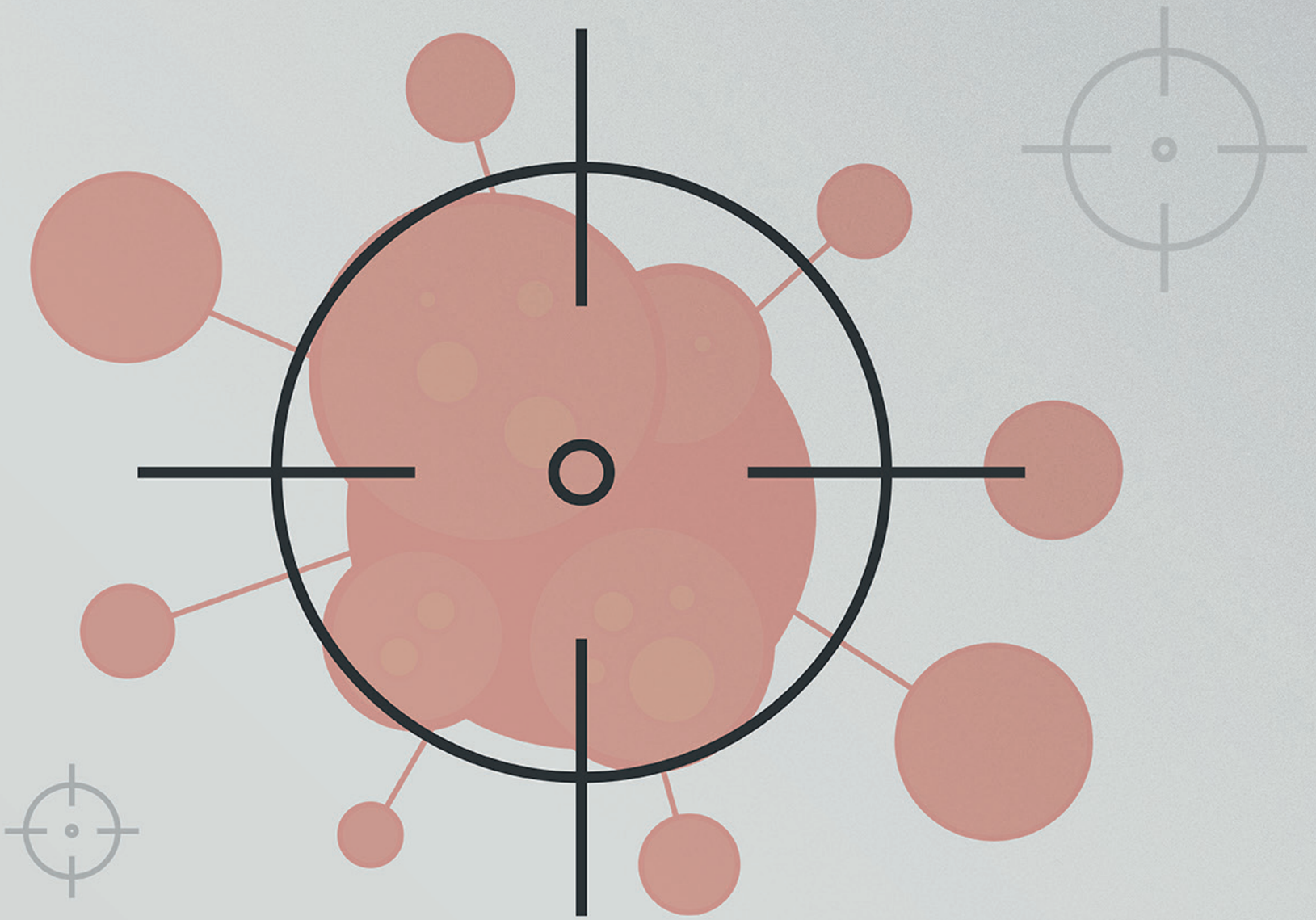
the Medicine Maker

SPECIAL SERIES:

Small Molecules



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IN MY VIEW

Complexity and its Questions

Pipelines are changing, and chemical complexity is on the rise. What can pharma manufacturers do about it?

Some people believe that the days of small molecule drugs are over – that pharma is now all about biologics and novel modalities, such as RNA and cell therapies. But this is far from reality. In 2021, the small molecule R&D pipeline was around 4 percent larger than it was the year before, with a record 8000 candidates in development. Interestingly, the increase is slightly skewed towards the earlier phases, with 5 percent growth for preclinical and phase I.

But complexity in all its forms is also increasing within the small molecule drug pipeline. Growing numbers of novel APIs are deemed highly potent. Some are used as drugs in their own right; others are used to make the linker payload component of an antibody–drug conjugate. Either way, their biological activity even at very low doses means that they must be carefully handled to ensure operator and environmental safety, which adds an additional layer of complexity.

Many new small molecule drugs have complex chemical structures, such as multiple chiral centers or tricky functional groups, which also pose manufacturing challenges. Opting for the synthetic route might demand reactions that require challenging reagents or conditions, such as very low temperatures.

Redesigning the synthetic route is sometimes an option. For example, while working on phase I API development for a potential sickle cell disease treatment, one company found that a bromide intermediate in the original route was unstable, requiring low temperatures and complicated purification. Installing and qualifying the new cryogenic equipment would have taken at least six months. Even then, the bromide's purity was only about 80 percent, which would have produced a low yield of below 60 percent when making the final API. By replacing bromide with chloride, the company fixed the problem; the modified intermediate fitted seamlessly into the route and was more stable. With 97 percent purity, the API yield was increased to 77 percent in the next step, using a simple isolation. And because no cryogenic step was needed, the six-month equipment delay was avoided.

Making the molecule is not the only challenge, however. A substantial – and growing – proportion of developmental drugs nowadays have poor solubility, with the knock-on effect of poor bioavailability. Some active molecules are so insoluble that they are commonly described as "brick dust" compounds. Solid form services experts can help improve the solubility of even these most insoluble compounds, enabling the creation of effective dosage forms with decent bioavailability. Sometimes, a more soluble stable polymorph, a salt form, or even a cocrystal can be found. Other times, smaller particles (via micronization) can help. Amorphous solid dispersions (often achieved via spray drying) are another common strategy. This latter process converts the API into a high-energy amorphous form, usually in combination with a performance-improving polymer. In my view, finding the best option is as much an art as it is a science.

Formulators responsible for designing the dosage forms may add further complexity with a wish list of essential properties. An inhaled drug, for example, will require a tight distribution of the optimal sized particles, which may be challenging to achieve.

In short, increased complexity and challenges go hand in hand. And smaller companies may not have the necessary in-house skills and capabilities to bring complex formulations to the market. Even large companies may need assistance from a niche specialist.

Responding to demand, CDMOs have invested in technology and capacity to enable these complex molecules to be made and modified effectively. Phase-specific, streamlined offerings provide the necessary flexibility for new chemistries to be incorporated seamlessly into a process

stream. Many CDMOs can now make and formulate highly potent APIs at more than one site. Some CDMOs are also putting a big focus on solid form services and their ability to overcome solubility issues.

When working with complex molecules and chemistries (especially where the prior art may be limited), you may need to accommodate changes to processes – and that requires flexibility and agility. The sooner a particular challenge is addressed, the less likely it is to cause a major delay in the development timeline. In fact, by integrating multiple technologies and teams into a single workflow, the timeline can often be accelerated.

As an example of the effectiveness of an integrated team working to solve complex chemistry problems, we recently worked on the development and kilo-scale manufacture for a phase I asset. The route was convoluted, with eight steps and an overall yield of just 14 percent – but the timeline for the delivery of the first batch was just six months! Our team in China optimized each of the eight steps in the process, while groups in Florida and Switzerland worked on particle engineering and API encapsulation. The result? A scalable kilo lab process delivered about 3 kg of the API with an overall yield of 29 percent inside the six-month deadline.

We dare say you'll agree that time is of the essence in drug development projects. And we hope you'll agree that using experts to solve tricky problems is key to getting complex small molecules over the finish line.

By Charles Johnson, Senior Director, Commercial Development, Lonza Small Molecules

UPFRONT

Fighting the Resistance – to Malaria

Researchers examine how combination therapies for malaria lead to drug resistance

Malaria has played a long and enduring role in human history. The disease is thought to have first emerged hundreds of thousands or millions of years ago. Although our understanding of this parasitic disease has drastically improved as contemporary science has advanced, researchers are still faced with the quandary of developing relevant medicines and therapeutics against it.

Many medicines against malaria have been developed, but all have lost efficacy due to the parasites' ability to evolve and develop drug resistance. In the first decade of the 2000s, we saw the most widely used antimalarial drugs, the artemisinins, begin to lose their efficacy as well. The resulting dearth of treatment options has left patients and those at risk of contracting the disease – particularly those in low- and middle-income countries (LMICs) – in a vulnerable state.

When artemisinin first emerged as a treatment option in the 1990s, it was welcomed by some countries' national malaria programs. Commenting on the importance of the drug, Maciej Boni, Associate Professor of Biology at Penn State University, says, "A small series of clinical trials were carried out in southern Vietnam in the 1990s. Though few were familiar with the drug prior to this, the studies proved its potency." Eventually artemisinin-combination therapies were recommended by the World Health Organization in 2005

Today, artemisinin is the leading treatment for malaria, but artemisinin resistance is now common in southeast Asia and emerging in eastern Africa. To slow this phenomenon and protect as many patient lives as possible (and for as long as possible), appropriate drug monitoring will make all the difference. "Typically, resistance emerges very slowly and requires constant surveillance. This means that we need dedicated networks of scientists working to collect and genotype samples," says Boni. "By creating rapid and responsive surveillance networks, we can help improve treatment in endemic countries and facilitate communication between public health institutions and patients."

But good surveillance relies on an understanding of resistance evolution in artemisinin and the partner drugs used alongside it in many regions of the world. Along with colleagues at Penn State University, the University of Oxford, and Imperial College London, Boni has found that resistance to partner drugs also encourages early resistance to artemisinin. He says, "We were looking at the conditions that affect resistance evolution. The reason it was previously so difficult to discern was that the earlier stages of resistance occur slowly. Therefore, it is a challenge for public health systems to detect."

In particular, the team's research focused on artemisinin partner drugs piperaquine, amodiaquine, and lumefantrine. To varying degrees, malaria has already developed some resistance to these drugs, but Boni and colleagues found that, when partner drug resistance levels are high, artemisinin resistance evolves even more quickly than expected.

The discovery is only more proof, he explains, that further surveillance is necessary to manage antimalarial resistance. In doing so, public health bodies and other healthcare stakeholders will be able to more appropriately respond to resistance as and when it occurs. "If we take Rwanda as an example, we already see signs of artemisinin resistance. With good surveillance in place, we would have a better idea of when to start enrolling patients in trials for alternative artemisinin-based combination therapies and see if the new treatment approach allows them to clear the parasite."

Boni and his Penn State colleagues are now working in collaboration with the World Health Organization and in-country national malaria control programs to assess the current situation in Rwanda, Burkina Faso, and other countries, and to make projections of what the next five to 10 years might look like.

"It's hard to say what things will look like in five years," he says. "Just like weather reporters can provide a forecast for the next few days, but can't tell you with certainty what the weather will be like over the next month, we don't know precisely how the future of malaria resistance will pan out. That's why it's so important to start thinking about drug resistance management early."

But effective management requires good funding. The better access researchers and national programs have to funding, the easier it will be to establish and strengthen management systems. Boni says, "In the next 10 to 15 years, we need to see more funding channeled into this area. We've come a long way when it comes to malaria; 15 years ago, it was considered a neglected disease. Although funding has massively increased in the last two decades, which was the right course of action, we need more of it to see a bigger impact in patient lives. Imagine how far we can go with the right tools and resources in place."

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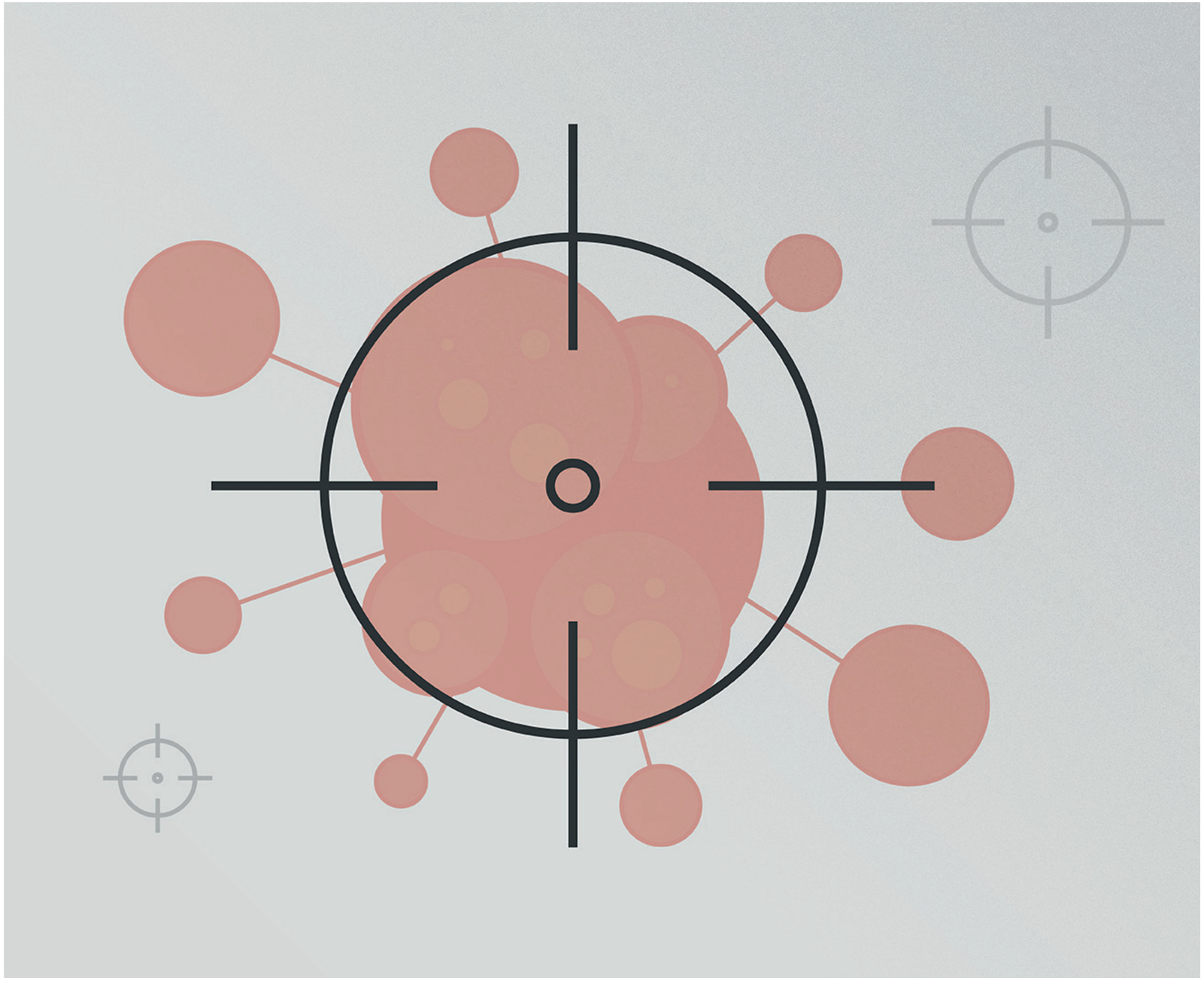
FEATURE

Lethal Weapon

Leaders at Artios explore the potential of expanding therapeutic opportunities in DDR beyond synthetic lethality

By Graeme Smith, Chief Scientific Officer at Artios, Cambridge, UK and Niall Martin, CEO of Artios, Cambridge, UK



Cancer often develops or becomes aggressive because of genomic instability that arises from mutations or aberrations in DNA. These lead to uncontrolled growth, proliferation, and metastatic spread of tumorigenic cells. The body employs a highly sophisticated and coordinated cellular network focused on preserving DNA integrity during states of cell replication or damage, known as the DNA damage response (DDR), designed to prevent the replication of cells with faulty DNA by either repairing the damage or triggering cell death.

Exploiting this inherent genomic protection is a therapeutic area that presents a challenging target for two main reasons. First, the multitude of enzymes important in the DDR have substantially different mechanisms of action to kinases, which have been the primary focus of most druggable targets in cancer cell biology. DDR targets include nucleases, helicases, and polymerases, which contain structural elements that are less characterized and accessible to drugging and therefore require novel mechanistic classes of drugs. A second challenge stems from initial concerns surrounding the blocking mechanisms that repair DNA damage in cells. These may inadvertently induce toxicity in normal cells, which raises the question of whether DNA repair systems that emerge selectively or are upregulated in cancer cells can be targeted to avoid impact on normal cells while specifically affecting cancer cells.

Killing cancer

In 2005, we were authors on one of two back-to-back papers in Nature demonstrating cancer-specific cell death in BRCA-mutated cancers via inhibitors of a DDR enzyme called PARP – poly (ADP-ribose) polymerase (1, 2). PARP inhibitors were shown to selectively kill cancer cells by targeting a PARP-mediated DDR backup mechanism on which cancer cells become dependent when normal BRCA1 and BRCA2 homologous recombination repair (HRR) mechanisms become deficient. These papers demonstrated a new concept in cancer therapeutics known as "synthetic lethality", which occurs when cell death is triggered by the loss of two key factors – such as DDR activity from both the PARP and BRCA1/2 processes – but not by the loss of either factor alone.

Following the Nature publications, Cambridge-based biotech firm KuDOS dosed the first patient with its new oral PARP inhibitor, KU-59436. In 2014, this product was approved and marketed as LYNPARZA (olaparib) by AstraZeneca for patients with advanced forms of hereditary BRCA-mutated ovarian cancer (3) and later for BRCA-mutated breast cancer (4). In 2019, olaparib was approved as a first-line maintenance treatment for germline BRCA-mutated metastatic pancreatic ductal adenocarcinoma.

PARP inhibitors' ability to selectively induce synthetic lethality in cancer cells now extends beyond mutations in BRCA1 and BRCA2 to include other homologous recombination repair deficiencies (HRDs). This led to the approval of olaparib in 2020 beyond BRCA mutations and into germline and somatic HRR gene mutations in metastatic castration-resistant prostate cancer. Olaparib is now approved in first-line maintenance of BRCA-mutated advanced ovarian cancer; HRD-positive advanced ovarian cancer in combination with bevacizumab; maintenance for recurrent ovarian cancer; adjuvant treatment of germline BRCA-mutated (gBRCAm), HER2-negative, high-risk early breast cancer; first-line maintenance of gBRCAm metastatic pancreatic cancer; and HRR gene-mutated metastatic castration-resistant prostate cancer (5).

Praise for PARP

Olaparib not only became the first approved PARP inhibitor, but also represented the first approved drug targeting the DDR. There are now four PARP inhibitors on the market: olaparib, niraparib, rucaparib, and talazoparib. Over 10 years of extensive clinical data have proven that this therapeutic approach is highly effective as a monotherapy and has the potential to synergize with several chemotherapies (chemopotentiation) and other agents, including checkpoint inhibitors. These combination approaches are designed to address PARP-mediated DDR mechanisms that become activated to enable tumor resistance to DNA-damaging treatments such as radiation and chemotherapy. The desire to combine PARP inhibitors with conventional chemotherapy has driven the search for highly selective next-generation PARP inhibitors with the potential for lower cytotoxicity and improved combination approaches.

Since olaparib gained market approval, mechanistic understanding in DDR biology has advanced, leading to a wave of companies exploring therapeutic opportunities that target new aspects of the DDR beyond synthetic lethality. Druggable opportunities under investigation focus on alternative DDR pathways upregulated under certain conditions that create therapeutic openings to exploit a tumor-selective target and ultimately drive tumor-specific death across diverse cancers. This next wave includes inhibitors targeting ataxia telangiectasia and Rad3-related protein kinase (ATR), which are being explored by AstraZeneca (6), Artios (7), Repare (8), and Merck KGaA (9). Potential opportunities include monotherapy, PARP inhibitor combinations, and immune checkpoint inhibitor combinations.

The research and drug development environment surrounding DDR has greatly matured over the last 20 years, enabling more sophisticated identification of new targets. DDR proteins and pathway relationships are not only better depicted and annotated, but can be interrogated in more advanced ways, including through artificial intelligence, high-content biological screening, gene editing technologies, and more physiologically relevant cancer models that include genetically


Graeme Smith

engineered mouse models and patient-derived xenografts. There have also been improvements in medicinal chemistry approaches to structurally target challenging proteins across the DDR pathway, as well as better ways to evaluate their clinical potential using more refined preclinical models.

An unfolding treatment landscape

A decade and a half later, the commercial picture surrounding the DDR has changed significantly. Our focus at Artios is to develop drugs that target pathways across the totality of the opportunities the DDR offers, with DNA polymerase-theta (Pol-theta) emerging as a novel target of particular interest. Resistance to first-generation PARP inhibitors is now well recognized in the clinical setting and has underscored the need for new DDR targets to overcome both de novo and acquired resistance. Pol-theta is a DNA repair enzyme involved in an alternative DNA double-strand break repair process that PARP-resistant cells can become dependent on, supporting the potential to prevent or address PARP resistance in different patient types. Interest in Pol-theta also stems from its minimal or no expression in normal cells and its observed upregulation in numerous cancers (associated with poor prognosis). This selective expression pattern suggests that Pol-theta inhibitors may have a favorable therapeutic index because of a more focused impact on tumor cells.

Clinical studies on Pol-theta inhibitors have recently begun, with Artios' ART4215 and ART6043 compounds – which we believe are the first specific, rationally designed Pol-theta polymerase inhibitor in clinical development. ART6043 is entering a first-in-human phase I clinical trial in patients with advanced or metastatic solid tumours (10). One part of the clinical trial development of these novel inhibitors is to test whether they can overcome PARP-inhibitor resistance as a single agent in particular patient types, and also in new studies for combination with PARP inhibitors such as AstraZeneca's Lynparza and Pfizer's TALZENNA. Other Pol-theta inhibitors in preclinical development have yet to enter clinical trials. Ideaya has a Pol-theta program targeting the helicase function, which is part of a strategic partnership with GSK signed in 2020 (11). Repare Therapeutics also has a Pol-theta program in partnership with ONO Pharmaceuticals (12).

Niall Martin

As the DDR treatment landscape continues to unfold, it is becoming increasingly clear that there are large untapped therapeutic opportunities beyond synthetic lethality. As scientists, we are dedicated to exploring novel approaches that target the totality of DDR to help address resistance, durability, and other unmet needs for difficult-to-treat cancers. As initial pioneers in targeting DDR with drugs, we are excited to help further evolve the field by applying the expertise and learnings we have acquired over the past two decades.

REFERENCES AVAILABLE ONLINE

FEATURE

The Microbiome Miner

Water is the giver of life, but could it be a giver of untold medicines too? Biosortia CEO Ross Youngs believes so

"Nature loves to hide" – a maxim attributed to Heraclitus (arguably Greece's strangest philosopher and a man adored by modern mystics), but do those four words have any bearing on the world of nature as studied by 21st century scientists? For Biosortia founder Ross Youngs, the answer is a resounding, "yes." Nature is full of secrets – and many are locked away in the microbiome. But now, argues Youngs, we have the means to more fully unlock the microbiome and apply the resulting knowledge to new medicines.

What led you to the world of pharma?

It's a long and convoluted story, so I'll give you the short version. After studying environmental science and industrial engineering in college, I ended up getting into the medical imaging field – a fast-growing, high-tech field at the time. It granted me a great deal of exposure to process technologies.

In 1988, I started my own company focused on making products for the optical disk industry, and it was a success – we ended up hitting the Inc. 500 five years in a row. As we explored bioplastics, we quickly realized there wasn't a lot of waste biomass that could be turned into plastics efficiently. To meet that gap in demand, we turned our attention to sourcing a form of biomass that would be cheap and environmentally friendly, which led us to algae. While working on algae, we came up with technologies that received millions of dollars in funding from various branches of the US military.

"When stacked against the full volume of a bay or a lake, eight Olympic swimming pools of water adds up to not much at all."

Though we had begun looking at algae as a means for producing biofuels, we were soon sure that the technology we had patented would have much further flung applications – including drug discovery!

How does algae connect with drug discovery?

The most important small molecules on the planet are found in microbes, but less than one percent of the microbes on the planet can be grown in a culture. From this less-than-one percent, mankind has derived half of the drugs that sit on shelves today – be it directly, indirectly, or through inspiration. Virtually all of today's research is focused on indirect access. Some readers may remember the days of combinatorial chemistry – and they'll remember that it failed pretty miserably.

Our technology has the potential to break through this deadlock by grabbing microorganisms on a massive scale from where they live in nature, without the need to culture anything. In the context of lab benchtop work, 100 milliliters would be considered a large sample size. Biosortia starts with sample sizes 200 million times larger than that. Our approach is to open up direct access to the most important hidden chemistry on the planet.

Why focus on nature's microbiome rather than the human microbiome?

It's true that there are tens of thousands of unknown small molecules in our bodies. Take a look at the human metabolome database and you'll see that fewer than 140 of the microbial small molecules in our blood have ever been identified. So, you may rightly ask – why does the natural microbiome matter? Well, believe it or not, the water of your nearest lake overlaps with your metabolic pathways and gut microbiome's genes by more than 73 percent. So that water is as about as valid a source as you are – and I'm sure you'll agree that extracting water at scale is preferable to extracting anything from you at scale!

What's your ideal source – river, pond, lake, or swamp?

The beauty of the natural world is that it is as diverse as we humans are. You might think that two lakes in Alabama, sitting side by side and used for exactly the same thing, would have identical or at least very similar microbiomes… But you'd be wrong. Upon testing, every aquatic environment shows up as unique. Temperature, pressure, sunlight, nutrients, pH, and oxygen content – all these variables shape the nature of the microbiome.

Few people realize that, for the last 4.2 billion years, microbiomes have dominated the Earth. Right now, the weight of all living microbes outweighs that of all other living organisms, if we exclude woody biomass.

In other words, we are in such early days that there could be 100 companies like ours mining the microbiome, and we would all be discovering new small molecules that are relevant to the signaling in our cells. We could be at the North Pole or the ocean floor, and we would still find microbiomes. There is no real need to be picky

That said, I would point to the tropical and subtropical environments of our country's southeast as especially interesting cases. There is a lot of water with a lot of diversity in salt and nutrient content, aerobic and anaerobic respiration, photosynthesis… The kind of variables that could keep a company like mine busy for a lifetime.

How do you find and then "mine" water?

We use a lot of collaborators – many of them from universities – to tell us about appropriate aquatic environments. From these sites, we are specifically looking for places that have not been extensively studied. When you can easily discern peaks of unknown molecules in your sample, you know there is a good chance that the microbiome in question may be of interest. The Biosortia Microbiomics approach is to start with a minimum of 100,000 grams of the dry-weight, high-quality microbiome and separate the desired molecules using extractions and fractionations. The goal is to amplify the hidden chemistry so it can be read by analytical equipment (LC/MS) at higher quantities than two parts per billion. Once we have found an optimal site, we scale up the harvest to large samples of around 20 liters. On these samples, we carry out an initial genomic analysis and then move on to what we call "scouting," which uses equipment that can easily process 10,000 liters in one day, allowing us to collect enough biomass to explore a living, active microbiome. Why genomic and not metabolomic? Because genomics, metabolic pathways, and gene clusters provide valuable relationship information. Metabolomics is also used to understand the actual molecules present, and direct analysis and indirect genomics lead to a greater understanding. If the larger sample also shows promise, we escalate once more to our full-scale unit, which can process over 20 million liters of the source – enough to capture the entire microbiome.

If that figure scares you, then let me put you at ease. The kind of harvest we typically carry out sits at around 20 million liters, which is equivalent to eight Olympic swimming pools. Though that may seem like a lot of water, when stacked against the full volume of a bay or a lake, eight Olympic swimming pools of water adds up to not much at all. I can also assure you that we work with and gain approval from the relevant state, local, and federal authorities who manage these water sources. After we finish conducting our prospecting, the microbiome will completely recover in less than one day.

We typically collect 1 million grams of the dry-weight microbiome from 20 million liters of the aquatic microbiome source, and that biomass is stored at a maximum of -20°C. Based on our initial analysis of the small molecules and references to data sets, we arrive at a greater understanding of the chemical novelty. Typically we find tens of thousands of addressable (i.e. obtainable) novel small molecules.

How exactly do you find those new and interesting small molecules?

Deep analytical data is collected on the fractions of the small molecules, including LC-MS/MS and other computational or analytical data.

This 2D data provides insight for projecting opportunities when coupled with training sets on known small molecules and activities for AI prioritization. For example, we may use a training set for antiviral activities, and artificial intelligence analysis may then help us to uncover new antiviral opportunities from the unknown small molecules of the microbiome. Just as the human microbiome holds a wealth of novelty in inflammation and neuromodulation, we can see that the microbiome has the potential to be the greatest untapped source for antiviral activity, once we take into consideration the wealth of microbes and viruses (or phages) that outnumber the microbes 10 to 1.

Additionally, we're developing several scanning strategies (some in-house and some with partners) to assess the potential of the many new small molecules we retrieve in every single harvest. Artificial intelligence (AI) is quite interesting in this context. When using machine learning for drug discovery, AI can work with either real or predicted data. Predicted data is easier to come by but when you are working with computations upon computations, errors can amplify. In short, using real data produces better output; we can see this in the application of machine learning on approved drugs to discover new ones.

In our approach, we can apply the power of AI to tens of thousands of unknown and untested small molecules to pinpoint potential opportunities, helping us prioritize our next steps for testing those molecules. Antivirals, immunology, and oncology are great places to start because the existing scientific literature has shown that the gut microbiome is key in modulating the immune systems of humans.

And what will you do with the promising molecules?

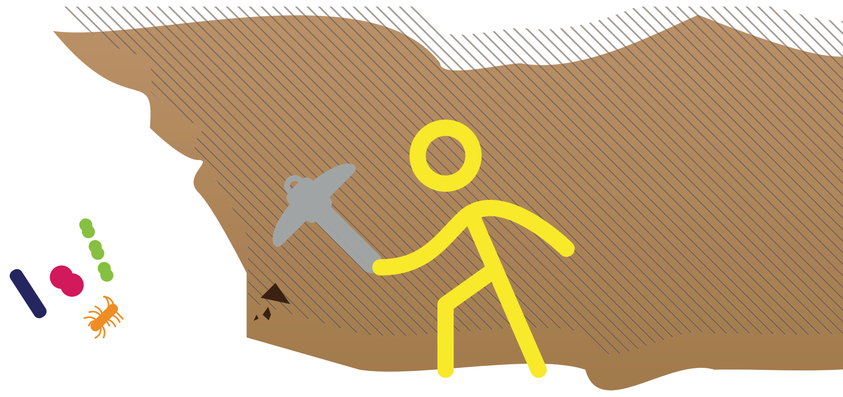
Our goal is to execute at full scale and be able to provide these molecules to partners; for example, private biotechs, pharmaceutical companies, or academic institutions. To enable those handovers, we plan to build a library of molecules, understand them, prioritize them, and curate them. We want to focus on the molecules as intellectual property, and let experts outside our company handle the medicine-making procedures. Though it's true that you cannot patent a natural molecule, you can patent its activity. Discover that activity and the patent can be yours.

What impact do you hope this work will have in the future?

I believe our work is a revolution in the making. I would say that ten years after we have begun executing at scale, you will hardly find an academic institution or an industrial company involved in life sciences that isn't directly mining microbiomes for what I'm not afraid to describe as "the hidden secrets of life".

"Though it's true that you cannot patent a natural molecule, you can patent its activity. Discover that activity and the patent can be yours."

In fact, I'll go further and say that this technique is so productive that in ten years, the vast majority of life science products will derive from it. If one percent of the world's microbes have given us half of our existing medicines, think about what the full 100 percent could do. Nature has had billions of years to create the cell signaling chemistry that runs through biology, and this shift could open it all up to us.



INTERVIEW

AI Versus AMR

MIT scientists studying AI computer programs' ability to identify drug mechanisms believe the future is bright for antibiotics discovery, but more work needs to be done

Known for being expensive and not particularly efficient, current methods for screening drug mechanisms of action have not performed well when it comes to the search for new antibiotics. Felix Wong and Aarti Krishnan, postdoctoral fellows in the Jim Collins lab at MIT and members of the Broad Institute of MIT and Harvard, hope to address that with a new study focusing on computer models' ability to identify drug mechanisms of action. Here, Wong and Krishnan discuss AlphaFold, a promising AI program they have used to accurately predict the behavior of bacterial proteins in interaction with antibacterial compounds. But is AlphaFold ready for the big leagues?

Why is AMR still such a key topic?

Global deaths due to drug-resistant bacterial infections are projected to reach 10 million per year by 2050 (1), almost twice the reported number of global COVID-19 deaths to date. The increased prevalence of AMR also means that there will be increased morbidity for even routine procedures such as surgeries and hospital care.

The lack of new antibiotics has been a longstanding challenge. How pressing is the need for innovation in this area?

It took 38 years for us to introduce a new class of antibiotics to the clinic – the oxazolidinones in 2000 – after the 1962 introduction of quinolones (2). There has been no shortage of innovation in antibiotic discovery, but finding clinically relevant antibiotics is hard. The major classes we discovered in the middle of the 20th century (which are

still in use today) came from empirical screens of natural products, notably from soil bacteria. Now, this pipeline has dried up and many of our efforts to invent new approaches have yielded molecules that are toxic to humans or for which resistance could evolve easily. One way forward may be to vastly augment the chemical libraries we are exploring to better sample more aspects of chemical space (3).

Why aren't many companies developing new antibiotics?

A major reason that companies hesitate to invest in new antibiotics is that bringing these drugs to market is generally not profitable. There is no financial incentive for a money-losing business. Governments, academia, and industry must take immediate action so that we can discover novel antibiotics against killer superbugs. A typical R&D cost for an antibiotic can be US$1.5 billion, but the revenue it generates is only about $46 million per year (4). This situation is partly due to the possibility that not many people might need that specific antibiotic and partly to the fact that the price one can charge for treatment is usually limited by government regulations.

Is the development of new antibiotics considered scientifically difficult?

Definitely. Finding chemical compounds that kill bacteria is not hard, but finding those that kill bacteria without being toxic to humans – and have enough favorable medicinal chemistry properties to inspire further testing – is rare. On top of this, bacteria might quickly evolve resistance to the compounds of interest and render them useless.

It wasn't always this difficult. The "golden age" of antibiotic discovery in the mid-20th century saw many chemical screens yield new, selective, and effective antibiotics. The problem now is that we've picked much of this low-hanging fruit. In the meantime, bacteria have evolved resistance. Our current chemical screens don't yield nearly enough lead compounds, which may be due to the fact that we can only explore so much chemical space. Developing new computational screening pipelines is one approach that could help us navigate chemical space and discover new lead compounds.

How are in silico drug discovery tools helping?

We now have computational approaches for virtual screening, so we can quickly and cheaply predict antibiotic activity from the chemical spaces of billions of compounds. We can go through these in weeks and use our models to prioritize which molecules we procure and test in the lab. The Collins lab pioneered this type of approach and it resulted in the discovery of a new antibiotic candidate, halicin, two years ago (5).

Predicting antibiotic activity is a coarse-grained approach, though. Toxic compounds often have antibiotic activity (which the model would recognize), but they don't make very good drugs. In our study, we wanted to go a step further and predict drug binding targets. This means that we could, in principle, predict how exactly an antibacterial compound works and whether or not its mechanism of action might have toxic liabilities. If our in silicoapproaches could successfully do this, we could more easily pick out real antibiotics from large chemical spaces, characterize how they select against bacteria, and maybe even design antibiotics de novo.

How does AlphaFold help – and what are its limitations?

AlphaFold is an AI system developed by DeepMind that uses the amino acid sequence from a protein to give us a three-dimensional structure. It can provide excellent predictions of the 3D structures of many proteins; those structures are freely available to the scientific community and can be used for molecular docking simulations – an in silicoapproach similar to assembling a jigsaw puzzle. This allows us to predict how a compound targets bacteria by simulating whether or not it binds to a specific protein of interest. Many antibiotics work in this way; for instance, quinolones bind specifically to bacterial DNA gyrase and topoisomerase, whereas β-lactams bind specifically to bacterial penicillin-binding proteins.

"We now have computational approaches for virtual screening, so we can quickly and cheaply predict antibiotic activity from the chemical spaces of billions of compounds."

In our research, we aimed to perform this sort of prediction at scale. We looked at the interactions between 296 proteins from Escherichia coli and 218 antibacterial compounds, or ligands (6). By simulating each of the 64,310 pairwise protein-ligand interactions using molecular docking on the corresponding AlphaFold-predicted protein structures, we could predict which binding interactions were likely and which were unlikely. We then performed experiments at the bench for 12 different proteins, empirically testing them for binding activity with respect to each of the 218 antibacterial compounds. After comparing our model predictions with our experimental results, we found that the model performed no better than chance; it correctly predicted a real interaction only about half of the time. Thus, one of the main takeaways of our study is that molecular docking needs to be improved so that we can correctly predict binding interactions and better leverage AlphaFold for antibiotic discovery. One known limitation of AlphaFold is that it predicts only static, rigid protein structures that are "stuck" in time, but the dynamic and disordered properties of these structures could be important for drug binding.

REFERENCES AND FULL INTERVIEW AVAILABLE ONLINE