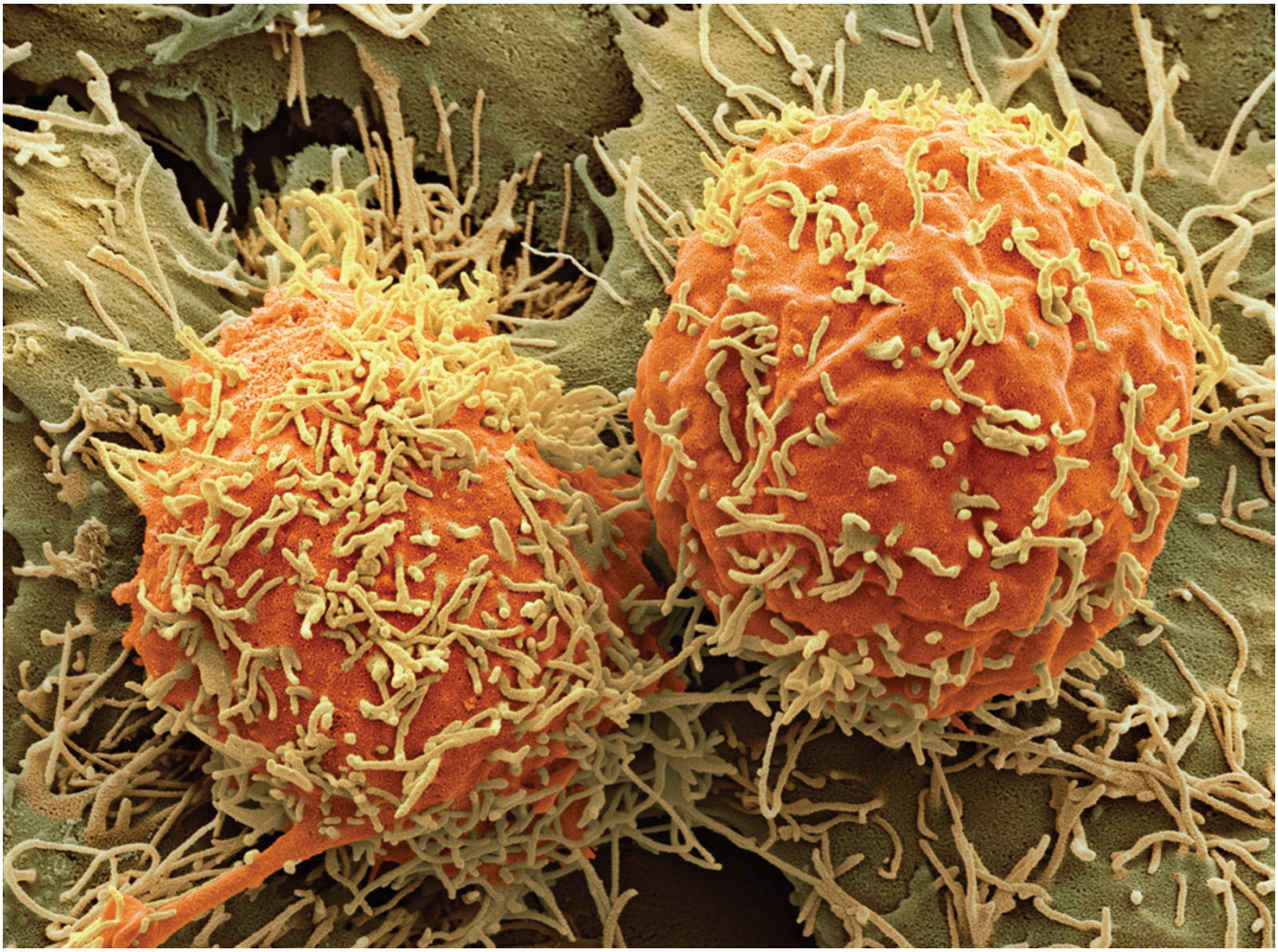

STEVE GSCHMEISSNER/SPL

A coloured scanning electron micrograph of two hepatocellular carcinoma cells. Patients with the disease typically live for only 6 to 20 months after diagnosis.

DRUG DEVELOPMENT

Try and try again

Drug companies have been fighting a losing battle against advanced liver cancer — but refinements of proven techniques along with radical new approaches could turn the tide.

BY MEGAN SCUDELLARI

At a subdued Friday afternoon poster session at a small conference in San Francisco, California, in January 2013, scientists from the pharmaceutical company AbbVie, based in Chicago, Illinois, quietly presented the findings from a phase III clinical trial for a highly anticipated liver-cancer drug. The drug had failed. Tested in more than 1,000 patients over 3 years, it offered no significant difference in survival over the standard drug therapy. The disappointment was palpable.

AbbVie had produced the latest lead balloon in a string of expensive, spectacular failures of advanced liver-cancer drugs: a year earlier, a therapy by the New York City-based pharma company Bristol-Myers Squibb had floundered in phase III, preceded by a 2011 flop from the drug giant Pfizer (also based in New York City). And this summer, the blows kept coming. In June, Eli Lilly, a pharma company headquartered in Indianapolis, Indiana, announced a phase III miss. Most recently, the Swiss drug company Novartis Pharmaceuticals, based in Basel, chalked up a failure in July. All told, five large, expensive phase III trials have failed in the past four years. "It's a bit of a graveyard right now," says Paul Lammers, the president and chief executive of Mirna Therapeutics, a biotech company based in Austin, Texas.

All five drugs were designed to treat hepatocellular carcinoma (HCC), which makes up 70–85% of all cases of liver cancer in most countries. HCC can be cured with surgery or a liver transplant if caught promptly, but only 20–30% of HCC cases are detected in the early stages. For most patients, the cancer is too advanced or underlying conditions, such as hepatitis or fatty liver disease, make the liver too fragile for invasive treatments. And without them, HCC is quick and deadly: patients typically live for only 6 to 20 months after diagnosis. According to data from the National Cancer Institute, in the United States just 15% of those with advanced disease survive one year after being diagnosed, highlighting the dire need for new therapies. So many recent

clinical-trial failures have brought a growing sense of urgency, and researchers are pushing hard to investigate therapeutic strategies that break new ground as well as those that borrow heavily from existing approaches.

Today, only one drug is approved to treat advanced HCC. Sorafenib, an oral medication marketed as Nexavar by the drug companies Bayer (Leverkusen, Germany) and Onyx Pharmaceuticals (San Francisco, California), is thought to work by binding to and shutting down key receptor tyrosine-kinase enzymes in two biological pathways that are implicated in cancer. When active, these kinases promote cell replication and the growth of new blood vessels, which are crucial for tumour survival and expansion. Sorafenib can buy patients time, but the drug is far from ideal: treatment costs US$5,400 per month, but it extends lifespan by an average of only 2.8 months and causes a range of side effects that include diarrhoea, nausea, fatigue and skin reactions.

When sorafenib was approved for HCC in 2007, many heralded it as the beginning of a new era of targeted HCC therapies, and look-alikes quickly appeared in pharmaceutical pipelines. Three of the four drugs in sorafenib's wake — AbbVie's linifanib, Bristol-Myers Squibb's brivanib and Pfizer's sunitinib — also inhibit multiple tyrosine kinases. Novartis's everolimus works in a similar fashion by blocking a protein called mTOR that activates the cell cycle and blood vessel growth. None worked better than sorafenib. Researchers also tried supplementing sorafenib with additional kinase inhibitors, such as erlotinib (developed by Genentech in San Francisco, California), but there was no improvement.

After the approval of sorafenib, companies expected to improve on it quickly and produce many more drugs for HCC that capitalized on the same mechanism. "People thought we'd be done tomorrow," says Jordi Bruix, head of the Oncology Liver Unit of the Hospital Clínic de Barcelona at the University of Barcelona in Spain, who designed and ran the phase III clinical trial of sorafenib. "This has all failed."

"There was a lot of expectation put into molecular medicine, but this has not been fulfilled."

Today, some drug developers wonder whether sorafenib even works in the way they originally thought. "Maybe we got tricked. While sorafenib hit that tyrosine-kinase pathway, it may not be the key mechanism by which it's working," says David Kirn, executive chairman of the biotech company SillaJen (based in Busan, South Korea), which is pursuing an HCC drug therapy. This leads to the next question, he says: what else is sorafenib hitting? It is a question that does not yet have an answer.

Some companies are continuing to target HCC with kinase inhibitors, but only in a subset of people with specific biomarkers that are thought to identify patients who will benefit most from that approach[1]. Other firms are beginning to look beyond sorafenib to new targets and new ways to hit them, such as by using RNA interference (RNAi) technology or cancer-killing viruses, or by attacking the cancer before it digs in.

MIRNA THERAPEUTICS

David Brown works on drug candidate MRX34 at the Mirna Therapeutics laboratory in Austin, Texas.

DIFFICULT DEVELOPMENTS

Molecular medicine — the identification and targeting of specific genetic errors and molecular changes that cause disease — has been highly successful in improving the understanding and treatment of many types of cancer. But liver cancer is not one of them[2]. The causes and progression of HCC are obscured by labyrinthine complexity.

There are three primary complexities. First, the disease is extremely diverse in both its causes and genetics. HCC develops from cirrhosis (that is, scarring of the liver due to chronic liver disease) in more than 90% of cases; but cirrhosis can result from any number of conditions, such as alcoholism, infection with hepatitis B or C, or the build-up of fat in the liver. "These patients have two major diseases; they have cancer, and they have major liver dysfunction," says Kirn.

Furthermore, whereas most cancers are driven by certain key genetic mutations, the mutations associated with HCC vary widely among patients and even within a single tumour — two different regions in the same tumour can have strikingly different mutations. Because of this dramatic diversity, finding a reliable drug is like trying to hit a moving target.

This variation also precludes the use of genetic testing to categorize patients for treatment: there are no clear genetic signatures by which to stratify them. "All the [genetic] classifications are useless to treat patients," says Bruix, whose research team developed the most widely used system for staging and treating HCC. "There was a lot of expectation put in molecular medicine, but this has not been fulfilled."

The second challenge for drug developers is that liver cells are a fortress against drugs. Liver-cancer cells show enhanced expression of proteins that confer drug resistance, and liver cancer is regularly resistant to chemotherapy. Moreover, attempting to overcome that resistance with combinations of drugs is risky: treatments that have any degree of toxicity can exacerbate the underlying liver disease.

Finally, patients with HCC who are eligible to enrol in phase III clinical trials — the true testing ground for a drug's efficacy — are typically very ill. To a large extent, this is the nature of cancer-drug development: phase III clinical trials generally enrol patients who have failed to respond to approved therapies and are in the advanced stages of disease. Therefore, "the chance to see a dramatic impact is very slim", Lammers says.

This is particularly evident in liver cancer, in which late-stage patients deteriorate quickly as their livers begin to fail. In fact, companies struggle to enrol sufficient numbers of patients into HCC clinical trials because many individuals do not have a long enough life expectancy to qualify for the trial.

A MIX OF OLD AND NEW

Today, the pharma industry is split between two camps with very different drug-development philosophies for advanced liver cancer: those who are continuing to pursue kinase inhibitors such as sorafenib, and those who are attempting to hit new drug targets using novel strategies (see 'Clinical trials for advanced hepatocellular carcinoma').

Industry veteran and physician Brian Schwartz, who led the initial development of sorafenib at Bayer, thinks that additional kinase inhibitors can succeed. Companies just have to be more careful about which patients receive which drugs, he says.

Now at the biotech company ArQule in Woburn, Massachusetts, Schwartz is overseeing a phase III trial of tivantinib. Tivantinib is a small-molecule inhibitor of c-MET, which is a receptor tyrosine kinase involved in cell proliferation and blood vessel growth. With the aim of boosting the drug's chance of success, the company is enrolling only patients who have high levels of c-MET on the surface of their tumour cells. Schwartz expects that such patient stratification will become the norm for HCC, as it is in other cancer fields. The recent failed trials might have got closer to success had they stratified them differently, he says. "That's why we spent a lot of time making sure we would stratify this trial correctly."

But other companies think that a more audacious approach is needed. Tekmira Pharmaceuticals, based in Burnaby, Canada, develops RNAi-based drug candidates. RNAi silences specific disease proteins by chopping up those proteins' messenger RNAs: molecules that carry

CLINICAL TRIALS FOR ADVANCED HEPATOCELLULAR CARCINOMA

Several prominent liver-cancer drug candidates have failed in recent years, but here are some therapies that are in clinical trials.

Treatment (company)	Trial phase	ClinicalTrials.gov identifier	Notes
Receptor tyrosine-kinase inhibitors			
Axitinib (Pfizer)	II	NCT01210495	Already approved for advanced kidney cancer
Lenvatinib (Eisai)	III	NCT01761266	Inhibits multiple receptor tyrosine kinases
Regorafenib (Bayer)	III	NCT01774344	Currently approved to treat colorectal cancer; inhibits multiple kinases
Tivantinib (ArQule)	III	NCT02029157	Inhibits a cell receptor called c-MET; might be most effective against patients with high levels of c-MET on their tumours
Other targets			
Cixutumumab (US National Cancer Institute)	II (completed)	NCT00639509	A monoclonal antibody that blocks a protein for cell growth
LY2157299 (Eli Lilly)	II	NCT02178358	Inhibits signalling by transforming growth factor-β
MRX34 (Mirna Therapeutics)	I	NCT01829971	The first microRNA to enter clinical trials for hepatocullar carcinoma
Pexa-Vec (JX-594) (SillaJen)	II (completed)	NCT00554372	Activates the immune system using an engineered vaccinia virus
TKM-PLK1 (Tekmira)	I/II	NCT02191878	Silences a protein involved in tumour-cell proliferation

genetic information for a specific protein from the nucleus to the cytoplasm. With RNAi, a small piece of RNA is delivered to a tumour cell, where it works with a protein complex to disable specific pieces of mRNA. In this way, a chosen mRNA — the product of a cancer-causing gene, for instance — can be targeted and destroyed without affecting other processes in the cell.

Mark Murray, president and chief executive of Tekmira, wanted to apply RNAi technology to cancer, so the company screened an array of oncology targets to see whether any could be effectively silenced with RNAi.

One target rose like cream to the top: polo-like kinase 1 (PLK1), a protein that is involved in tumour-cell proliferation and is overproduced in liver-cancer cells. Other companies have tried to target PLK1 with small-molecule inhibitors, but these drugs travelled to the bone marrow and interrupted normal cell division, causing dangerous side effects. To avoid this, Tekmira enclosed its interfering RNA in a small bubble of lipids that accumulate only in the liver. "We've learned from previous attempts to inhibit PLK1 and built a better mousetrap, if you will," says Murray. The final drug candidate, TKM-PLK1, is currently in a phase I/II safety and efficacy trial that is expected to conclude next year.

A different type of small RNA, microRNA, is also under investigation. In 2002, biochemist David Brown (then at Ambion in Foster City, California) and his colleagues began experimenting with these tiny strands of RNA, which do not carry instructions for creating specific proteins as mRNAs do, but instead directly coordinate activities in cells. Brown compared healthy human cells with tumour cells and identified a set of 20 microRNAs that are involved in cancer: 6 that are overabundant in cancer cells and 14 that are found at reduced levels or are absent in cancer cells.

One of the missing microRNAs, a 23-nucleotide snippet called miR-34, blocked the activity of some 25 oncogenes. Brown, now at Mirna Therapeutics, and his team have now designed a synthetic version of miR-34. When they injected it into animal models of liver cancer, the drug shrank tumours until they vanished. Today, Mirna's drug is in a phase I safety trial and is the first microRNA to enter clinical trials for HCC.

Others are investigating whether the growing field of cancer immunotherapy, which uses the body's own immune system to attack the disease, might be a better fit for tackling liver cancer. In March, SillaJen acquired the San Francisco-based biotherapeutics firm Jennerex, which has been attempting to use a virus to direct the immune system against liver-tumour cells. The company's drug, Pexa-Vec, is an engineered vaccinia virus — the same virus that is the main component in the smallpox vaccine. The virus preferentially infects cancer cells because it is activated only by certain enzymes produced by tumours. On activation, the virus multiplies inside tumour cells and causes them to burst open. In the process, it releases antigens that attract immune-system cells to the tumour site. The activated virus also attracts adaptive immune cells, using both its own viral proteins and proteins generated by a synthetic gene inserted into the virus.

In a randomized phase II trial of 30 patients with advanced liver cancer, high doses of Pexa-Vec resulted in a median survival time of more than 14 months: twice the survival time for patients on placebo[3]. A phase III trial is slated to begin next year in partnership with the biotech company Transgene (based in Strasbourg, France) and Lee's Pharmaceutical (based in Hong Kong).

REMOVING THE ROOTS

Another small yet vocal contingent of academic researchers advocates targeting HCC much earlier than today's drug candidates do, before the disease progresses.

One tantalizing target is the protein that seems to cause HCC in people infected with hepatitis. At Temple University in Philadelphia, Pennsylvania, Mark Feitelson has spent the past 30 years studying how hepatitis B leads to liver cancer. He found that the hepatitis B virus causes cancer through the action of a small protein known as hepatitis Bx (HBx). HBx is one of four proteins produced by the hepatitis B genome and is the only one involved in the regulation of viral gene expression (the other three are structural). It also seems to be a cancer-causing brute: HBx binds to and inactivates tumour suppressors, upregulates oncogenes and blocks the immune system from killing viral-infected cells.

If a drug could target HBx in hepatitis B-infected livers, Feitelson says, it could pull out the cancer by its roots, instead of waiting until the cancer was established and then trying to simply trim its branches. "If someone gets a cut, do you wait until it is infected and amputate the arm?" he asks. "Or do you put on a band aid with a topical antibiotic and be done with it?" Targeting HCC early could also help to limit the genetic variety that develops over time, resulting in more-uniform cancer cells that are easier to identify and kill.

Enough early markers for liver cancer exist — such as hepatitis infection and cirrhosis — that companies should attempt to use drugs far earlier than they do, Feitelson argues.

For now, however, drug development in HCC remains focused on late-stage cancer and the incremental steps that could extend patients' lives beyond the extra three months offered by sorafenib. "If we can make [HCC] into a more manageable disease with a quality of life that fits, that would be great," Lammers says. "We have high hopes, but nobody is talking about a cure." ■

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2. Bruix, J., Gores, G. J. & Mazzaferro, V. *Gut* **63,** 844–855 (2014).
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