? In our modern world, information is often one of the most lucrative (and sought-after) assets. Without strict protection from loopholes and bad actors, there are genuine concerns for the buying and selling of personal medical data. As author and speaker Bernard Marr poignantly put it, "Unregulated data-mining causes a whole different set of problems – privacy issues as well as the imbalance of power which is caused by information being in the hands of the few, rather than the many," (9). Interestingly, the WHO highlights that "even informed consent may be insufficient to compensate for the power dissymmetry between the collectors of data and the individuals who are the sources," (10). Many have suggested that AI governance in pathology and healthcare should be

established at national and institutional levels to safeguard patient interests.

Privacy may become a bigger issue as we progress into multimodal AI, where systems pull in data from many different sources; everything from extensive biobanks, health records, and even your smart watch. In an effort to stay impartial, let's call this whirlpool of information a "privacy bad dream" rather than a full-blown nightmare. Protection does exist, of course – for example, the Health Insurance Portability and Accountability Act in the US – but it currently does not extend to all types of healthcare data, such as the user-generated and de-identified kinds. European legislation is further reaching; the General Data Protection Regulation casts a much wider net, including a public release on how AI systems use and process people's data to make decisions (11).



Solutions for this technical tangle of privacy protections are already being developed. Some propose federated learning, where algorithms are trained on data supplied by multiple decentralized servers. Others suggest differential privacy, where general patterns of information are shared, but individual identities are kept hidden. Methods like this seem to address the problem, but obscuring the granular accuracy and detail of data in the name of ethics does ultimately pose a difficult question: what do we value more – performance or privacy?

Transparency

Another major issue for healthcare AI is transparency. When, if ever, is it appropriate to let a patient know their diagnosis was determined using AI? Does the patient have a right to know? What if the system simply confirmed the pathologist's initial impressions? Perhaps the answers depend on the diagnosis and the patient's level of involvement.

But it's hard to ignore the potential damage to patient–practitioner trust caused by a failure to explicitly disclose the use of AI support. On the other hand, it is important to remember that most patients are not AI experts. To gain informed consent from patients, practitioners first need to be able to provide them with the knowledge they need to make informed decisions in the most appropriate and useful format.

Accountability

Is AI considered a product? The answer is unclear – and this means that the question of who is liable if AI does not work as intended is a complicated one. The urge to pin liability on the developers of AI software may provide patients with a route for compensation but may ultimately encourage companies to leave the field rather than shoulder the financial risk. Similarly, holding practitioners liable for the failings of third-party software feels unfair and would almost certainly minimize widespread use by medical professionals. Should we leave the debate for the courts to decide – enjoying an agreeable status quo while it lasts?

Sustainability

With a future of continued anthropogenic climate change ahead, there are nearly endless ways we can (and must) adapt to become better stewards of the planet. Healthcare is responsible for its fair share of resource-guzzling and emissions-belching, but more intensive technologies often need increasing amounts of energy to run – and AI is no exception. The promise of a technological future is often sobered by its potential environmental impact on an already strained planet.

What carbon emissions lie at the foot of AI? It's hard

to gauge, as the numbers are highly dependent on location, time, and energy sources. But one study found that carbon dioxide emissions for one AI system reached 28 kilograms in a single month (12). Another concluded that training an AI model can emit more carbon emissions than six cars across their lifetimes, even factoring in the emissions used to manufacture them (13). But how do we decide which parts of healthcare are worth the emissions needed to fuel them? Although more pathologists are becoming aware of the impact of their work, and the move toward more sustainable labs is laudable, we may run the risk of over-egging the size of healthcare emissions compared to other, perhaps less altruistic, sectors.

Speaking on the matter in its ethical guidelines, the WHO said that "AI systems should be designed to minimize their environmental consequences and increase energy efficiency. That is, use of AI should be consistent with global efforts to reduce the impact of human beings on the Earth's environment, ecosystems and climate (10)."

Though our five-point primer is far from exhaustive, I hope it at least offers a springboard for further enlightening discussion.

"Use of AI should be consistent with global efforts to reduce the impact of human beings on the Earth's environment."

LET'S NOT THROW THE BOT OUT WITH THE BATHWATER…

Despite me starting this piece with a sarcastic tone, it really does feel like AI will change the face of pathology. Its potential is far too great to ignore – so our question must become: How can we ensure that AI usage is both useful and ethical?

Well, if I've learned anything in my research into this topic, it's that AI implementation needs to be a two-way street. First, any company who is active in this space must reach out to pathologists and laboratory medicine professionals to understand their daily workflows, needs, and pain points in as much detail as possible. Second, pathologists, laboratory medicine professionals, and educators must all play their important part – willingly offering their time and expertise when it is sought or proactively getting involved. And finally, it's clear that there is an imbalanced focus on certain issues – with privacy, respect, and sustainability falling by the wayside.

Pathology is a field stymied by increasingly high volumes of work being loaded onto an already strained workforce. AI tools, if properly, safely – and ethically – integrated into existing workflows, could help pathologists better manage better ones, creating more time for patients, difficult cases, and academic research.

Pathology's AI adoption and its integration with genomics, radiology, and clinician notetaking could enable precision medicine in the truest sense of the buzzword – ushering in an era that really does look like the future of healthcare. But as the ethical foundations for artificial intelligence are still being laid, we are probably best to remember that AI is just a tool – and it doesn't always have to come out of the bag.

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FENDING OFF THE AI WINTER

Without adequate education for professionals and the public, AI risks being left out in the cold

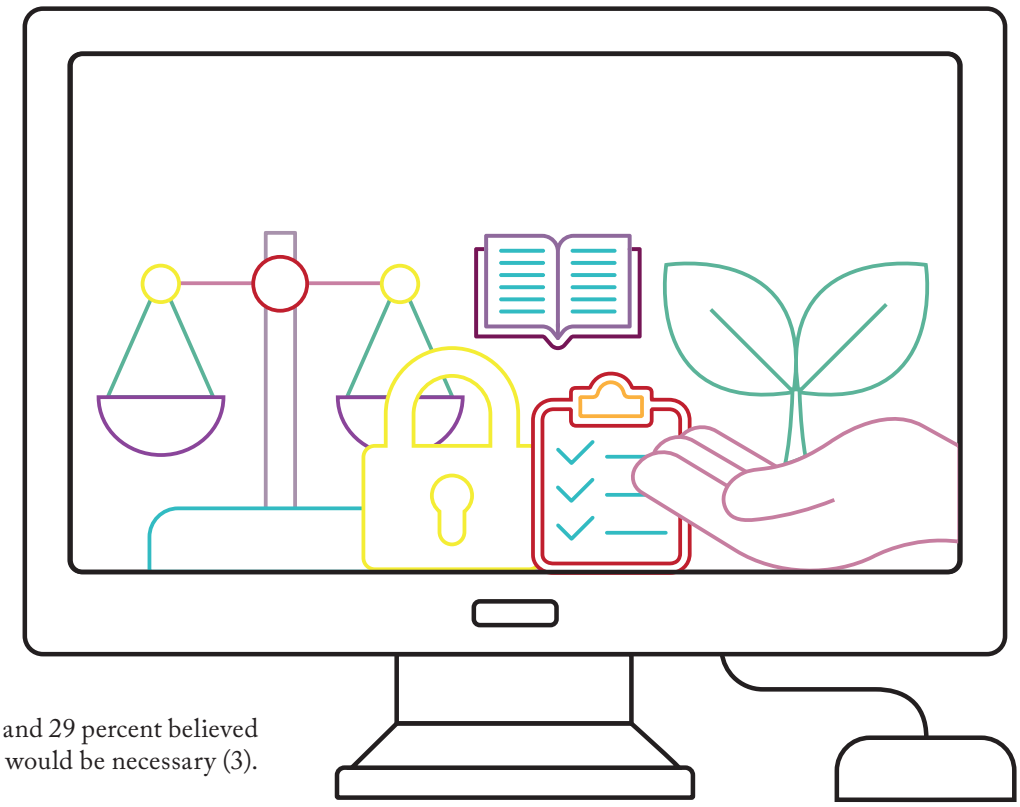
In the early 1970s, the field of AI froze over. An academic paper known as the Lighthill Report emerged, filled with scathing criticism of AI development and its history of overhype and under-delivery. The report marked the start of the first AI winter – a period in which interest, funding, and general faith in AI development hit an all-time low. It would take several years for this technological ice age to thaw and for AI to once again bask in the warm glow of positive public opinion – until it found itself in a second winter of frozen progress in the late 1980s (1).

Thus far, AI's hype/hate cycles follow a recurring theme: broken promises – the reality of AI's potential overshadowing its actual ability. In a world where celebrities are embarrassed to have been associated with NFTs, Elon Musk's image as mega-rich tech-lord is disintegrating, and Meta insists that its Metaverse is definitely – 100 percent – going to be a big deal, it doesn't seem all that unlikely that public opinion of AI could once again turn sour.

Could this history of broken promises be tackled with education on AI to give us more realistic expectations? It seems possible. To most, AI is a nebulous concept. Do you know what it is? Is it the same as deep learning? Natural language processing? And what's all this you've been hearing about neural networks? Don't worry if you're confused. According to one survey on AI in digital pathology, there was "a uniformly low perceived knowledge of AI" among respondents (2). In another study, 44.1 percent of respondents felt that training from an AI platform representative would be of help for its future implementation in the lab, and 29 percent believed that a dedicated course or workshop would be necessary (3).

This shouldn't come as a surprise; pathologists are experts in healthcare, not technology. However, the gap in knowledge on the pathologist level leaves AI-powered healthcare susceptible to forces both well-intentioned and nefarious – forces that could be deliberate or completely unconscious.

"Pathologists no doubt want what's best for their patients and making sure AI tools are safe and ethical is essential for that," says Francis McKay, Research Fellow in ethics and social implications of digital health at the University of Oxford. McKay is something of an AI activist. He has recently co-authored a paper on the ethics challenges of AI in pathology (4), and co-curated (the now offline) digitisingdisease.com, a website-cum-exhibition designed to bring critical AI studies to the general public. "That said, there may not be a deep or applied understanding of what the ethical issues are or how to solve them, which is understandable given their novelty and complexity," he continues. "Admittedly, whether pathologists actually need such knowledge for their work is unclear – there are a lot of potential ethical issues and some may be more relevant than others depending on the work they do. A broad overview of key issues and solutions will help situate their work in context, however, and allow them to use AI with confidence going forward."



To find out how education may lead to a more ethical AI future, I sat down with McKay to discuss critical AI studies and how they relate to pathology and laboratory medicine

First of all, what motivates your interest in this topic?

It's clear that healthcare is going to be an important domain for AI research over the coming years, but we know from ongoing work in critical AI studies that it can pose significant ethical challenges for individuals and communities. We must learn from that work and investigate how it applies to healthcare.

I'm also motivated by the general need to improve professional and public understanding of applied ethical issues around medical AI. For instance, research suggests that there is a lack of understanding among pathologists about the ethical challenges of digital pathology, as well as a desire for more understanding (5). Based on my own research with patients and the public, I'd say there's also a sense of poor understanding and acceptance of AI in the general public – even more so when it comes to its application to healthcare – and a need to be reassured over its use.

The National Pathology Imaging Cooperative has been working to develop an infrastructure for ethical development of AI in pathology over the past few years. We thought it would be a good idea to communicate some of the things we've learned from that process, both to help improve that understanding and to be transparent in our own ethical decision-making. Our most recent article (4) is a general summary of the key issues we encountered and how we responded to them to help guide others interested in developing similar systems.

With such a strong push toward AI and digital technology, are ethical factors a big enough part of the equation?

I think increasing numbers of people are interested in AI, both in pathology and in healthcare more generally. Whether the focus on ethics is "enough" is hard to say given the novelty of the situation. We must remember that AI is relatively new

as medical technologies go (indeed, in many ways, it is more of a promissory technology in the hospital at the moment than one patients and professionals encounter regularly). So it is bound to take time for ethical awareness to mature.

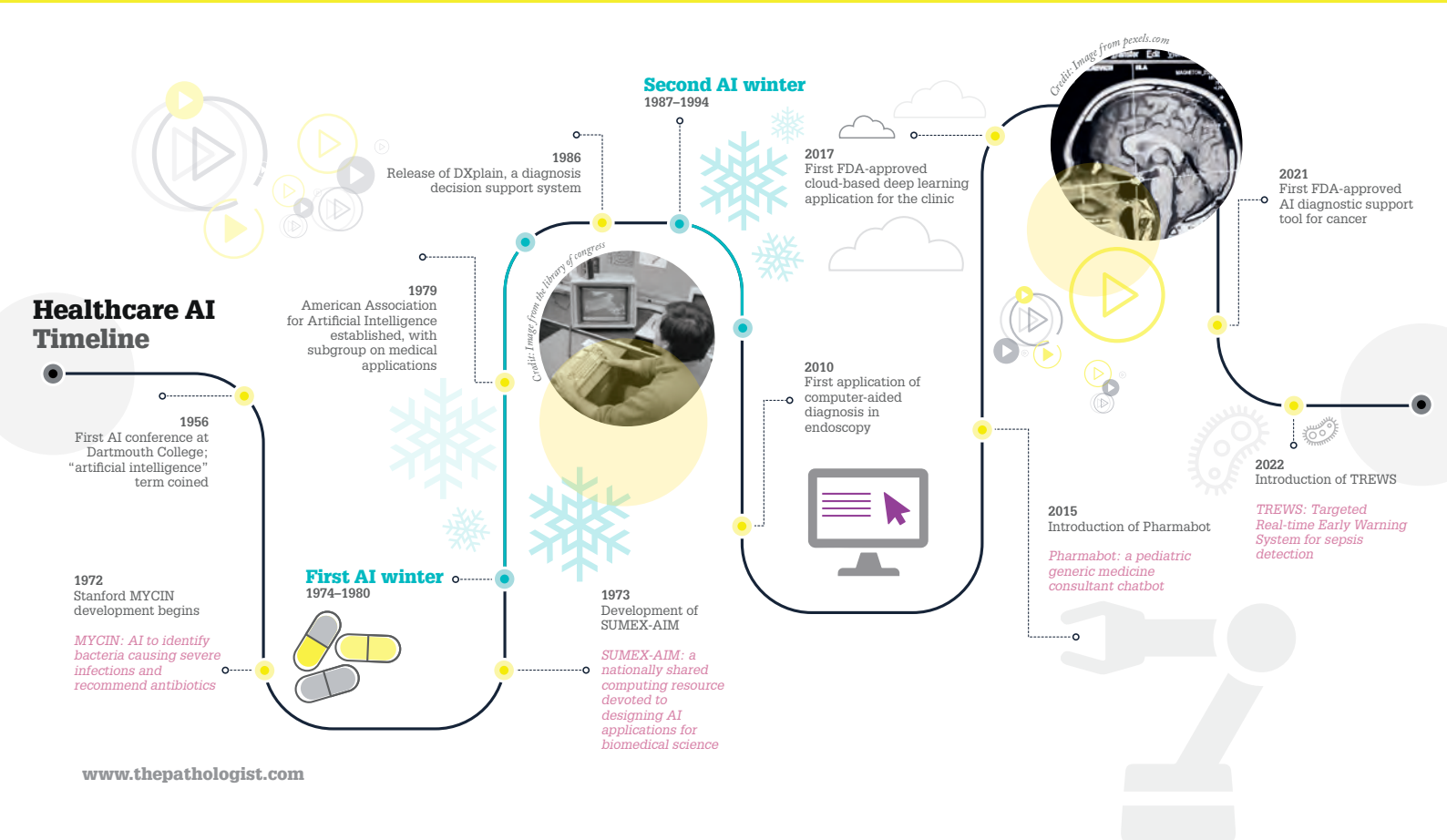
That said, I don't think ethical factors have been entirely absent; rather, certain ethical issues may have dominated the conversation more than others. For instance, there is a strong discourse on data privacy and there are numerous media narratives about the apocalyptic and existential threats of AI. Both have done much to steer public and professional understanding of the ethics in a particular direction and have consequently led, especially in the case of data privacy, to multiple ways of addressing them. But these can sometimes eclipse awareness of other ethical issues concerning the downstream social impacts of AI, such as how it might contribute to bias or the appropriate limits of commercial involvement. Part of the concern is that, if we don't widen the discourse, we might curtail the kinds of ethical interventions we can develop.

In 2021, the WHO outlined six ethical principles for health AI. Clearly, conversations are being had. Are they being heard?

There are a lot of ethical frameworks out there (6) and it can be easy to get lost in it all. Moreover, those general principles can be pitched at such a level of abstraction that it can be hard to figure out how they apply to everyday contexts. And that's why pathology-specific frameworks are useful; it allows those frameworks to be translated to applied domains with which pathologists are more familiar. Our article hopefully responds to that need by providing a general heuristic of the key issues to prioritize right now (that is, as digital pathology systems are being deployed). The novelty, complexity, and dominance of certain ethical narratives also affects our ability to develop that ethical awareness. And we probably shouldn't overlook a possible impact of the pandemic, which has arguably focused our attention on some issues over others and left us with little capacity to reflect at length on AI ethics.

What effects might AI ethics have on the patient journey or ultimate outcomes?

I think it's fair to say that we won't be able to get professional and public support for AI without being able to show that medical AI tools are developed safely and ethically. In that sense, it's an essential part of the care infrastructure – just as important as putting scanners into hospitals or training AI on



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histopathology data. On that point, it should be noted that there's also a real problem around obtaining a social license for big data and AI-driven health research in general, which we've seen in responses to things like care.data and General Practice Data for Planning and Research. All this means that evidencing the ethical underpinnings of the work is essential for patients and professionals to accept these new technologies. Without that social license, we may see another AI winter in which no public benefit can be derived from medical AI because researchers are too wary of developing them or funding dries up.

That answer addresses the most general level, but there are also more specific possibilities; for instance, if part of developing ethical AI is ensuring equity of service by limiting things like algorithmic bias, it will have a direct effect on ensuring that all communities, not just a subset, can share in the technology.

How much responsibility for awareness of these issues falls on pathologists and laboratory medicine professionals?

There is value in pathologists and laboratory professionals cultivating an awareness of the ethical issues for a couple of reasons. One is to help further the discourse of the ethical issues from an applied perspective. In many ways, we don't know what all the issues are, so pathologists can play a role by highlighting other problem spaces or developing more nuanced solutions once they reflect on ethical challenges. Another reason is to communicate with and reassure others, including patients and the public, about the use of AI in the service of healthcare. There's a great deal of public concern around AI, and there's also a great deal of discussion on the importance of explainable AI – in other words, making its internal workings clear to provide the public with the reassurance they need. But how much information do patients need to be informed in their own care options? And do healthcare professionals currently possess enough knowledge of AI to explain it when asked? Greater technical and ethical understanding of AI can only support efforts to communicate with others who need reassurance.

> "Pathologists no doubt want what's best for their patients and making sure AI tools are safe and ethical is essential for that."

How do we effectively safeguard in terms of misdiagnosis or misleading conclusions by AI?

It's not yet clear what role AI will play in diagnosis. There's a spectrum of possibilities from providing optional overlay information when assessing a histopathology slide all the way up to full automation. As far as I see it, AI is most likely to be used as an assistive technology, rather than a fully automated process, though some lightweight tasks may be automated with little concern. Nonetheless, keeping a human in the loop is one crucial way to prevent machine misdiagnosis. Interestingly, however, it works both ways – pathologists are also fallible in their diagnoses and can have differences in opinion in their clinical judgments. AI could play a role in that regard by standardizing diagnosis, suggesting alternatives, or highlighting things that might otherwise be overlooked.

In addition to having both a human and AI in the loop, ensuring that datasets are representative of the population and the range of cancers and rigorously validating AI tools will go far toward safeguarding against misdiagnosis.

What one thing can AI users (or future users) do now to address potential ethical issues?

The answer to that question depends on who is considered an "AI user." In some ways, it's everyone – health data researchers, pathologists, patients, and more. In that case, there's probably no one thing that captures them all, because there are different ethical demands based on different users' relationships to the technology. All that said, a general first step is to be informed about what the ethical issues are and to reflect on what emergent issues might be. Fortunately, there's a growing body of literature on the topic. Our article offers one entryway specifically for pathologists and our website serves a non-expert audience. And for anyone who wants to take it further, a whole field of critical AI studies awaits!

See references online at .txp.to/fend-off-ai

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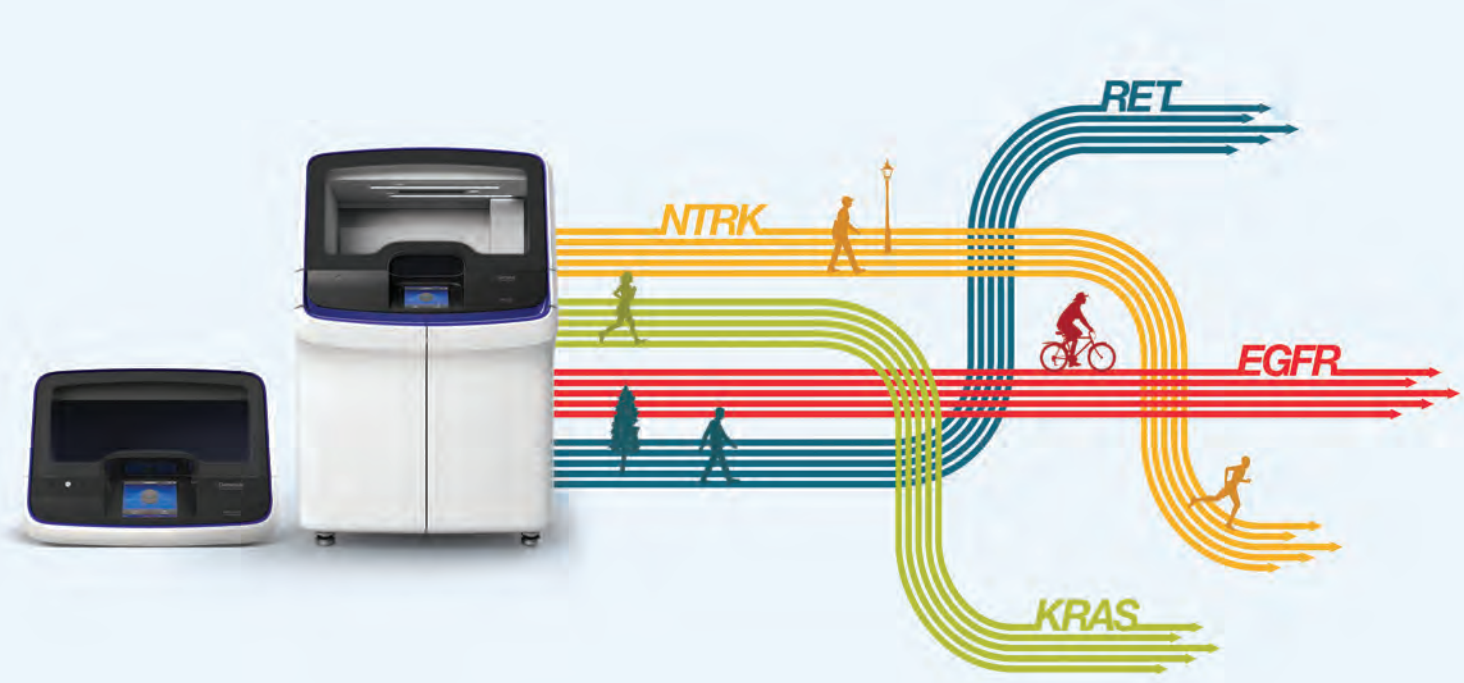

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Leveling Up Outcomes. A trial of more than 1,400 breast cancer patients has shown that genomic-led targeted therapies are able to improve outcomes for patients (1). Patients whose genomic alterations were classified as level I/II according to the ESMO Scale for Clinical Actionability of Molecular Targets were given drug matches that led ultimately to improved outcomes. However, improvements did not occur for patients presenting beyond level I/II when using the same therapy selection process.

Genomic Deep Dive. Analysis of roughly 30,000 Crohn's disease (CD) patients has spotlighted 10 associated genes. The research found numerous genes linked with CD onset and susceptibility, four of which lie within established CD loci. A single coding variant was significantly associated in nine of the genes; the 10th shows an increase in very rare coding variants (2).

Global Susceptibility. A meta analysis of 61,047 lung cancer cases has revealed five new susceptibility loci across diverse populations via genome-wide association studies performed across continental populations (3). DNA damage assays suggested that some of the genes, including *IRF4,* have an influence by promoting endogenous DNA damage.

CAD You Believe It? A genome-wide association study of close to 250,000 cases of coronary artery disease has revealed close to equal heritability across ancestral groups (4). In total, 95 novel loci were detected, eight of which were significant in Black and Hispanic groups. Moreover, the team found 15 loci across populations that overlap with established loci for clinical coronary artery disease.

The Cancer Detector. A novel assay is able to create comprehensive profiles of the epigenetics of plasma-isolated nucleosomes, DNA methylation, and cancer-specific biomarkers with high accuracy, a new study has shown (5). The system detects six active and repressive histone modifications at high-resolution and offers insight into their ratios and combinatorial patterns via single-molecule imaging.

A Long Read. A large dataset of human long-read RNA sequences has been built from a collection of 88 samples from Genotype-Tissue Expression tissues, allowing a team to identify over 70,000 novel transcripts for annotated genes, validating protein expression for 10 percent of them (6). The work provides new insights into specific transcript alterations caused by common and rare genetic variants.

See references online at: tp.txp.to/new-in-molecular-pathology

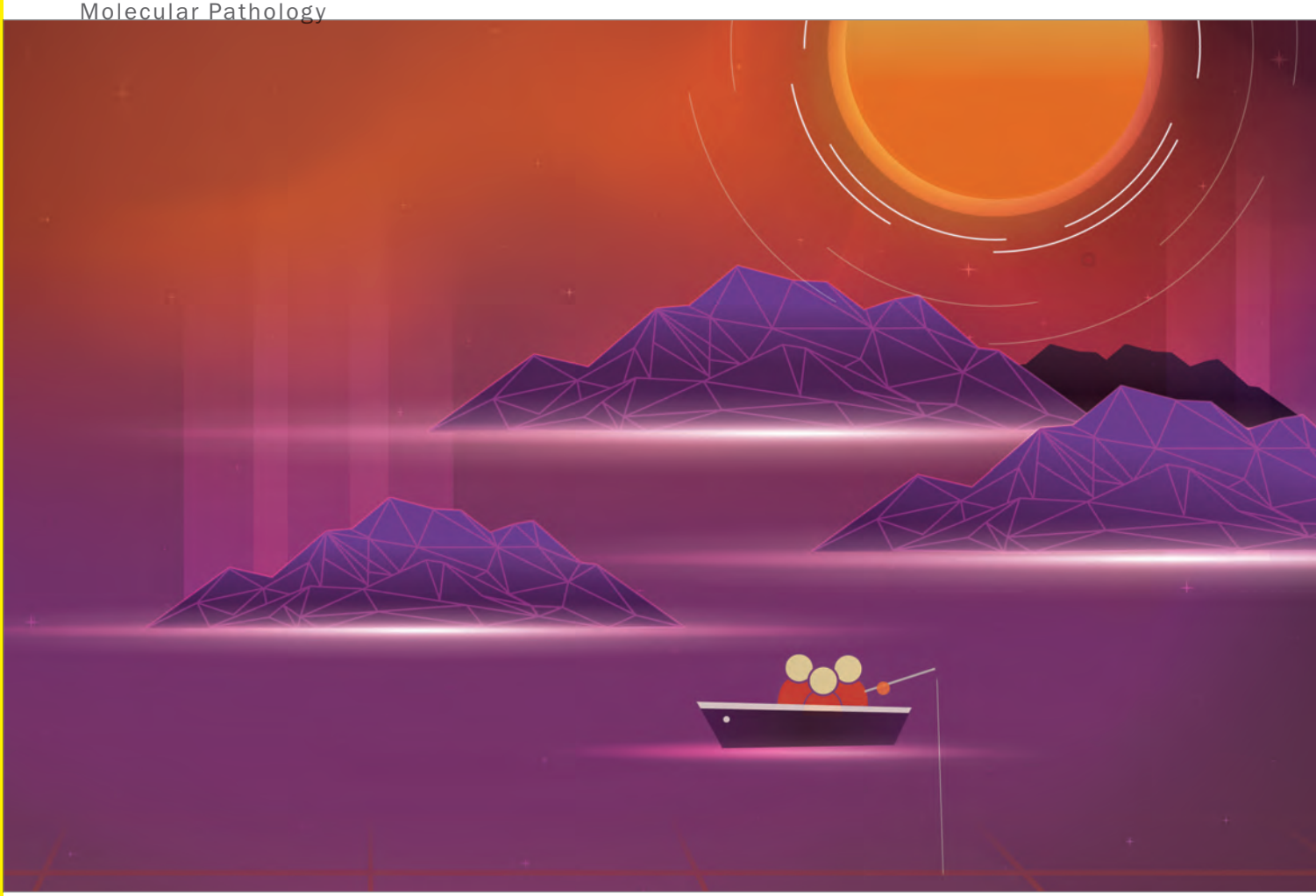
IN OTHER NEWS

In the right circles. *The CHRONOS clinical trial has shown that genotyping tumor DNA in blood can help inform metastatic colorectal cancer patient care (7), supporting circulating tumor DNA as a valid strategy to help determine treatment efficacy.*

Hey, St. Jude. *A tool created by scientists at St. Jude Children's Research Hospital can find safe locations to slot genes into human DNA, potentially offering an opportunity to improve the safety and efficacy of cell and gene therapies (8).*

Myocardial map. *A new high-resolution map of human cardiac models after myocardial infarction identifies disease-specific cardiac cell states (9). The data are publicly available for future studies (10).*

Genetic jeopardy. *Information based on over 735,000 individuals indicates that rare genetic variants have a significant effect on disability-adjusted life years (11). Some common variants' effects on risk are similar to that of high sodium intake and low exercise.*



Future Trends in Spatial Biology

The use of spatial biology in laboratory medicine is on the rise – but what does the future hold?

By Tian Yu

Pathologists are on the front lines of precision medicine, working to diagnose and treat diseases with ever-increasing accuracy – and, as our understanding of the human body improves, so must our diagnostic tools and techniques.

A new and developing area, spatial biology, holds promise for creating novel and someday clinically significant insights. In the past few decades, the study of cell morphology and molecular biology have followed separate paths – microscopy was used to examine cellular structure and dynamics, whereas genomics and transcriptomic methods were used to study gene expression in homogenized tumor samples containing millions of cells and lacking in situ context. Recent technological advancements have revolutionized our capacity to quantify cellular heterogeneity with spatial context, opening the doors to advanced study of the tumor microenvironment and treatment responses.

Spatial biology is a relatively new field of study in which cells and tissues are observed in more or less intact 2D or 3D surroundings. In the same way that GPS records location coordinates to generate a map and track specific targets of interest, cellular- and molecular-level applications can also follow a similar logic. We can use these techniques to help map out a cell's spatial architecture and understand how it interacts with its surroundings, allowing us to see things that would be unobtainable using bulk sequencing or other technologies.

Going forward – and as technology matures – spatial biology will play an increasingly vital role in untangling the complexities of diseases. What does the near future hold? In 2022, I anticipate the following trends will make headway in the field.

1. Automation

Spatial biology may surprise those who are new to the technology because it

involves a wide range of methodologies (such as cyclic immunostaining, in situ sequencing via barcodes, or imaging mass cytometry) and different target analytes (proteins, RNA, and more) (1). However, many current spatial biology approaches have stages that must be completed by hand, making them low-throughput, time-consuming, and unscalable.

In recent years, a few industry solution providers and academic research groups have focused on the end-to-end workflow needed for high-throughput, multi-omics spatial tissue profiling with minimum user input (2, 3, 4, 5). The procedures for each of these automated solutions vary largely – from expensive, closed-system instruments, liquid-handling robots, and specialized equipment to open-source protocols that use existing laboratory equipment.

2. Resolution

There has been great demand for cellular – or even subcellular – spatial resolution for molecular targets in biological sciences, driven by both scientific curiosity and the potential to gain important new insights into subcellular components and biologically significant interactions between neighboring cells. It is worth noting that, in many cases, particularly those using spatial barcoding, high resolution is not required – in fact, research shows that significant discoveries were made with spatially barcoded technologies with 55–100-micron resolution, the equivalent of cellular "neighborhoods" (6, 7). Nonetheless, spatial resolution has become a common metric for prospective users to compare the performances of different technologies and test developers are highly motivated to make improvements in this area.

In general, pathologists will favor single cell level resolution. Current spatially barcoded array techniques are at a disadvantage compared with image-based techniques in spatial resolution, but they are moving quickly to catch up – dropping the 100 µm resolution (6) to a few hundred nanometers (8).

3. Multi-omics and multiplex

Individuals have unique genomic, transcriptomic, and proteomic profiles – all of which can play a role in disease progression and treatment response. Though studying the transcriptome provides gene expression data on a temporal snapshot of potentially labile RNA molecules present in a cell or tissue at a given point in time, proteomic detections provide more accurate phenotypic information about present and active proteins.

Studies have often demonstrated a disconnect between changes happening at the RNA level and those at the protein level. One explanation is that many post-transcriptional events, including translation, mRNA decay, and splicing, can affect gene expression. A multi-omic approach that integrates spatial proteomics data with spatial transcriptomics can provide a more comprehensive understanding of tissue biology. Additionally, more biomarkers can now be detected within their natural spatial confines, thanks to advanced technologies such as multiplex fluorescence, DNA, RNA, and metal isotope labeling.

The number of protein targets may be expanded by iterative techniques that include repeating rounds of antibody labeling and detection with a multiplex count of more than 50 targets (1), but such cyclic processes can be labor and time-intensive. This can be avoided by relying on secondary readouts such as deep sequencing, which could quantify up to 100 proteins in a single staining and scanning procedure. Sequencing-based spatial transcriptomic methods provide the highest level of multiplexing, demonstrating spatially resolved information on 10,000 or more genes (1).

4. Artificial intelligence

Spatial biology techniques generate a large amount of data, often in the form of images – but with the sheer volume and complexity of data comes a range of new analysis challenges. Multi-omics, multiplexing, and multimodal data integration (e.g., processing the same piece of tissue for single-cell RNA-seq and spatial biology in parallel) offer a wealth of information, but much of it is left untapped or underused.

There is an unmet need in the community for improved computational tools to extract quantitative information from images and sequencing data. The resulting data points and spatial features need to be first linked by tissue morphology, most commonly the H&E-stained tissue slide, and then with clinical information to produce new insights. In recent years, AI has been used in image analysis for various tasks such as classification, segmentation, and tracking. In particular, it is increasingly used in histopathology to help pathologists with disease detection and prognosis. The best part? AI can also be used for spatial data analysis.

5. Sample quality

One of the key challenges in spatial biology is tissue acquisition. To acquire high-quality tissue samples, we must pay attention to how the tissue is collected, processed, and stored. Advances in the multiplex analysis of proteins on FFPE sections are bringing patients one step closer to next-generation pathology, in which companion diagnostic tests suggest therapeutic actions for individualized medicine. However, moving nascent transcriptomic technologies from the lab to clinics has several drawbacks, including variations encountered in fixation and potential

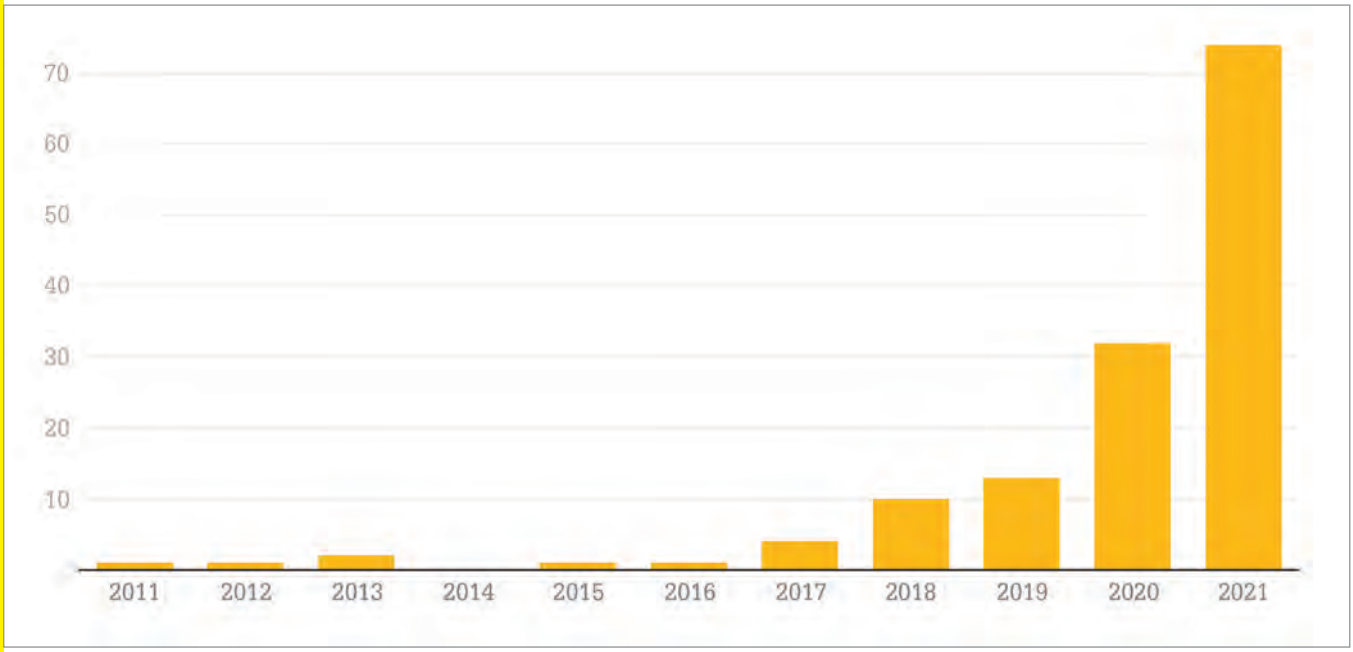


Table 1. The number of spatial multi-omics articles per year on PubMed. Search query: spatial multi-omics.

analyte degradation.

Under environmental conditions, RNA is an unstable molecule that is easily degraded by RNase. FFPE samples are typically fixed for a minimum of 24 hours in 10 percent neutral buffered formalin, which cross links and thus damages the molecule – and the high temperatures used during FFPE processing can further degrade it. The integrity of RNA can vary because clinical samples are frequently stored in fixative and the time between taking the biopsy and fixation (cold ischemic time) may differ from sample to sample. To be able to translate spatial omics methods to the clinic, sample quality must improve in terms of analyte preservation and workflow robustness.

6. Standardized diagnostic biomarkers

Thousands of biomarkers can be detected from the same tissue, but what does that mean to the pathologist who has to arrive at a diagnosis? It is important to remember that spatial data should not be viewed in isolation; rather, they should be considered in the context of clinical and complementary molecular information. The problem is that it often proves difficult to understand how biomarker data may be used in clinical applications due to the lack of cross-modality standardization of cell phenotype definitions. Keith Wharton, Vice President and Medical Director of Ultivue and a board-certified pathologist with a background in molecular biology, said, "Right now, everybody has a different opinion about the meaning, for example, of T cell exhaustion versus dysfunction versus other terms to indicate T effector cells that don't kill the tumor," he says, referring to the lack of consensus in T lymphocyte biomarkers and their clinical application. "Can we use multiple modalities, perhaps on samples from multiple species, to agree as a community on which standardized cell phenotypes should be measured and how?" Of potential relevance, the Partnership for Accelerating Cancer Therapies (PACT) collaborates with the pharmaceutical industry to facilitate systematic and uniform clinical testing of biomarkers to enable consistent generation of data, standardized assays to support data reproducibility and comparability of findings across studies, and the discovery and validation of new biomarkers.

Spatial biology is a powerful new tool that we are just beginning to harness for clinical benefit, but it has the potential to unlock many of the mysteries of disease and cellular interactions. Exciting progress has already been made in the field, and it's clear that spatial biology is here to stay. Is there a role for it in clinical practice? "The connection is indirect," said Richard Levenson, Professor of Pathology and Laboratory Medicine at UC Davis, who made significant contributions to the development of multiple spatial multiplexed imaging technologies. "The role of spatial biology for pathologists is on the research side, so they can understand disease and the biology behind it." In pathology, the objective is to find practical, inexpensive solutions that lead to a diagnosis and prognosis – and, although spatial biology is heading in the right direction, it still has a long road ahead in its transition from bench to bedside.

Tian Yu is Chief Scientific Officer of Truckee Applied Genomics, Reno, Nevada, USA.

See references online at: tp.txp.to/future-trends

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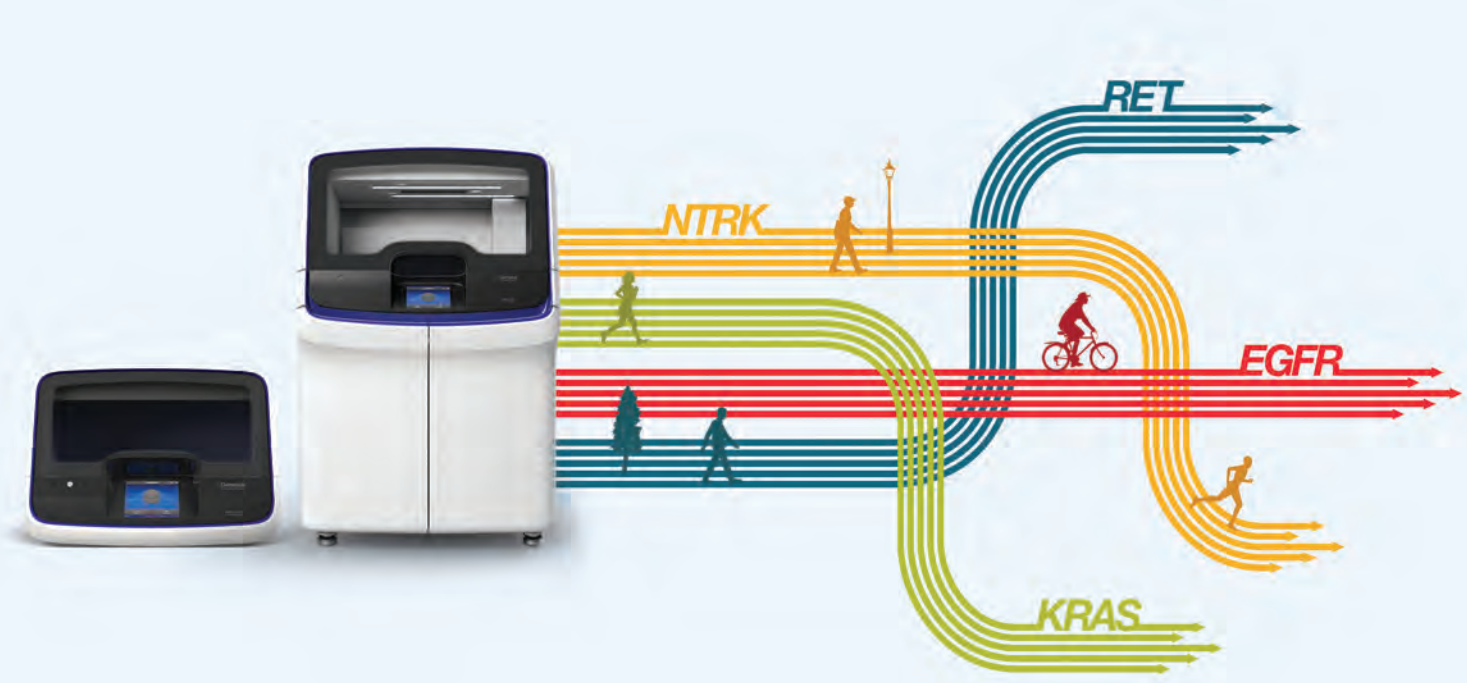

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Into the Unknown. Scientists have scrambled to identify the cause of rising cases of acute hepatitis with unknown etiology in children. Now, two studies have found high adeno-associated virus 2 (AAV2) levels in blood or liver cells in cases of unexplained hepatitis (1). The class II HLA-DRB1*04:01 allele was also identified in pediatric cases, suggesting increased genetic susceptibility (2).

Spotting Sepsis. Use of machine learning-based Targeted Real-time Early Warning Systems (TREWS) is associated with reduced mortality rate and improved outcomes in sepsis patients (3). What factors drive adoption? Researchers suggest that knowledge of, experience with, and positive attitudes toward TREWS are essential to increase their clinical impact (4).

New Symptoms, New Course. A descriptive report of monkeypox cases in central London has identified new clinical presentations of the current outbreak, including rectal pain and penile edema (5). A variable temporal association between mucocutaneous and systemic features was also observed, suggesting a new clinical course to the disease.

Something in the Genes. Analyzing 125,584 cases across 60 studies from 25 countries, the COVID-19 Host Genetics Initiatives has provided the first update on its efforts to map the human genetic architecture of COVID-19 (6). The project has reported 23 genome-wide significant loci associated with disease susceptibility and severity, including *SFTPD, MUC5B,* and *ACE2*.

Taming of the Shrew. A new virus, Langya henipavirus, has been identified in China, causing symptoms ranging from a cough to severe pneumonia (7). It is thought to be transmitted via contact with wild shrews, but so far there has been no indication of human-to-human transmission.

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IN OTHER NEWS

Cha cha SLIDE. *A saliva-based self-test with RT-LAMP in a mobile device platform detects SARS-CoV-2 and delivers results to a smartphone app within 45 minutes (8). The test achieved a limit of detection of 5 copies/μL and, using clinical samples, demonstrated good agreement with RT-PCR results.*

Global need. *A lack of evidence-based clinical management guidelines for monkeypox may interfere with safe and effective intervention worldwide (9). Existing guidelines lack sufficient detail and are not inclusive of diverse groups.*

Under the weather. *Over half of all human pathogenic diseases have been aggravated by climate change, with research revealing 1,006 unique pathways of climatic hazards leading to infectious disease spread (10).*

My test on paper. *An inexpensive paper-based assay detects SARS-CoV-2 variants in under 30 minutes, offering streamlined wide-scale testing outside the laboratory setting (11).*

Something in the Water

Seeking out DNA-based tools for effective wastewater epidemiology

By Francisco Bizouarn

Scientists agree that infectious diseases are one of the most critical threats to global public health today (1) – an opinion borne out by COVID-19, monkeypox, and more. To help mitigate the spread of disease, communities have begun to rely more heavily on early detection through wastewater-based epidemiological testing using molecular techniques.

Water testing has historically focused on testing sources for the presence of parasites, bacteria, and viruses upstream of human contact – but the COVID-19 pandemic spotlighted the potential of using wastewater as a sentinel system to monitor and predict outbreaks by detecting and quantifying pathogens shed from infected individuals. This type of surveillance enables scientists to detect and quantify small changes in pathogen concentrations in wastewater from a pooled community such as a building, campus, or geographic region. With this information, researchers can potentially forecast ebbs and flows in disease levels and help local authorities prepare accordingly.

The go-to technology for detecting and quantifying nucleic acids is quantitative PCR (qPCR). However, when analyzing low target molecule concentrations, many scientists have found that qPCR is not sensitive enough for wastewater testing and that other techniques are better suited to the task. For example, droplet digital PCR (ddPCR) is a molecular counting technology, related to classic qPCR, that partitions a PCR reaction into tens of thousands of sub-reactions and analyzes each partition for the target molecule of interest. This technique is particularly attractive for wastewater testing because of its precision at low levels, multiplexing capability, and tolerance to inhibition.

Because it's a direct counting method combined with distribution analysis, ddPCR is not dependent on standards for its precision and accuracy. At low concentrations (a few molecules per reaction), quantification is practically only limited by stochastic effects from sampling.

The partitioning process also simplifies the chemistry surrounding multiplexing. Higher-abundance targets no longer "starve out" lower-abundance targets, so four, eight, or even 12 targets can be amplified and analyzed in the same reaction. This allows for larger panels and the addition of recovery reference targets.

As a post-amplification analysis reaction, ddPCR is not dependent on amplification efficiency for its accuracy and can therefore tolerate moderate inhibition. This is advantageous when testing wastewater samples because, even after purification, they can contain significant inhibitors of the PCR process.

Now more than ever, government authorities are relying on wastewater testing, leveraging molecular techniques to monitor infectious disease outbreaks in our communities. Technologies such as ddPCR permit more refined monitoring and allow efficient, definitive localized surveillance, enabling authorities to predict future surges and enact policies to mitigate outbreaks and save lives.

Francisco Bizouarn is Market Development Manager, Digital Biology Group, Bio-Rad Laboratories, Tampa, Florida, USA.

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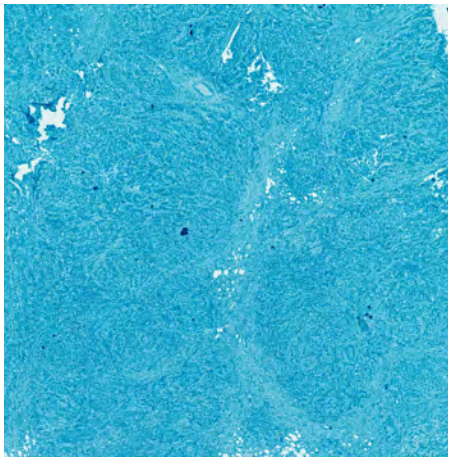
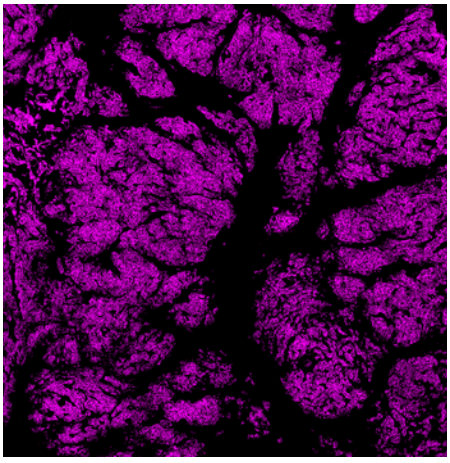
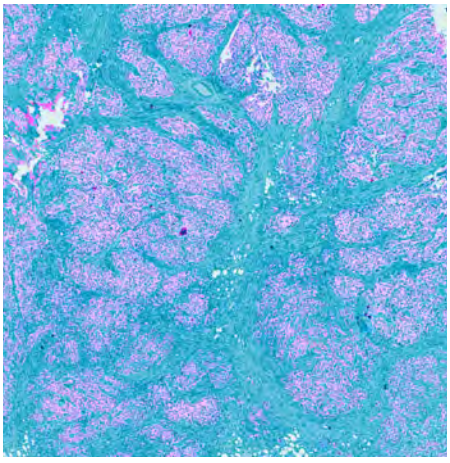
Credit: Bio-Rad

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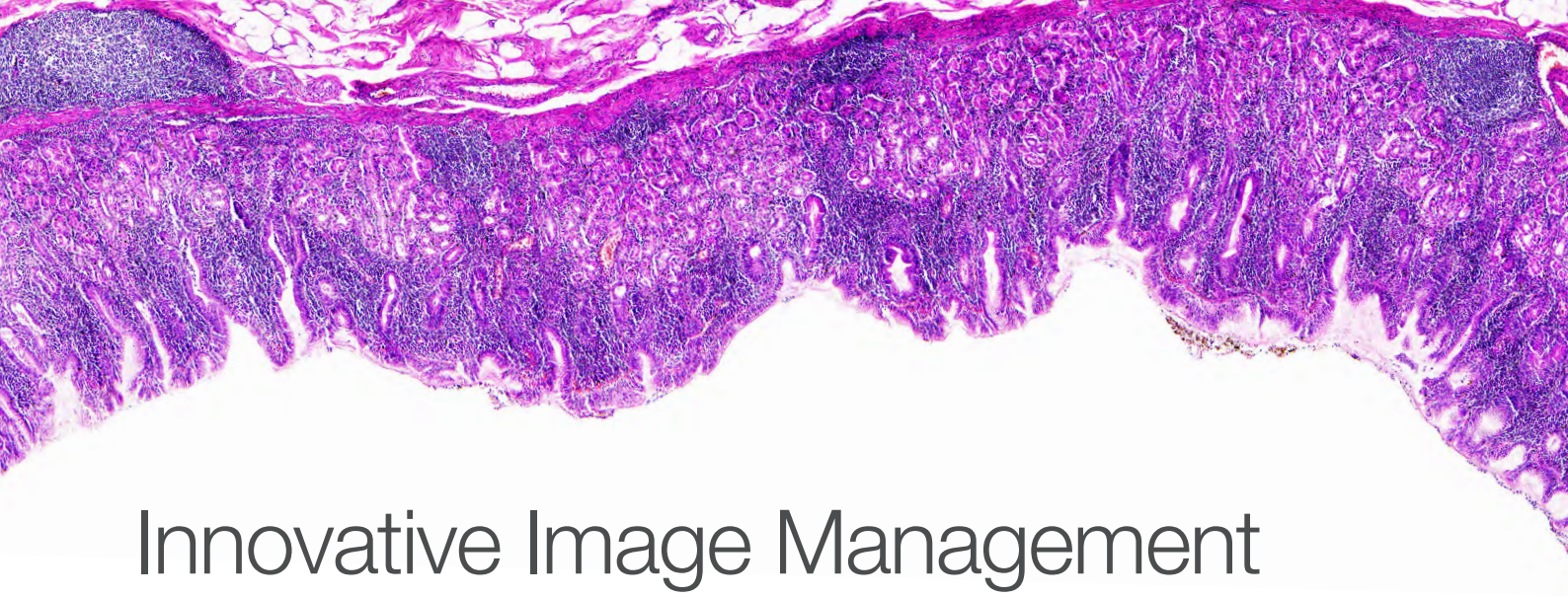

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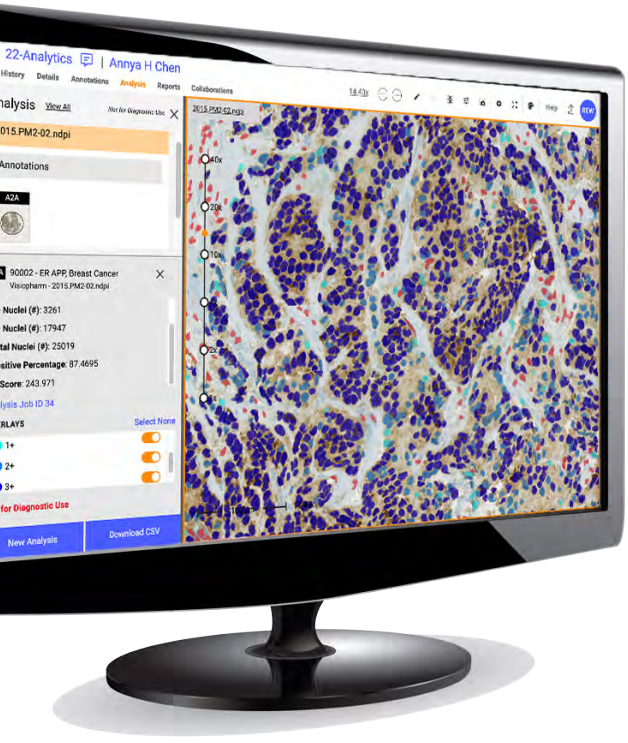

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Solving the Challenge of COVID-19 with Whole-Slide Imaging

Examining the capabilities of automated slide scanners that help accelerate COVID-19 research

By Wei Juan Wong

Two years into the pandemic, COVID-19 still presents a challenge for pathologists and researchers seeking to better understand how the disease affects the human body. Whole-slide imaging is central to this work because high-resolution images allow researchers to visualize and evaluate damage to tissues and cells. Though whole-slide images can be captured manually, the high volume of images needed for research requires a faster approach – so many researchers are turning to automated slide scanners to speed up the process and obtain higher-quality images. For example, Si Wang and colleagues used an automated slide scanner to study the lungs of patients with COVID-19 (1), whereas Ni Huang and others did the same to research COVID-19 infection of the oral cavity and saliva (2).

Automated slide scanners include many features to help researchers study infectious diseases – and a better understanding of COVID-19 means better patient outcomes. Automated slide scanners play an important role in disease research by providing fast, high-resolution imaging for quantitative analysis and publication. Here are six key scanner features I've found helpful in my own work.

1. Automated detection of samples during the scanning process.

Scanning whole slides of tissue sections and other samples for COVID-19 research can be time-consuming if the software scans both sample and background; automated sample detection speeds up the process by only detecting and scanning the sample. With accurate autofocus and high magnification on just the sample area, researchers can obtain all the information they need from the image while saving time scanning.

2. Intuitive software for an easy scan setup.

Easy-to-use scanner software makes it simple for researchers of all experience levels to obtain high-quality virtual slide images of tissues and cells. For example, software that saves project settings for specific samples enables researchers to start a quick batch scan with minimal supervision. This greatly reduces setup time and increases efficiency, especially for fluorescence imaging – a method often used in COVID-19 research to label SARS-CoV-2 RNA.

3. Accurate color reproduction of tissues and stains.

In COVID-19 research and other pathology applications, accurate color reproduction is vital to view stained samples as they are seen with the naked eye. Various technologies – such as modern, energy-efficient LEDs that can match the spectral characteristics of a halogen lamp – support accurate color reproduction. Furthermore, color-corrected cameras and ICC profiles facilitate accurate color and intensity reproduction on computer monitors. These features enable the purple, cyan, and pink stains often used in COVID-19 research to show up correctly in whole-slide images.

4. Automated immersion oil dispensing.

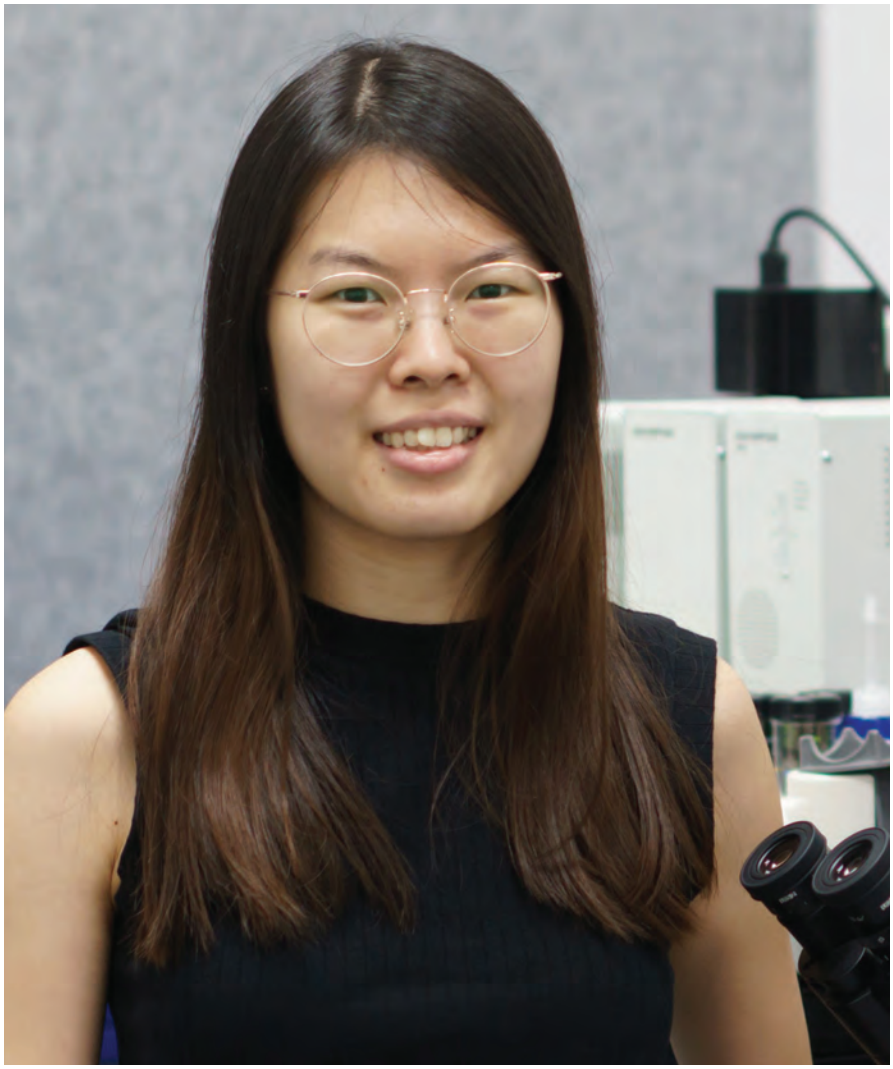
High-resolution images let researchers see small details, such as viruses. Scanning slides with immersion oil can provide high-resolution images to help researchers visualize infections;

however, this process is typically done manually and can be time-consuming. An automated oil dispenser integrated into the scanner software can add the correct amount of oil to the scan area and remember the slides that have already been scanned with oil. This automated feature gives oil scanning the same convenience as using a dry objective.

"Automated slide scanners include many features to help researchers study infectious diseases – and a better understanding of COVID-19 means better patient outcomes."

5. Flexible imaging to visualize infections.

Depending on the sample and application, COVID-19 researchers may need a range of observation methods to visualize infections or stains. Scanners with multiple imaging modes enable researchers to clearly see different details of the sample, such as tissue or RNA. Having the option to mix and match methods in a batch scan offers even greater flexibility and efficiency, because researchers can process multiple sample types in one scan.



Wei Juan Wong

6. Links to image management systems.

COVID-19 research requires a large number of high-resolution images to understand how the virus affects the human body. Multiple images are stitched together into one large, tiled image to enable easy visualization of tissues or cells. Without an easy way to store and view large files, image management can become cumbersome and disorganized, but automated slide scanners that link to an image management system can enable image sharing and collaboration via a simple web viewer. This feature eliminates the need to transfer large images while retaining the ability to view high-resolution data.

Wei Juan Wong is an Application Specialist, Digital Slide Scanning Systems, Olympus Soft Imaging Solutions, Germany. Evident Corporation is a new, wholly owned subsidiary of Olympus and is comprised of its former Life Science and Industrial divisions.

References

1. *S Wang et al., "A single-cell transcriptomic landscape of the lungs of patients with COVID-19," Nat Cell Biol, 23, 1314 (2021). PMID: 34876692.*
2. *N Huang et al., "SARS-CoV-2 infection of the oral cavity and saliva," Nat Med, 27, 892 (2021). PMID: 33767405.*

Cloud-Based Cancer Care

Could the key to better cancer care lie outside conventional approaches and thinking?

By Jurgi Camblong

Traditional cancer research cannot move fast enough to keep up with the evolution and prevalence of the disease. The answers we need live inside each of us at a molecular level and we are now beginning to explore this new dimension of information. But humans cannot always process biological data as quickly or efficiently as deep learning machines and algorithms. The vast knowledge base stored within the human body cannot be seen through a microscope; it requires deep exploration into the 0s and 1s of biological data. In short, if we want to make urgently needed progress in cancer care, we need to embrace emerging technologies and data-driven approaches.

Despite noble goals, researchers undoubtedly face challenges in breaking free of a siloed approach through the adoption of cloud-based technologies. Certainly, one major barrier to the widespread application of data analytics in healthcare is that, unlike many other industries, healthcare decisions deal with highly sensitive information, require timely data and action, and may have life-or-death consequences. As a result, research labs often do their own data analysis and keep the data in-house.

Through cloud computing and insight-sharing initiatives, an individual's anonymized health data can be analyzed and compared in the context of a much larger, more complete health data network. Traditionally, though a provider may be able to compare a single patient's current health status with their past medical records or clinical industry guidelines, they do not always have access to active data or "living" examples of effective treatments or strategies that evolve in real time. The COVID-19 pandemic was an extremely apparent example of how much collaborative global medical strategies are lacking and where regional hospital networks can benefit from sharing emerging trends and effective therapies worldwide via a single portal.

We must all engage in clinical collaboration on a global scale – we cannot stay working in individual siloes. Here, technology – namely, cloud-based platforms – can allow significant clinical insights to be instantly shared among researchers so that individual clinicians are no longer alone in their fight to improve cancer care.

As we gather increasingly large amounts of data, bioinformaticians can be extremely helpful to medical professionals. They can optimize technologies, such as machine learning, to find relevant (and previously undiscoverable) data to support new findings. For example, a single variant discovered among the data may indicate a possible treatment option, but another biomarker may contradict its usefulness as a viable therapy. By offering a macroscopic view of the data, healthcare professionals can better classify and interpret biomarkers – allowing better decision-making for their patients.

Cancer care is continually evolving, thanks to technology platforms that record and combine longitudinal data with treatment information and outcomes. The next step – or leap – is to use artificial intelligence and machine learning to extract new insights from the data, improve disease prevention, and bring faster, more effective drugs to market. There are already two biomolecular approaches being implemented in the field that show great promise in supporting research for new therapies: the detection of homologous recombination deficiency and RNA fusions (RNA targeting). Clinical studies are starting to reveal how effective these biomarkers can be at not only identifying cancers, but also tracking the effectiveness of treatment for malignancies associated with these molecular abnormalities.

To improve cancer care, we must fine-tune the complicated research process behind clinical discoveries – something I believe we can accomplish with a data-driven medicine approach and advanced analytical tools. At the same time, we must provide oncologists with better insights into patients' reactions to different treatments, as well as the outcomes achieved, to support more informed decisions. In my view, we can only achieve these two goals when health data are not siloed. Access to better data through the cloud enables broader perspectives and makes us smarter as a community.

Profession

Your career
Your business
Your life

Peer-to-Peer, Featuring Mark Wick

Mark Wick reflects on his extensive career, how far pathology has come, and why it remains a leading discipline in medicine

Ivan Damjanov interviews Mark Wick

You are a great advocate for pathology – but, if you could start over, would you choose it again?

Without a doubt! To me, pathology is the perfect blend of basic science and clinical medicine (though I did consider pursuing psychiatry during medical school). At that point, both disciplines involved analytical thinking, but I'm glad I chose to pursue pathology and I would do the same again. I was trained in the "generalist" model of pathology, rather than as a subspecialist. I have always disliked learning something and then not using it, so I try to keep everything I learn practical in some way. Subspecialty pathology is here to stay, but it has its drawbacks as well as strengths.

Though I've always been an active practitioner, my experience in medical writing and editing has also had a significant impact on me. Contributing to and learning from the pathology literature has given me a great deal of satisfaction. My 22 years as Editor of the American Journal of Clinical Pathology were particularly valuable – so much so that I encourage all physicians to strengthen their language and writing skills in any way they can.

"We currently stand at a point where pathologists can begin to paint complete molecular portraits of various diseases – and that will only continue to improve diagnosis and guide personalized treatment."

Tell me about your early days…

I completed my postgraduate training in 1978 at the Mayo Clinic in Rochester, Minnesota, during which time there were several illustrious people on the pathology faculty: David Dahlin, George Farrow, Louis Weiland, and others. I had no idea how fortunate I was to have them as teachers, but I quickly realized. The common thread was their firm grasp of the literature and how to practically apply it to hospital pathology. My love for doing projects and writing papers began during residency and definitely helped me in my future career. Every week, the Mayo Publication Office would issue a list of registered manuscript topics. People then had the option of contacting the responsible parties and discussing possible conflicts and cooperative ventures before the paper was finally formulated. My residency year group of five people collectively published over 50 manuscripts during our training. When I joined Juan Rosai and Louis "Pepper" Dehner on the faculty at the University of Minnesota, that love of publishing grew even more. We had a very stimulating and cooperative group there, as well as the time and facilities to follow our interests.

What were the strengths of your department at the University of Virginia?

Excellence in diagnostic work, availability of laboratory resources, good leadership,

and an enthusiasm for doing professional projects and publications were just some of the department's positive traits. The house staff were also exceptionally talented. We used the "generalist" sign-out system for most cases and every faculty member took on general cases as and when they came in. However, our main strength was an open-door atmosphere. If I was stuck on a case, my resident and I walked down the hall to show the staff member with a special interest in that area; both the resident and I learned and our colleagues got to see interesting and challenging cases.

You were one of the early promoters of evidence-based medical practice. Tell us more about it…

Evidence-based medical practice (EBMP) is based on the critical analysis and application of published literature, rather than on customs, habits, or personality traits. EBMP is the reason that, wherever possible, I try to provide pertinent references in my consultation reports. I believe pathology has made progress in implementing the principles of EBMP, but we still have work to do – for instance, teaching residents EBMP through journal clubs and interdepartmental conferences.

Throughout your career, you have tried to solve pathology's technical problems. Was this out of interest or necessity?

It was both by inclination and necessity. Looking back on my time in practice, I am astonished at how effectively and extensively technology has been integrated into pathology. The issue now is to know when to use a particular technique – and when not to. For example, we have all experienced the "immunoconfusion" brought on by shotgunning immunohistochemical workups of difficult cases.

Do you think molecular biology will eventually replace immunohistochemistry?

Just as electron microscopy and traditional histochemistry still have (or should have) their places in pathology practice, I think immunohistochemistry will continue to provide value. Technologies are best used together and judiciously, rather than simply swapping one for another. For molecular biology, data management will be key. Medical statisticians are crucial to making decisions on the value of molecular methods and, although many genetic aberrations can now be identified, their biological significance is often still uncertain. We currently stand at a point where pathologists can begin to paint complete molecular portraits of various diseases – and that will only continue to improve diagnosis and guide personalized treatment.

What topics do you think will define pathology in the 21st century?

Several topics are disappearing from pathology practice for various reasons, but I believe the following traits will continue to define the field:

- excellence in morphologic analysis
- skills in medical statistics and medical economics
- critical evaluation of published literature, especially on "new" technology
- consolidation of resources and efforts
- renewed efforts to revive pathology education during medical school and beyond
- a return to honest, critical presentations of "new" information at pathology meetings
- efforts to return physicians to leadership positions in laboratory medicine and individual hospital laboratories
- finding innovative ways to give academics the time and resources to succeed professionally

The future looks bright for our profession – it remains only for us to lead the way!

Mark Wick is Professor Emeritus of Pathology, Division of Surgical Pathology, University of Virginia School of Medicine, Charlottesville, Virginia, USA.

Ivan Damjanov is Professor Emeritus of Pathology at the University of Kansas, Kansas City, USA.

A Stain on the Lab

When it comes to advanced staining, we can do better

Advanced Staining: Is It an Art or a Science?

Laboratory professionals, let's address the elephant in the room: the staining status quo is simply not enough. The world of advanced staining is often a complicated, delayed, and inaccurate place. It's been this way for so long that to many it may not even register as a problem, but it is likely making everyone's work harder than it needs to be. We've been questioning this status quo – and we think it's time for change.

Pathologists know better than most the importance of a diagnosis. When the diverging roads of treatment lie ahead – one path leading to recovery and the other the opposite – it's the pathologist who must point the way. But with no standard quality for staining, it has always been difficult for pathologists to give those vital directions with confidence.

For decades, quality has been key in staining and immunohistochemistry (IHC) – but everything from less-successful antibodies to insufficient dilutions has meant that one in three slides aren't accurate enough for a correct diagnosis (1). That's every other person you walk by on the street whose diagnosis might be compromised. Add an abundance of variables to the mix and you get the current status quo – a situation where many patients are losing out.

The vast majority of unsuitable slides are the result of weak staining that results in false negatives; the remainder are caused by poor signal-to-noise ratio that generates false positives (1). And yet, policies and procedures for staining are largely decided on a lab-by-lab basis. This failure is a result of delayed standardization, which makes suboptimal lab performance all the more likely.

At Sakura Finetek Europe, we're questioning the situation in which laboratory medicine finds itself. Better yet, we're offering a solution – one that could see staining become the fast, accurate, trustworthy process it needs to be. With a growing interest in digital pathology, there is an equally growing need for IHC standardization and optimization, but we have decades' worth of catching up to do. Likewise, recent IVDR regulation in the EU means that compliance is required now, not at some distant future point.

To help labs catch up, we have been hard at work creating solutions such as our Tissue-Tek Genie® that yield reliable, standardization-powered results through closed-loop technology. These systems use single protocols for over 130 optimal antibodies, offering the convenience of reduced IHC assay calibration and validation. We combine this with third-party quality control and by only offering optimal scoring antibodies – giving reassurance to pathologists and patients who need quick, reliable, and accurate results more than ever.

We know that pathologists love the flexibility in advanced staining, but it's that exact same flexibility that is causing confusion. Our aim is to rid the advanced staining market of its blemishes, to rub away the variables and the subjectivity – but the work has to start now. What we all have to do is provide a standard for optimal stain quality. For us, that's achieved through offering automated start-to-end solutions with Advanced SMART Automation for tissue diagnostics that set a new standard – enabling you to achieve optimal results.

It's time to wipe away this stain on the lab. Join us in creating a new staining standard, so that we can improve the lives of patients and lab professionals everywhere. Optimal only – make it simple.

Reference

1. *M Vyberg, S Nielsen, "Proficiency testing in immunohistochemistry—experiences from Nordic Immunohistochemical Quality Control (NordiQC)," Virchows Arch, 468, 19 (2016). PMID: 26306713.*

ENHERTU®
fam-trastuzumab deruxtecan-nxki
20 mg/mL INJECTION FOR INTRAVENOUS USE

HER2-low is a new classification of HER2 expression in mBC[1,2]

A targeted treatment for eligible patients with HER2-low metastatic breast cancer is now available[1]

Score and report HER2-low expression (defined as IHC 1+ or IHC 2+/ISH–).[1]

~60% of patients with HER2-negative mBC may now be classified as HER2-low[3]

Learn more at ScoreHER2Low.com

Important Safety Information

Indication

ENHERTU is a HER2-directed antibody and topoisomerase inhibitor conjugate indicated for the treatment of adult patients with unresectable or metastatic:

- HER2-low (IHC 1+ or IHC 2+/ISH-) breast cancer who have received a prior chemotherapy in the metastatic setting or developed disease recurrence during or within 6 months of completing adjuvant chemotherapy

WARNING:
INTERSTITIAL LUNG DISEASE and EMBRYO-FETAL TOXICITY

- **Interstitial lung disease (ILD) and pneumonitis, including fatal cases, have been reported with ENHERTU. Monitor for and promptly investigate signs and symptoms including cough, dyspnea, fever, and other new or worsening respiratory symptoms. Permanently discontinue ENHERTU in all patients with Grade 2 or higher ILD/pneumonitis. Advise patients of the risk and to immediately report symptoms.**
- **Exposure to ENHERTU during pregnancy can cause embryo-fetal harm. Advise patients of these risks and the need for effective contraception.**

Contraindications

None.

Warnings and Precautions

Interstitial Lung Disease / Pneumonitis

Severe, life-threatening, or fatal interstitial lung disease (ILD), including pneumonitis, can occur in patients treated with ENHERTU. Advise patients to immediately report cough, dyspnea, fever, and/or any new or worsening respiratory symptoms. Monitor patients for signs and symptoms of ILD. Promptly investigate evidence of ILD. Evaluate patients with suspected ILD by radiographic imaging. Consider consultation with a pulmonologist. For asymptomatic ILD/pneumonitis (Grade 1), interrupt ENHERTU until resolved to Grade 0, then if resolved in ≤28 days from date of onset, maintain dose. If resolved in >28 days from date of onset, reduce dose one level. Consider corticosteroid treatment as soon as ILD/pneumonitis is suspected (e.g., ≥0.5 mg/kg/day prednisolone or equivalent). For symptomatic ILD/pneumonitis (Grade 2 or greater), permanently discontinue ENHERTU. Promptly initiate systemic corticosteroid treatment as soon as ILD/pneumonitis is suspected (e.g., ≥1 mg/kg/day prednisolone or equivalent) and continue for at least 14 days followed by gradual taper for at least 4 weeks.

Metastatic Breast Cancer and HER2-Mutant Solid Tumors (5.4 mg/kg)

In patients with metastatic breast cancer and other solid tumors treated with ENHERTU 5.4 mg/kg, ILD occurred in 12% of patients. Fatal outcomes due to ILD and/or pneumonitis occurred in 1.0% of patients treated with ENHERTU. Median time to first onset was 5 months (range: 0.9 to 23).

Neutropenia

Severe neutropenia, including febrile neutropenia, can occur in patients treated with ENHERTU. Monitor complete blood counts prior to initiation of ENHERTU and prior to each dose, and as clinically indicated. For Grade 3 neutropenia (Absolute Neutrophil Count [ANC] <1.0 to 0.5 x 10^9/L), interrupt ENHERTU until resolved to Grade 2 or less, then maintain dose. For Grade 4 neutropenia (ANC <0.5 x 10^9/L), interrupt ENHERTU until resolved to Grade 2 or less, then reduce dose by one level. For febrile neutropenia (ANC <1.0 x 10^9/L and temperature >38.3° C or a sustained temperature of ≥38° C for more than 1 hour), interrupt ENHERTU until resolved, then reduce dose by one level.

Metastatic Breast Cancer and HER2-Mutant Solid Tumors (5.4 mg/kg)

In patients with metastatic breast cancer and other solid tumors treated with ENHERTU 5.4 mg/kg, a decrease in neutrophil count was reported in 65% of patients. Sixteen percent had Grade 3 or 4 decreased neutrophil count. Median time to first onset of decreased neutrophil count was 22 days (range: 2 to 664). Febrile neutropenia was reported in 1.1% of patients.

Left Ventricular Dysfunction

Patients treated with ENHERTU may be at increased risk of developing left ventricular dysfunction. Left ventricular ejection fraction (LVEF) decrease has been observed with anti-HER2 therapies, including ENHERTU. Assess LVEF prior to initiation of ENHERTU and at regular intervals during treatment as clinically indicated. Manage LVEF decrease through treatment interruption. When LVEF is >45% and absolute decrease from baseline is 10-20%, continue treatment with ENHERTU. When LVEF is 40-45% and absolute decrease from baseline is <10%, continue treatment with ENHERTU and repeat LVEF assessment within 3 weeks. When LVEF is 40-45% and absolute decrease from baseline is 10-20%, interrupt ENHERTU and repeat LVEF assessment within 3 weeks. If LVEF has not recovered to within 10% from baseline, permanently discontinue ENHERTU. If LVEF recovers to within 10% from baseline, resume treatment with ENHERTU at the same

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; mBC, metastatic breast cancer.

Please see accompanying Brief Summary of Prescribing Information, including Boxed WARNINGS, and Medication Guide.

Important Safety Information (cont'd)

Warnings and Precautions (cont'd)

dose. When LVEF is <40% or absolute decrease from baseline is >20%, interrupt ENHERTU and repeat LVEF assessment within 3 weeks. If LVEF of <40% or absolute decrease from baseline of >20% is confirmed, permanently discontinue ENHERTU. Permanently discontinue ENHERTU in patients with symptomatic congestive heart failure. Treatment with ENHERTU has not been studied in patients with a history of clinically significant cardiac disease or LVEF <50% prior to initiation of treatment.

Metastatic Breast Cancer and HER2-Mutant Solid Tumors (5.4 mg/kg)

In patients with metastatic breast cancer and other solid tumors treated with ENHERTU 5.4 mg/kg, LVEF decrease was reported in 3.6% of patients, of which 0.4% were Grade 3.

Embryo-Fetal Toxicity

ENHERTU can cause fetal harm when administered to a pregnant woman. Advise patients of the potential risks to a fetus. Verify the pregnancy status of females of reproductive potential prior to the initiation of ENHERTU. Advise females of reproductive potential to use effective contraception during treatment and for at least 7 months following the last dose of ENHERTU. Advise male patients with female partners of reproductive potential to use effective contraception during treatment with ENHERTU and for at least 4 months after the last dose of ENHERTU.

Additional Dose Modifications

Thrombocytopenia

For Grade 3 thrombocytopenia (platelets <50 to 25 x 10^9/L) interrupt ENHERTU until resolved to Grade 1 or less, then maintain dose. For Grade 4 thrombocytopenia (platelets <25 x 10^9/L) interrupt ENHERTU until resolved to Grade 1 or less, then reduce dose by one level.

Adverse Reactions

Metastatic Breast Cancer and HER2-Mutant Solid Tumors (5.4 mg/kg)

The pooled safety population reflects exposure to ENHERTU 5.4 mg/kg intravenously every 3 weeks in 984 patients in Study DS8201-A-J101 (NCT02564900), DESTINY-Breast01, DESTINY-Breast03, DESTINY-Breast04, and NCT04644237. Among these patients 65% were exposed for >6 months and 39% were exposed for >1 year. In this pooled safety population, the most common (≥20%) adverse reactions, including laboratory abnormalities, were nausea (76%), decreased white blood cell count (71%), decreased hemoglobin (66%), decreased neutrophil count (65%), decreased lymphocyte count (55%), fatigue (54%), decreased platelet count (47%), increased aspartate aminotransferase (48%), vomiting (44%), increased alanine aminotransferase (42%), alopecia (39%), increased blood alkaline phosphatase (39%), constipation (34%), musculoskeletal pain (32%), decreased appetite (32%), hypokalemia (28%), diarrhea (28%), and respiratory infection (24%).

HER2-Low Metastatic Breast Cancer

DESTINY-Breast04

The safety of ENHERTU was evaluated in 371 patients with unresectable or metastatic HER2-low (IHC 1+ or IHC 2+/ISH-) breast cancer who received ENHERTU 5.4 mg/kg intravenously every 3 weeks in DESTINY-Breast04. The median duration of treatment was 8 months (range: 0.2 to 33) for patients who received ENHERTU.

Serious adverse reactions occurred in 28% of patients receiving ENHERTU. Serious adverse reactions in >1% of patients who received ENHERTU were ILD/pneumonitis, pneumonia, dyspnea, musculoskeletal pain, sepsis, anemia, febrile neutropenia, hypercalcemia, nausea, pyrexia, and vomiting. Fatalities due to adverse reactions occurred in 4% of patients including ILD/pneumonitis (3 patients); sepsis (2 patients); and ischemic colitis, disseminated intravascular coagulation, dyspnea, febrile neutropenia, general physical health deterioration, pleural effusion, and respiratory failure (1 patient each).

ENHERTU was permanently discontinued in 16% of patients, of which ILD/pneumonitis accounted for 8%. Dose interruptions due to adverse reactions occurred in 39% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose interruption were neutropenia, fatigue, anemia, leukopenia, COVID-19, ILD/pneumonitis, increased transaminases, and hyperbilirubinemia. Dose reductions occurred in 23% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose reduction were fatigue, nausea, thrombocytopenia, and neutropenia.

The most common (≥20%) adverse reactions, including laboratory abnormalities, were nausea (76%), decreased white blood cell count (70%), decreased hemoglobin (64%), decreased neutrophil count (64%), decreased lymphocyte count (55%), fatigue (54%), decreased platelet count (44%), alopecia (40%), vomiting (40%), increased aspartate aminotransferase (38%), increased alanine aminotransferase (36%), constipation (34%), increased blood alkaline phosphatase (34%), decreased appetite (32%), musculoskeletal pain (32%), diarrhea (27%), and hypokalemia (25%).

Use in Specific Populations

- **Pregnancy:** ENHERTU can cause fetal harm when administered to a pregnant woman. Advise patients of the potential risks to a fetus. There are clinical considerations if ENHERTU is used in pregnant women, or if a patient becomes pregnant within 7 months following the last dose of ENHERTU.
- **Lactation:** There are no data regarding the presence of ENHERTU in human milk, the effects on the breastfed child, or the effects on milk production. Because of the potential for serious adverse reactions in a breastfed child, advise women not to breastfeed during treatment with ENHERTU and for 7 months after the last dose.
- **Females and Males of Reproductive Potential:** Pregnancy testing: Verify pregnancy status of females of reproductive potential prior to initiation of ENHERTU. Contraception: *Females*: ENHERTU can cause fetal harm when administered to a pregnant woman. Advise females of reproductive potential to use effective contraception during treatment with ENHERTU and for at least 7 months following the last dose. *Males*: Advise male patients with female partners of reproductive potential to use effective contraception during treatment with ENHERTU and for at least 4 months following the last dose. Infertility: ENHERTU may impair male reproductive function and fertility.
- **Pediatric Use:** Safety and effectiveness of ENHERTU have not been established in pediatric patients.
- **Geriatric Use:** Of the 883 patients with breast cancer treated with ENHERTU 5.4 mg/kg, 22% were ≥65 years and 3.6% were ≥75 years. No overall differences in efficacy within clinical studies were observed between patients ≥65 years of age compared to younger patients. There was a higher incidence of Grade 3-4 adverse reactions observed in patients aged ≥65 years (60%) as compared to younger patients (48%).
- **Renal Impairment:** A higher incidence of Grade 1 and 2 ILD/pneumonitis has been observed in patients with moderate renal impairment. Monitor patients with moderate or severe renal impairment.
- **Hepatic Impairment:** In patients with moderate hepatic impairment, due to potentially increased exposure, closely monitor for increased toxicities related to the topoisomerase inhibitor.

To report SUSPECTED ADVERSE REACTIONS, contact Daiichi Sankyo, Inc. at 1-877-437-7763 or FDA at 1-800-FDA-1088 or fda.gov/medwatch.

Please see accompanying Brief Summary of Prescribing Information, including Boxed WARNINGS, and Medication Guide.

References: 1. ENHERTU [prescribing information]. Daiichi Sankyo Inc., Basking Ridge, NJ and AstraZeneca Pharmaceuticals LP, Wilmington, DE, 2022. **2.** Modi S, Jacot W, Yamashita T, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *N Engl J Med*. 2022;387(1):9-20. doi:10.1056/NEJMoa2203690 **3.** Schettini F, Chic N, Brasó-Maristany F, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. Supplementary tables. *NPJ Breast Cancer*. 2021;7(1):1. Accessed August 4, 2022. https://static-content.springer.com/esm/art%3A10.1038%2Fs41523-020-00208-2/MediaObjects/41523_2020_208_MOESM1_ESM.pdf

Daiichi-Sankyo | AstraZeneca

PP-US-ENB-1626 08/22

ENHERTU® (fam-trastuzumab deruxtecan-nxki) for injection, for intravenous use
Initial U.S. Approval: 2019

BRIEF SUMMARY: See package insert for full prescribing information.

WARNING: INTERSTITIAL LUNG DISEASE and EMBRYO-FETAL TOXICITY

- **Interstitial Lung Disease (ILD) and pneumonitis, including fatal cases, have been reported with ENHERTU. Monitor for and promptly investigate signs and symptoms including cough, dyspnea, fever, and other new or worsening respiratory symptoms. Permanently discontinue ENHERTU in all patients with Grade 2 or higher ILD/pneumonitis. Advise patients of the risk and the need to immediately report symptoms *[see Dosage and Administration (2.3) in the full prescribing information, Warnings and Precautions (5.1)]*.**
- **Embryo-Fetal Toxicity: Exposure to ENHERTU during pregnancy can cause embryo-fetal harm. Advise patients of these risks and the need for effective contraception *[see Warnings and Precautions (5.4), Use in Specific Populations (8.1, 8.3)]*.**

1 INDICATIONS AND USAGE

1.1 HER2-Positive Metastatic Breast Cancer

ENHERTU is indicated for the treatment of adult patients with unresectable or metastatic HER2-positive breast cancer who have received a prior anti-HER2-based regimen either:

- in the metastatic setting, or
- in the neoadjuvant or adjuvant setting and have developed disease recurrence during or within six months of completing therapy.

1.2 HER2-Low Metastatic Breast Cancer

ENHERTU is indicated for the treatment of adult patients with unresectable or metastatic HER2-low (IHC 1+ or IHC 2+/ISH-) breast cancer who have received a prior chemotherapy in the metastatic setting or developed disease recurrence during or within 6 months of completing adjuvant chemotherapy *[see Dosage and Administration (2.1) in the full prescribing information]*.

1.3 Unresectable or Metastatic HER2-Mutant Non-Small Cell Lung Cancer

ENHERTU is indicated for the treatment of adult patients with unresectable or metastatic non-small cell lung cancer (NSCLC) whose tumors have activating HER2 (ERBB2) mutations, as detected by an FDA-approved test, and who have received a prior systemic therapy.

This indication is approved under accelerated approval based on objective response rate and duration of response *[see Clinical Studies (14.3) in the full prescribing information]*. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

1.4 Locally Advanced or Metastatic Gastric Cancer

ENHERTU is indicated for the treatment of adult patients with locally advanced or metastatic HER2-positive gastric or gastroesophageal junction (GEJ) adenocarcinoma who have received a prior trastuzumab-based regimen.

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Interstitial Lung Disease/Pneumonitis

Severe, life-threatening, or fatal interstitial lung disease (ILD), including pneumonitis, can occur in patients treated with ENHERTU *[see Adverse Reactions (6.1)]*. A higher incidence of Grade 1 and 2 ILD/pneumonitis has been observed in patients with moderate renal impairment.

Advise patients to immediately report cough, dyspnea, fever, and/or any new or worsening respiratory symptoms. Monitor patients for signs and symptoms of ILD. Promptly investigate evidence of ILD. Evaluate patients with suspected ILD by radiographic imaging. Consider consultation with a pulmonologist. For asymptomatic (Grade 1) ILD, consider corticosteroid treatment (e.g., ≥0.5 mg/kg/day prednisolone or equivalent). Withhold ENHERTU until recovery *[see Dosage and Administration (2.3) in the full prescribing information]*. In cases of symptomatic ILD (Grade 2 or greater), promptly initiate systemic corticosteroid treatment (e.g., ≥1 mg/kg/day prednisolone or equivalent) and continue for at least 14 days followed by gradual taper for at least 4 weeks. Permanently discontinue ENHERTU in patients who are diagnosed with symptomatic (Grade 2 or greater) ILD *[see Dosage and Administration (2.3) in the full prescribing information]*.

Metastatic Breast Cancer and HER2-Mutant NSCLC (5.4 mg/kg)
In patients with metastatic breast cancer and HER2-mutant NSCLC treated with ENHERTU 5.4 mg/kg, ILD occurred in 12% of patients. Fatal outcomes due to ILD and/or pneumonitis occurred in 1.0% of patients treated with ENHERTU. Median time to first onset was 5 months (range: 0.9 to 23).

Locally Advanced or Metastatic Gastric Cancer (6.4 mg/kg)
In patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma treated with ENHERTU 6.4 mg/kg, ILD occurred in 10% of patients. Median time to first onset was 2.8 months (range: 1.2 to 21).

5.2 Neutropenia

Severe neutropenia, including febrile neutropenia, can occur in patients treated with ENHERTU.

Monitor complete blood counts prior to initiation of ENHERTU and prior to each dose, and as clinically indicated. Based on the severity of neutropenia, ENHERTU may require dose interruption or reduction *[see Dosage and Administration (2.3) in the full prescribing information]*.

Metastatic Breast Cancer and HER2-Mutant NSCLC (5.4 mg/kg)
In patients with metastatic breast cancer and HER2-mutant NSCLC treated with ENHERTU 5.4 mg/kg, a decrease in neutrophil count was reported in 65% of patients. Sixteen percent had Grade 3 or 4 decreased neutrophil count. Median time to first onset of decreased neutrophil count was 22 days (range: 2 to 664). Febrile neutropenia was reported in 1.1% of patients.

Locally Advanced or Metastatic Gastric Cancer (6.4 mg/kg)
In patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma treated with ENHERTU 6.4 mg/kg, a decrease in neutrophil count was reported in 72% of patients. Fifty-one percent had Grade 3 or 4 decreased neutrophil count. Median time to first onset of decreased neutrophil count was 16 days (range: 4 to 187). Febrile neutropenia was reported in 4.8% of patients.

5.3 Left Ventricular Dysfunction

Patients treated with ENHERTU may be at increased risk of developing left ventricular dysfunction. Left ventricular ejection fraction (LVEF) decrease has been observed with anti-HER2 therapies, including ENHERTU.

Assess LVEF prior to initiation of ENHERTU and at regular intervals during treatment as clinically indicated. Manage LVEF decrease through treatment interruption. Permanently discontinue ENHERTU if LVEF of less than 40% or absolute decrease from baseline of greater than 20% is confirmed. Permanently discontinue ENHERTU in patients with symptomatic congestive heart failure (CHF) *[see Dosage and Administration (2.3) in the full prescribing information]*.

Treatment with ENHERTU has not been studied in patients with a history of clinically significant cardiac disease or LVEF less than 50% prior to initiation of treatment.

Metastatic Breast Cancer and HER2-Mutant NSCLC (5.4 mg/kg)
In patients with metastatic breast cancer and HER2-mutant NSCLC treated with ENHERTU 5.4 mg/kg, LVEF decrease was reported in 3.6% of patients, of which 0.4% were Grade 3.

Locally Advanced or Metastatic Gastric Cancer (6.4 mg/kg)
In patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma treated with ENHERTU 6.4 mg/kg, no clinical adverse events of heart failure were reported; however, on echocardiography, 8% were found to have asymptomatic Grade 2 decrease in LVEF.

5.4 Embryo-Fetal Toxicity

Based on its mechanism of action, ENHERTU can cause fetal harm when administered to a pregnant woman. In postmarketing reports, use of a HER2-directed antibody during pregnancy resulted in cases of oligohydramnios manifesting as fatal pulmonary hypoplasia, skeletal abnormalities, and neonatal death. Based on its mechanism of action, the topoisomerase inhibitor component of ENHERTU, DXd, can also cause embryo-fetal harm when administered to a pregnant woman because it is genotoxic and targets actively dividing cells *[see Use in Specific Populations (8.1), Clinical Pharmacology (12.1), Nonclinical Toxicology (13.1) in the full prescribing information]*. Advise patients of the potential risks to a fetus.

Verify the pregnancy status of females of reproductive potential prior to the initiation of ENHERTU. Advise females of reproductive potential to use effective contraception during treatment and for 7 months after the last dose of ENHERTU. Advise male patients with female partners of reproductive potential to use effective contraception during treatment with ENHERTU and for 4 months after the last dose of ENHERTU *[see Use in Specific Populations (8.1, 8.3)]*.

6 ADVERSE REACTIONS

The following clinically significant adverse reactions are described elsewhere in the labeling:

- Interstitial Lung Disease/Pneumonitis *[see Warnings and Precautions (5.1)]*
- Neutropenia *[see Warnings and Precautions (5.2)]*
- Left Ventricular Dysfunction *[see Warnings and Precautions (5.3)]*

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Metastatic Breast Cancer and HER2-Mutant NSCLC (5.4 mg/kg)
The pooled safety population described in WARNINGS and PRECAUTIONS reflects exposure to ENHERTU 5.4 mg/kg intravenously every 3 weeks in 984 patients in Study DS8201-A-J101 (NCT02564900), DESTINY-Breast01,

DESTINY-Breast03, DESTINY-Breast04, and DESTINY-Lung02. Among these patients, 65% were exposed for greater than 6 months and 39% were exposed for greater than one year. In this pooled safety population, the most common (≥20%) adverse reactions (including laboratory abnormalities) were nausea (76%), decreased white blood cell count (71%), decreased hemoglobin (66%), decreased neutrophil count (65%), decreased lymphocyte count (55%), fatigue (54%), decreased platelet count (47%), increased aspartate aminotransferase (48%), vomiting (44%), increased alanine aminotransferase (42%), alopecia (39%), increased blood alkaline phosphatase (39%), constipation (34%), musculoskeletal pain (32%), decreased appetite (32%), hypokalemia (28%), diarrhea (28%), and respiratory infection (24%).

Locally Advanced or Metastatic Gastric Cancer (6.4 mg/kg)

The data described in WARNINGS and PRECAUTIONS reflect exposure to ENHERTU 6.4 mg/kg intravenously every 3 weeks in 125 patients in DESTINY-Gastric01.

HER2-Positive Metastatic Breast Cancer

DESTINY-Breast03

The safety of ENHERTU was evaluated in 257 patients with unresectable or metastatic HER2-positive breast cancer who received at least one dose of ENHERTU 5.4 mg/kg in DESTINY-Breast03 *[see Clinical Studies (14.1) in the full prescribing information]*. ENHERTU was administered by intravenous infusion once every three weeks. The median duration of treatment was 14 months (range: 0.7 to 30) for patients who received ENHERTU and 7 months (range: 0.7 to 25) for patients who received ado-trastuzumab emtansine.

Serious adverse reactions occurred in 19% of patients receiving ENHERTU. Serious adverse reactions in >1% of patients who received ENHERTU were vomiting, interstitial lung disease, pneumonia, pyrexia, and urinary tract infection. Fatalities due to adverse reactions occurred in 0.8% of patients including COVID-19 and sudden death (one patient each).

ENHERTU was permanently discontinued in 14% of patients, of which ILD/pneumonitis accounted for 8%. Dose interruptions due to adverse reactions occurred in 44% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose interruption were neutropenia, leukopenia, anemia, thrombocytopenia, pneumonia, nausea, fatigue, and ILD/pneumonitis. Dose reductions occurred in 21% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose reduction were nausea, neutropenia, and fatigue.

The most common (≥20%) adverse reactions, including laboratory abnormalities, were nausea, decreased white blood cell count, decreased neutrophil count, increased aspartate aminotransferase, decreased hemoglobin, decreased lymphocyte count, increased alanine aminotransferase, decreased platelet count, fatigue, vomiting, increased blood alkaline phosphatase, alopecia, hypokalemia, constipation, musculoskeletal pain, diarrhea, decreased appetite, headache, respiratory infection, abdominal pain, increased blood bilirubin, and stomatitis.

Tables 3 and 4 summarize common adverse reactions and laboratory abnormalities observed in DESTINY-Breast03.

Table 3: Common Adverse Reactions (≥10% All Grades or ≥2% Grades 3-4) in Patients Treated with ENHERTU in DESTINY-Breast03

Adverse Reactions	ENHERTU 5.4 mg/kg N=257		Ado-trastuzumab emtansine 3.6 mg/kg N=261	
	All Grades %	Grades 3-4 %	All Grades %	Grades 3-4 %
Gastrointestinal Disorders				
Nausea	76	7	30	0.4
Vomiting	49	1.6	10	0.8
Constipation	34	0	20	0
Diarrhea	29	1.2	7	0.4
Abdominal pain[a]	21	0.8	8	0.4
Stomatitis[b]	20	0.8	5	0
Dyspepsia	11	0	6	0
General Disorders and Administration Site Conditions				
Fatigue[c]	49	6	35	0.8
Blood and Lymphatic System Disorders				
Anemia[d]	33	7	17	6
Skin and Subcutaneous Tissue Disorders				
Alopecia[e]	37	0.4	3.1	0
Musculoskeletal and Connective Tissue Disorders				
Musculoskeletal pain[f]	31	1.2	25	0.4
Metabolism and Nutrition Disorders				
Decreased appetite	29	1.6	17	0.4
Investigations				
Decreased weight	17	1.2	6	0.4
Respiratory, Thoracic and Mediastinal Disorders				
Respiratory infection[g]	22	0.8	12	1.1
Epistaxis	11	0	16	0.4
Cough	11	0.4	10	0
Interstitial lung disease[h]	11	0.8	1.9	0
Nervous System Disorders				
Headache[i]	22	0.4	16	0
Peripheral neuropathy[j]	13	0.4	14	0.4
Dizziness	13	0.4	8	0

Events were graded using NCI CTCAE version 5.0.

a Including abdominal pain, abdominal discomfort, lower abdominal pain, and upper abdominal pain

b Including stomatitis, aphthous ulcer, mouth ulceration, oral mucosa erosion, and oral mucosal eruption

c Including fatigue, asthenia, malaise, and lethargy

d Including anemia, decreased hemoglobin, and decreased red blood cell count

e This Grade 3 event was reported by the investigator. Per NCI CTCAE v.5.0, the highest NCI CTCAE grade for alopecia is Grade 2.

f Including back pain, myalgia, pain in extremity, musculoskeletal pain, muscle spasms, bone pain, neck pain, musculoskeletal chest pain, and limb discomfort

g Including respiratory tract infection, lower and upper respiratory tract infection, pneumonia, influenza, influenza-like illness, viral upper respiratory infection, bronchitis, and respiratory syncytial virus infection

h Interstitial lung disease includes events that were adjudicated as ILD for ENHERTU: pneumonitis, interstitial lung disease, organizing pneumonia, pneumonia, and pulmonary mass. For ado-trastuzumab emtansine: pneumonitis, interstitial lung disease, organizing pneumonia, and pulmonary embolism.

i Including headache and migraine

j Including peripheral neuropathy, peripheral sensory neuropathy, and paresthesia

Other clinically relevant adverse reactions reported in less than 10% of patients in the ENHERTU-treated group were:

- *Respiratory, Thoracic and Mediastinal Disorders:* dyspnea (8%)
- *Skin and Subcutaneous Tissue Disorders:* pruritus (8%) and skin hyperpigmentation (6%) [including skin hyperpigmentation, skin discoloration, and pigmentation disorder]
- *Nervous System Disorders:* dysgeusia (6%)
- *Metabolism and Nutrition Disorders:* dehydration (4.3%)
- *Eye Disorders:* blurred vision (3.5%)
- *Cardiac Disorders:* asymptomatic left ventricular ejection fraction decrease (2.7%) *[see Warnings and Precautions (5.3)]*
- *Injury, Poisoning and Procedural Complications:* infusion-related reactions (2.3%) [including hypersensitivity and infusion-related reactions]
- *Blood and Lymphatic System Disorders:* febrile neutropenia (0.8%)

(continued)

Table 4: Selected Laboratory Abnormalities in Patients in DESTINY-Breast03

Laboratory Parameter	ENHERTU 5.4 mg/kg N=257		Ado-trastuzumab emtansine 3.6 mg/kg N=261	
	All Grades %	Grades 3-4 %	All Grades %	Grades 3-4 %
Hematology				
Decreased white blood cell count	74	8	24	0.8
Decreased neutrophil count	70	18	30	2.3
Decreased hemoglobin	64	7	38	6
Decreased lymphocyte count	55	14	23	3.9
Decreased platelet count	52	7	79	24
Chemistry				
Increased aspartate aminotransferase	67	0.8	83	5
Increased alanine aminotransferase	53	1.6	67	6
Increased blood alkaline phosphatase	49	0.8	46	0.8
Hypokalemia	35	4.7	39	1.5
Increased blood bilirubin	20	0	14	0
Increased blood creatinine	16	0.8	8	0.4

Percentages were calculated using patients with worsening laboratory values from baseline and the number of patients with both baseline and post-treatment measurements as the denominator.
Frequencies were based on NCI CTCAE v.5.0 grade-derived laboratory abnormalities.

DESTINY-Breast01 and Study DS8201-A-J101

The safety of ENHERTU was evaluated in a pooled analysis of 234 patients with unresectable or metastatic HER2-positive breast cancer who received at least one dose of ENHERTU 5.4 mg/kg in DESTINY-Breast01 and Study DS8201-A-J101 (NCT02564900) *[see Clinical Studies (14.1) in the full prescribing information]*. ENHERTU was administered by intravenous infusion once every three weeks. The median duration of treatment was 7 months (range: 0.7 to 31).

In the pooled 234 patients, the median age was 56 years (range: 28-96), 74% of patients were <65 years, 99.6% of patients were female, and the majority were White (51%) or Asian (42%). Patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 (58%) or 1 (42%) at baseline. Ninety-four percent had visceral disease, 31% had bone metastases, and 13% had brain metastases.

Serious adverse reactions occurred in 20% of patients receiving ENHERTU. Serious adverse reactions in >1% of patients who received ENHERTU were interstitial lung disease, pneumonia, vomiting, nausea, cellulitis, hypokalemia, and intestinal obstruction. Fatalities due to adverse reactions occurred in 4.3% of patients including interstitial lung disease (2.6%), and the following events occurred in one patient each (0.4%): acute hepatic failure/acute kidney injury, general physical health deterioration, pneumonia, and hemorrhagic shock.

ENHERTU was permanently discontinued in 9% of patients, of which ILD accounted for 6%. Dose interruptions due to adverse reactions occurred in 33% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose interruption were neutropenia, anemia, thrombocytopenia, leukopenia, upper respiratory tract infection, fatigue, nausea, and ILD. Dose reductions occurred in 18% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose reduction were fatigue, nausea, and neutropenia.

The most common (≥20%) adverse reactions, including laboratory abnormalities, were nausea, decreased white blood cell count, decreased hemoglobin, decreased neutrophil count, fatigue, vomiting, alopecia, increased aspartate aminotransferase, increased alanine aminotransferase, decreased platelet count, constipation, decreased appetite, diarrhea, hypokalemia, and cough.

Tables 5 and 6 summarize common adverse reactions and laboratory abnormalities observed in ENHERTU-treated patients in DESTINY-Breast01 and Study DS8201-A-J101.

Table 5: Common Adverse Reactions (≥10% All Grades or ≥2% Grades 3 or 4) in Patients in DESTINY-Breast01 and Study DS8201-A-J101

Adverse Reactions	ENHERTU 5.4 mg/kg N=234	
	All Grades %	Grades 3 or 4 %
Gastrointestinal Disorders		
Nausea	79	7
Vomiting	47	3.8
Constipation	35	0.9
Diarrhea	29	1.7
Abdominal pain[a]	19	1.3
Stomatitis[b]	14	0.9
Dyspepsia	12	0
General Disorders and Administration Site Conditions		
Fatigue[c]	59	6
Skin and Subcutaneous Tissue Disorders		
Alopecia	46	0.4[d]
Rash[e]	10	0
Metabolism and Nutrition Disorders		
Decreased appetite	32	1.3
Blood and Lymphatic System Disorders		
Anemia[f]	31	7
Respiratory, Thoracic and Mediastinal Disorders		
Cough	20	0
Dyspnea	13	1.3
Epistaxis	13	0
Interstitial lung disease[g]	9	2.6[h]
Nervous System Disorders		
Headache[i]	19	0
Dizziness	10	0
Infections and Infestations		
Upper respiratory tract infection[j]	15	0
Eye Disorders		
Dry eye	11	0.4[k]

Events were graded using NCI CTCAE version 4.03.
a Including abdominal discomfort, gastrointestinal pain, abdominal pain, lower abdominal pain, and upper abdominal pain
b Including stomatitis, aphthous ulcer, mouth ulceration, oral mucosa erosion, and oral mucosa blistering. One Grade 1 event of aphthous ulcer was not included in the summary of grouped term stomatitis (from DESTINY-Breast01).
c Including fatigue and asthenia
d This Grade 3 event was reported by the investigator. Per NCI CTCAE v.4.03, the highest NCI CTCAE grade for alopecia is Grade 2.
e Including rash, pustular rash, and maculo-papular rash
f Including anemia, decreased hemoglobin, decreased hematocrit, and decreased red blood cell count
g Interstitial lung disease includes events that were adjudicated as ILD: pneumonitis, interstitial lung disease, respiratory failure, organizing pneumonia, acute respiratory failure, lung infiltration, lymphangitis, and alveolitis.
h All events had fatal outcomes (n=6).
i Including headache, sinus headache, and migraine
j Including influenza, influenza-like illness, and upper respiratory tract infection
k This Grade 4 event was reported by the investigator. Per NCI CTCAE v.4.03, the highest NCI CTCAE grade for dry eye is Grade 3.

Other clinically relevant adverse reactions reported in less than 10% of patients were:

- *Injury, Poisoning and Procedural Complications:* infusion-related reactions (2.6%)
- *Blood and Lymphatic System Disorders:* febrile neutropenia (1.7%)

Table 6: Selected Laboratory Abnormalities in Patients with Unresectable or Metastatic HER2-positive Breast Cancer Treated with ENHERTU in DESTINY-Breast01 and Study DS8201-A-J101

Laboratory Parameter	ENHERTU 5.4 mg/kg N=234	
	All Grades %	Grades 3 or 4 %
Hematology		
Decreased white blood cell count	70	7
Decreased hemoglobin	70	7
Decreased neutrophil count	62	16
Decreased platelet count	37	3.4
Chemistry		
Increased aspartate aminotransferase	41	0.9
Increased alanine aminotransferase	38	0.4
Hypokalemia	26	3

Percentages were calculated using patients with worsening laboratory values from baseline and the number of patients with both baseline and post-treatment measurements as the denominator.
Frequencies were based on NCI CTCAE v.4.03 grade-derived laboratory abnormalities.

HER2-Low Metastatic Breast Cancer

The safety of ENHERTU was evaluated in 371 patients with unresectable or metastatic HER2-low (IHC 1+ or IHC 2+/ISH-) breast cancer who received ENHERTU 5.4 mg/kg in DESTINY-Breast04 *[see Clinical Studies (14.2) in the full prescribing information]*. ENHERTU was administered by intravenous infusion once every three weeks. The median duration of treatment was 8 months (range: 0.2 to 33) for patients who received ENHERTU.

Serious adverse reactions occurred in 28% of patients receiving ENHERTU. Serious adverse reactions in >1% of patients who received ENHERTU were ILD/pneumonitis, pneumonia, dyspnea, musculoskeletal pain, sepsis, anemia, febrile neutropenia, hypercalcemia, nausea, pyrexia, and vomiting. Fatalities due to adverse reactions occurred in 4.0% of patients including ILD/pneumonitis (3 patients); sepsis (2 patients); and ischemic colitis, disseminated intravascular coagulation, dyspnea, febrile neutropenia, general physical health deterioration, pleural effusion, and respiratory failure (1 patient each).

ENHERTU was permanently discontinued in 16% of patients, of which ILD/pneumonitis accounted for 8%. Dose interruptions due to adverse reactions occurred in 39% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose interruption were neutropenia, fatigue, anemia, leukopenia, COVID-19, ILD/pneumonitis, increased transaminases, and hyperbilirubinemia. Dose reductions occurred in 23% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose reduction were fatigue, nausea, thrombocytopenia, and neutropenia.

The most common (≥20%) adverse reactions, including laboratory abnormalities, were nausea, decreased white blood cell count, decreased hemoglobin, decreased neutrophil count, decreased lymphocyte count, fatigue, decreased platelet count, alopecia, vomiting, increased aspartate aminotransferase, increased alanine aminotransferase, constipation, increased blood alkaline phosphatase, decreased appetite, musculoskeletal pain, diarrhea, and hypokalemia.

Tables 7 and 8 summarize common adverse reactions and laboratory abnormalities observed in DESTINY-Breast04.

Table 7: Common Adverse Reactions (≥10% All Grades or ≥2% Grades 3 or 4) in Patients Treated with ENHERTU in DESTINY-Breast04

Adverse Reactions	ENHERTU 5.4 mg/kg N=371		Chemotherapy N=172	
	All Grades %	Grades 3 or 4 %	All Grades %	Grades 3 or 4 %
Gastrointestinal Disorders				
Nausea	76	4.6	30	0
Vomiting	40	1.6	13	0
Constipation	34	0.8	22	0
Diarrhea	27	1.3	22	1.7
Abdominal pain[a]	18	0.5	13	0
Stomatitis[b]	13	0.3	12	0.6

(continued)

Table 7: Common Adverse Reactions (≥10% All Grades or ≥2% Grades 3 or 4) in Patients Treated with ENHERTU in DESTINY-Breast04

Adverse Reactions	ENHERTU 5.4 mg/kg N=371		Chemotherapy N=172	
	All Grades %	Grades 3 or 4 %	All Grades %	Grades 3 or 4 %
General Disorders and Administration Site Conditions				
Fatigue[c]	54	9	48	4.7
Pyrexia	12	0.3	13	0
Skin and Subcutaneous Tissue Disorders				
Alopecia	40	0	33	0
Rash[d]	13	0	23	4.7
Blood and Lymphatic System Disorders				
Anemia[e]	39	10	27	5
Metabolism and Nutrition Disorders				
Decreased appetite	32	2.4	19	1.2
Musculoskeletal and Connective Tissue Disorders				
Musculoskeletal pain[f]	32	1.3	31	0.6
Investigations				
Decreased weight	16	0.3	8	0
Vascular Disorders				
Hemorrhage[g]	16	0	3.5	0
Nervous System Disorders				
Headache[h]	15	0.3	6	0
Peripheral neuropathy[i]	13	0	29	5
Dizziness[j]	11	0.5	6	0
Infections and Infestations				
Upper respiratory tract infection[k]	14	0.3	5	0
Respiratory, Thoracic and Mediastinal Disorders				
Interstitial lung disease[l]	12	1.3	0.6	0
Dyspnea	10	1.3	9	1.2

Events were graded using NCI CTCAE version 5.0.

a Including abdominal pain, abdominal discomfort, lower abdominal pain, and upper abdominal pain
b Including stomatitis, aphthous ulcer, mouth ulceration, and pharyngeal inflammation
c Including fatigue, asthenia, and malaise
d Including rash, pustular rash, pruritic rash, maculo-papular rash, palmar-plantar erythrodysesthesia syndrome, papular rash, macular rash, eczema, erythema multiforme, dermatitis, urticarial dermatitis, drug eruption, and dermatitis bullous
e Including anemia, decreased hemoglobin, and decreased red blood cell count
f Including back pain, myalgia, pain in extremity, musculoskeletal pain, bone pain, musculoskeletal chest pain, arthralgia, noncardiac chest pain, musculoskeletal stiffness, arthritis, spinal pain, and neck pain
g Including esophageal varices, hemorrhage, hemorrhoidal hemorrhage, epistaxis, hematuria, conjunctival hemorrhage, vaginal hemorrhage, gingival bleeding, genital hemorrhage, eye hemorrhage, hemoptysis, hemorrhagic cystitis, pharyngeal hemorrhage, rectal hemorrhage, upper gastrointestinal hemorrhage, and esophageal hemorrhage
h Including headache and migraine
i Including peripheral neuropathy, peripheral sensory neuropathy, peripheral motor neuropathy, polyneuropathy, paresthesia, hypoesthesia, dysesthesia, and neuralgia
j Including dizziness, postural dizziness, and vertigo
k Including upper respiratory tract infection, influenza, influenza-like illness, nasopharyngitis, pharyngitis, sinusitis, and rhinitis
l Interstitial lung disease includes events that were adjudicated as ILD for ENHERTU: interstitial lung disease, pneumonitis, organizing pneumonia, pneumonia, and radiation pneumonitis.

Other clinically relevant adverse reactions reported in less than 10% of patients treated with ENHERTU:

- *Nervous System Disorders:* dysgeusia (10%)
- *Respiratory, Thoracic and Mediastinal Disorders:* cough (10%)
- *Gastrointestinal Disorders:* abdominal distension (5%), gastritis (2.7%), flatulence (2.4%)
- *Eye Disorders:* blurred vision (4.9%) [including blurred vision and visual impairment]
- *Skin and Subcutaneous Tissue Disorders:* pruritus (3.2%) and skin hyperpigmentation (2.7%) [including skin hyperpigmentation, skin discoloration, and pigmentation disorder]

- *Metabolism and Nutrition Disorders:* dehydration (1.9%)
- *Blood and Lymphatic System Disorders:* febrile neutropenia (1.1%)
- *Injury, Poisoning and Procedural Complications:* infusion-related reactions (0.5%) [including injection site reaction and chills]

Table 8: Selected Laboratory Abnormalities in Patients in DESTINY-Breast04

Laboratory Parameter	ENHERTU 5.4 mg/kg N=371		Chemotherapy N=172	
	All Grades %	Grades 3 or 4 %	All Grades %	Grades 3 or 4 %
Hematology				
Decreased white blood cell count	70	9	78	25
Decreased hemoglobin	64	8	53	6
Decreased neutrophil count	64	14	73	38
Decreased lymphocyte count	55	18	40	11
Decreased platelet count	44	6	21	0.6
Chemistry				
Increased aspartate aminotransferase	38	2.2	38	4.1
Increased alanine aminotransferase	36	0.8	38	4.1
Increased blood alkaline phosphatase	34	0.3	24	0
Hypokalemia	25	3.3	17	1.2
Increased blood bilirubin	16	2.7	15	0.6
Increased blood creatinine	15	1.1	9	0.6

Percentages were calculated using patients with worsening laboratory values from baseline and the number of patients with both baseline and post-treatment measurements as the denominator.
Frequencies were based on NCI CTCAE v.5.0 grade-derived laboratory abnormalities.

Unresectable or Metastatic HER2-Mutant NSCLC

DESTINY-Lung02 evaluated two dose levels (5.4 mg/kg [n=101] and 6.4 mg/kg [n=50]); however, only the results for the recommended dose of 5.4 mg/kg intravenously every 3 weeks are described below due to increased toxicity observed with the higher dose in patients with NSCLC, including ILD/pneumonitis.

The safety of ENHERTU was evaluated in 101 patients in DESTINY-Lung02 *[see Clinical Studies (14.3) in the full prescribing information]*. Patients received ENHERTU 5.4 mg/kg intravenously once every three weeks until disease progression or unacceptable toxicity. Nineteen percent of patients were exposed for greater than 6 months. The median age was 59 years (range 30 to 83); 64% were female; 23% were White, 64% were Asian, and 14% were other races.

Serious adverse reactions occurred in 30% of patients receiving ENHERTU. Serious adverse reactions in >1% of patients who received ENHERTU were ILD/pneumonitis, thrombocytopenia, dyspnea, nausea, pleural effusion, and increased troponin I. Fatality occurred in 1 patient with suspected ILD/pneumonitis (1%).

ENHERTU was permanently discontinued due to an adverse reaction in 8% of patients. Adverse reactions which resulted in permanent discontinuation of ENHERTU were ILD/pneumonitis, diarrhea, hypokalemia, hypomagnesemia, myocarditis, and vomiting. Dose interruptions of ENHERTU due to adverse reactions occurred in 23% of patients. Adverse reactions which required dose interruption (>2%) included neutropenia and ILD/pneumonitis. Dose reductions due to an adverse reaction occurred in 11% of patients.

The most common (≥20%) adverse reactions, including laboratory abnormalities, were nausea, decreased white blood cell count, decreased hemoglobin, decreased neutrophil count, decreased lymphocyte count, decreased platelet count, decreased albumin, increased aspartate aminotransferase, increased alanine aminotransferase, fatigue, constipation, decreased appetite, vomiting, increased alkaline phosphatase, and alopecia.

Tables 9 and 10 summarize common adverse reactions and laboratory abnormalities observed in DESTINY-Lung02.

Table 9: Common Adverse Reactions (≥10% All Grades or ≥2% Grades 3 or 4) in Patients with Unresectable or Metastatic HER2-Mutant NSCLC in DESTINY-Lung02

Adverse Reactions	ENHERTU 5.4 mg/kg N=101	
	All Grades %	Grades 3 or 4 %
Gastrointestinal Disorders		
Nausea	61	3.0
Constipation	31	1.0
Vomiting[a]	26	2.0
Diarrhea	19	1.0
Stomatitis[b]	12	0
Blood and Lymphatic System Disorders		
Anemia	34	10
General Disorders and Administration Site Conditions		
Fatigue[c]	32	4.0
Metabolism and Nutrition Disorders		
Decreased appetite	30	1.0
Skin and Subcutaneous Tissue Disorders		
Alopecia	21	0
Musculoskeletal and Connective Tissue Disorders		
Musculoskeletal pain[d]	15	1.0

Events were graded using NCI CTCAE version 5.0.
a Including vomiting and retching
b including mucosal inflammation and stomatitis
c Including asthenia, fatigue, and malaise
d Including back pain, musculoskeletal stiffness, musculoskeletal chest pain, arthralgia, musculoskeletal pain, myalgia, and pain in extremity

Other clinically relevant adverse reactions reported in less than 10% of patients were:
- *Respiratory, Thoracic and Mediastinal Disorders:* interstitial lung disease (6%) [including interstitial lung disease that was adjudicated as ILD including pneumonitis, interstitial lung disease, pulmonary toxicity, and respiratory failure], dyspnea (5%), and epistaxis (3%)
- *Gastrointestinal Disorders:* abdominal pain (9%) [including abdominal discomfort, abdominal pain, and upper abdominal pain]
- *Skin and Subcutaneous Disorders:* rash (3%) [including rash and maculo-papular rash]
- *Infections and Infestations:* upper respiratory tract infection (4%) [including upper respiratory tract infection, pharyngitis, and laryngitis]
- *Nervous System Disorders:* headache (4%) [including headache and migraine]

Table 10: Select Laboratory Abnormalities in Patients with Unresectable or Metastatic HER2-Mutant NSCLC in DESTINY-Lung02

Laboratory Parameter	ENHERTU 5.4 mg/kg N=101[a]	
	All Grades[b] %	Grades 3 or 4[b] %
Hematology[c]		
Decreased white blood cell count	60	4.0
Decreased hemoglobin	58	10
Decreased neutrophil count	52	12
Decreased lymphocyte count	43	16
Decreased platelet count	40	4.0
Chemistry		
Decreased albumin	39	0
Increased aspartate aminotransferase	35	1.0
Increased alanine aminotransferase	34	2.0
Increased alkaline phosphatase	22	0
Hypokalemia	17	2.0

a Percentages were calculated using patients with worsening laboratory values from baseline and the number of patients with both baseline and post-treatment measurements as the denominator.
b Frequencies were based on NCI CTCAE v.5.0 grade-derived laboratory abnormalities.

c The denominator used to calculate the rate varied from 98 to 99 based on the number of patients with a baseline value and at least one post-treatment value.

Locally Advanced or Metastatic Gastric Cancer

The safety of ENHERTU was evaluated in 187 patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma in DESTINY-Gastric01 *[see Clinical Studies (14.4) in the full prescribing information]*. Patients intravenously received at least one dose of either ENHERTU (N=125) 6.4 mg/kg once every three weeks or either irinotecan (N=55) 150 mg/m^2 biweekly or paclitaxel (N=7) 80 mg/m^2 weekly for 3 weeks. The median duration of treatment was 4.6 months (range: 0.7 to 22.3) in the ENHERTU group and 2.8 months (range: 0.5 to 13.1) in the irinotecan/paclitaxel group.

Serious adverse reactions occurred in 44% of patients receiving ENHERTU 6.4 mg/kg. Serious adverse reactions in >2% of patients who received ENHERTU were decreased appetite, ILD, anemia, dehydration, pneumonia, cholestatic jaundice, pyrexia, and tumor hemorrhage. Fatalities due to adverse reactions occurred in 2.4% of patients: disseminated intravascular coagulation, large intestine perforation, and pneumonia occurred in one patient each (0.8%).

ENHERTU was permanently discontinued in 15% of patients, of which ILD accounted for 6%. Dose interruptions due to adverse reactions occurred in 62% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose interruption were neutropenia, anemia, decreased appetite, leukopenia, fatigue, thrombocytopenia, ILD, pneumonia, lymphopenia, upper respiratory tract infection, diarrhea, and hypokalemia. Dose reductions occurred in 32% of patients treated with ENHERTU. The most frequent adverse reactions (>2%) associated with dose reduction were neutropenia, decreased appetite, fatigue, nausea, and febrile neutropenia.

The most common (≥20%) adverse reactions, including laboratory abnormalities, were decreased hemoglobin, decreased white blood cell count, decreased neutrophil count, decreased lymphocyte count, decreased platelet count, nausea, decreased appetite, increased aspartate aminotransferase, fatigue, increased blood alkaline phosphatase, increased alanine aminotransferase, diarrhea, hypokalemia, vomiting, constipation, increased blood bilirubin, pyrexia, and alopecia.

Tables 11 and 12 summarize adverse reactions and laboratory abnormalities observed in patients receiving ENHERTU 6.4 mg/kg in DESTINY-Gastric01.

Table 11: Adverse Reactions in ≥10% All Grades or ≥2% Grades 3 or 4 of Patients Receiving ENHERTU in DESTINY-Gastric01

	ENHERTU 6.4 mg/kg N=125		Irinotecan or Paclitaxel N=62	
Adverse Reactions	**All Grades %**	**Grades 3 or 4 %**	**All Grades %**	**Grades 3 or 4 %**
Gastrointestinal Disorders				
Nausea	63	4.8	47	1.6
Diarrhea	32	2.4	32	1.6
Vomiting	26	0	8	0
Constipation	24	0	23	0
Abdominal pain[a]	14	0.8	15	3.2
Stomatitis[b]	11	1.6	4.8	0
Metabolism and Nutrition Disorders				
Decreased appetite	60	17	45	13
Dehydration	6	2.4	3.2	1.6
Blood and Lymphatic System Disorders				
Anemia[c]	58	38	31	23
Febrile neutropenia	4.8	4.8	3.2	3.2
General Disorders and Administration Site Conditions				
Fatigue[d]	55	9	44	4.8
Pyrexia	24	0	16	0
Peripheral edema	10	0	0	0
Skin and Subcutaneous Tissue Disorders				
Alopecia	22	0	15	0
Respiratory, Thoracic and Mediastinal Disorders				
Interstitial lung disease[e]	10	2.4	0	0
Hepatobiliary Disorders				
Abnormal hepatic function	8	3.2	1.6	1.6

Events were graded using NCI CTCAE version 4.03.

[a] Including abdominal discomfort, gastrointestinal pain, abdominal pain, lower abdominal pain, and upper abdominal pain

[b] Including stomatitis, aphthous ulcer, mouth ulceration, oral mucosa erosion, and oral mucosal blistering

[c] Including anemia, decreased hemoglobin, decreased red blood cell count, and decreased hematocrit

[d] Including fatigue, asthenia, and malaise

[e] Interstitial lung disease includes events that were adjudicated as ILD: pneumonitis, interstitial lung disease, respiratory failure, organizing pneumonia, acute respiratory failure, lung infiltration, lymphangitis, and alveolitis.

Other clinically relevant adverse reactions reported in less than 10% of patients were:

- *Cardiac Disorders:* asymptomatic left ventricular ejection fraction decrease (8%) *[see Warnings and Precautions (5.3)]*
- *Infections and Infestations:* pneumonia (6%)
- *Injury, Poisoning and Procedural Complications:* infusion-related reactions (1.6%)

Table 12: Selected Laboratory Abnormalities Occurring in Patients Receiving ENHERTU in DESTINY-Gastric01

Laboratory Parameter	ENHERTU 6.4 mg/kg N=125		Irinotecan or Paclitaxel N=62	
	All Grades %	**Grades 3 or 4 %**	**All Grades %**	**Grades 3 or 4 %**
Hematology				
Decreased hemoglobin	75	38	55	23
Decreased white blood cell count	74	29	53	13
Decreased neutrophil count	72	51	45	23
Decreased lymphocyte count	70	28	53	12
Decreased platelet count	68	12	12	5
Chemistry				
Increased aspartate aminotransferase	58	9	32	8
Increased blood alkaline phosphatase	54	8	34	10
Increased alanine aminotransferase	47	9	17	1.7
Hypokalemia	30	4.8	18	8
Increased blood bilirubin	24	7	5	3.4

Percentages were calculated using patients with worsening laboratory values from baseline and the number of patients with both baseline and post-treatment measurements as the denominator.
Frequencies were based on NCI CTCAE v.4.03 grade-derived laboratory abnormalities.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Based on its mechanism of action, ENHERTU can cause fetal harm when administered to a pregnant woman. There are no available data on the use of ENHERTU in pregnant women. In postmarketing reports, use of a HER2-directed antibody during pregnancy resulted in cases of oligohydramnios manifesting as fatal pulmonary hypoplasia, skeletal abnormalities, and neonatal death (*see Data*). Based on its mechanism of action, the topoisomerase inhibitor component of ENHERTU, DXd, can also cause embryo-fetal harm when administered to a pregnant woman because it is genotoxic and targets actively dividing cells *[see Clinical Pharmacology (12.1), Nonclinical Toxicology (13.1) in the full prescribing information]*. Advise patients of the potential risks to a fetus.

There are clinical considerations if ENHERTU is used in pregnant women, or if a patient becomes pregnant within 7 months after the last dose of ENHERTU (*see Clinical Considerations*).

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

Clinical Considerations

Fetal/Neonatal Adverse Reactions

Monitor women who received ENHERTU during pregnancy or within 7 months prior to conception for oligohydramnios. If oligohydramnios occurs, perform fetal testing that is appropriate for gestational age and consistent with community standards of care.

Data

Human Data

There are no available data on the use of ENHERTU in pregnant women. In postmarketing reports in pregnant women receiving a HER2-directed antibody, cases of oligohydramnios manifesting as fatal pulmonary hypoplasia, skeletal abnormalities, and neonatal death have been reported. These case reports described oligohydramnios in pregnant women who received a HER2-directed antibody either alone or in combination with chemotherapy. In some case reports, amniotic fluid index increased after use of a HER2-directed antibody was stopped.

Animal Data

There were no animal reproductive or developmental toxicity studies conducted with fam-trastuzumab deruxtecan-nxki.

8.2 Lactation

Risk Summary

There is no data regarding the presence of fam-trastuzumab deruxtecan-nxki in human milk, the effects on the breastfed child, or the effects on milk production. Because of the potential for serious adverse reactions in a breastfed child, advise women not to breastfeed during treatment with ENHERTU and for 7 months after the last dose.

8.3 Females and Males of Reproductive Potential

Pregnancy Testing

Verify pregnancy status of females of reproductive potential prior to initiation of ENHERTU.

Contraception

Females

ENHERTU can cause fetal harm when administered to a pregnant woman *[see Use in Specific Populations (8.1)]*. Advise females of reproductive potential to use effective contraception during treatment with ENHERTU and for 7 months after the last dose.

Males

Because of the potential for genotoxicity, advise male patients with female partners of reproductive potential to use effective contraception during treatment with ENHERTU and for 4 months after the last dose *[see Nonclinical Toxicology (13.1) in the full prescribing information]*.

Infertility

Based on findings in animal toxicity studies, ENHERTU may impair male reproductive function and fertility *[see Nonclinical Toxicology (13.1) in the full prescribing information]*.

8.4 Pediatric Use

Safety and effectiveness of ENHERTU have not been established in pediatric patients.

8.5 Geriatric Use

Of the 883 patients with breast cancer treated with ENHERTU 5.4 mg/kg, 22% were 65 years or older and 3.6% were 75 years or older. No overall differences in efficacy within clinical studies were observed between patients ≥65 years of age compared to younger patients. There was a higher incidence of Grade 3-4 adverse reactions observed in patients aged 65 years or older (60%) as compared to younger patients (48%).

Of the 101 patients with unresectable or metastatic HER2-mutant NSCLC treated with ENHERTU 5.4 mg/kg, 40% were 65 years or older and 8% were 75 years or older. No overall differences in efficacy or safety were observed between patients ≥65 years of age compared to younger patients.

Of the 125 patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma treated with ENHERTU 6.4 mg/kg in DESTINY-Gastric01, 56% were 65 years or older and 14% were 75 years or older. No overall differences in efficacy or safety were observed between patients ≥65 years of age compared to younger patients.

8.6 Renal Impairment

No dose adjustment of ENHERTU is required in patients with mild (creatinine clearance [CLcr] ≥60 and <90 mL/min) or moderate (CLcr ≥30 and <60 mL/min) renal impairment *[see Clinical Pharmacology (12.3) in the full prescribing information]*. A higher incidence of Grade 1 and 2 ILD/pneumonitis has been observed in patients with moderate renal impairment *[see Warnings and Precautions (5.1)]*. Monitor patients with moderate renal impairment more frequently. The recommended dosage of ENHERTU has not been established for patients with severe renal impairment (CLcr <30 mL/min) *[see Clinical Pharmacology (12.3) in the full prescribing information]*.

8.7 Hepatic Impairment

No dose adjustment of ENHERTU is required in patients with mild (total bilirubin ≤ULN and any AST >ULN or total bilirubin >1 to 1.5 times ULN and any AST) or moderate (total bilirubin >1.5 to 3 times ULN and any AST) hepatic impairment. In patients with moderate hepatic impairment, due to potentially increased exposure, closely monitor for increased toxicities related to the topoisomerase inhibitor, DXd *[see Dosage and Administration (2.3) in the full prescribing information]*. The recommended dosage of ENHERTU has not been established for patients with severe hepatic impairment (total bilirubin >3 times ULN and any AST) *[see Clinical Pharmacology (12.3) in the full prescribing information]*.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Medication Guide).

Interstitial Lung Disease

- Inform patients of the risks of severe or fatal ILD. Advise patients to contact their healthcare provider immediately for any of the following: cough, shortness of breath, fever, or other new or worsening respiratory symptoms *[see Warnings and Precautions (5.1)]*.

Neutropenia

- Advise patients of the possibility of developing neutropenia and to immediately contact their healthcare provider should they develop a fever, particularly in association with any signs of infection *[see Warnings and Precautions (5.2)]*.

Left Ventricular Dysfunction

- Advise patients to contact their healthcare provider immediately for any of the following: new onset or worsening shortness of breath, cough, fatigue, swelling of ankles/legs, palpitations, sudden weight gain, dizziness, loss of consciousness *[see Warnings and Precautions (5.3)]*.

Embryo-Fetal Toxicity

- Inform female patients of the potential risk to a fetus. Advise female patients to contact their healthcare provider of a known or suspected pregnancy *[see Warnings and Precautions (5.4), Use in Specific Populations (8.1)]*.
- Advise females of reproductive potential to use effective contraception during treatment with ENHERTU and for 7 months after the last dose *[see Use in Specific Populations (8.3)]*.
- Advise male patients with female partners of reproductive potential to use effective contraception during treatment with ENHERTU and for 4 months after the last dose *[see Use in Specific Populations (8.3)]*.

Lactation

- Advise women not to breastfeed during treatment and for 7 months after the last dose of ENHERTU *[see Use in Specific Populations (8.2)]*.

Infertility

- Advise males of reproductive potential that ENHERTU may impair fertility *[see Use in Specific Populations (8.3)]*.

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Editing Pathology

Sitting Down With… Catherine Ketcham, Managing Editor of Laboratory Investigations and Modern Pathology at the United States and Canadian Academy of Pathology, Palm Springs, California, USA

What's involved in your role as an editor?
The word "editor" is a broad term – and editing documents is just a tiny part of it. I think of myself as the information hub for the journals. Communication comes to me, then goes out to other people. For example, if an author or reviewer has a question for the editor, it has to come through me; they're not allowed to contact one another directly – both to avoid bothering busy faculty members and to ensure that there's a record of everything we discuss with manuscripts.

What's it like to immerse yourself in the world of pathology without being a pathologist yourself?
It's a wonderful community. After getting to know people, going to USCAP meetings, and dealing with repeat authors and reviewers, I felt right at home. I also love being an integral part of a group as prestigious as USCAP.

Tell us about your two journals – Laboratory Investigation and Modern Pathology.
They are completely different journals. Laboratory Investigation started in 1952 and covers basic and translational science; I'm very comfortable with this area due to my experience as a biochemist. In 2010, the managing editor of Modern Pathology left and I was asked to step in until they found someone else, but the Editor-in-Chief and I got along so well that I kept the position. Modern Pathology is clinical diagnostic pathology, which is not subject matter I know well, but we have experts to help.

Typically, when I'm extra busy with one of the journals, the other one is going well – but I do have to be very organized. A lot of my scientific friends tell me that they would never want to be editors because they wouldn't like the deadlines and the amount of juggling, but it becomes second nature. Two issues must come out every month no matter what's going on in my life, so I make sure they do. And it's not just the publications; there's social media, podcasts, altmetrics scores, how the non-scientific media cover you… there's so much to worry about and so much to do to stay competitive these days.

What's more important for the work that you do – a strong understanding of the field or a familiarity with the medical publishing world?
These days, it is absolutely the medical publishing world because it has changed – and is changing – so fast. We have institutions, organizations, and even whole countries demanding open access; the old subscription model won't hold much longer in scientific publishing. Right now, our journals are hybrid, meaning that readers and contributors can choose between open access and subscription. Inevitably, though, everything will become open access and researchers who don't have a way to fund that will lose out – so it's going to be a rough transition for a lot of journals.

We're actively thinking about when to flip the switch for each journal. I think that Laboratory Investigations, because it's basic research, will be ready first. Basic science authors frequently have grant funding that can cover publication charges. Clinical pathologists do much of their work without funding (and, of course, we still want to publish their work), so it may take longer for Modern Pathology to make the transition.

Are there any goals you'd still like to accomplish?
I like traveling for work so when Laboratory Investigations started to receive more papers from China, I wanted to connect personally with Chinese researchers. I've made it a goal to travel throughout China, meet people at all the important institutions, and develop personal relationships, because they are the future of science. I'm also trying to learn a little Mandarin to understand what they go through when writing scientific papers in English. I can't imagine how hard that is. The least I can do is learn to say hello!

"[Pathology] is a wonderful community. After getting to know people, going to USCAP meetings, and dealing with repeat authors and reviewers, I felt right at home."

What do you wish all pathologists and laboratory medicine professionals knew about publishing?
I wish they knew that editors and publishers are not perfect. Please check your proofs really, really carefully. I have made mistakes; proofreaders make mistakes; publishers make mistakes; typesetters make mistakes. Once you send that proof back and it's published, we can't change it (though we can issue a correction). Be kind to your co-authors – spell their names right! We all want the published manuscript to be perfect, so look over your proofs carefully and save yourself a lot of trouble.

If you could give yourself one piece of advice at the start of your career, what would you say?
Be flexible – and don't be afraid of change. Try something; if it doesn't work, try something else.

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