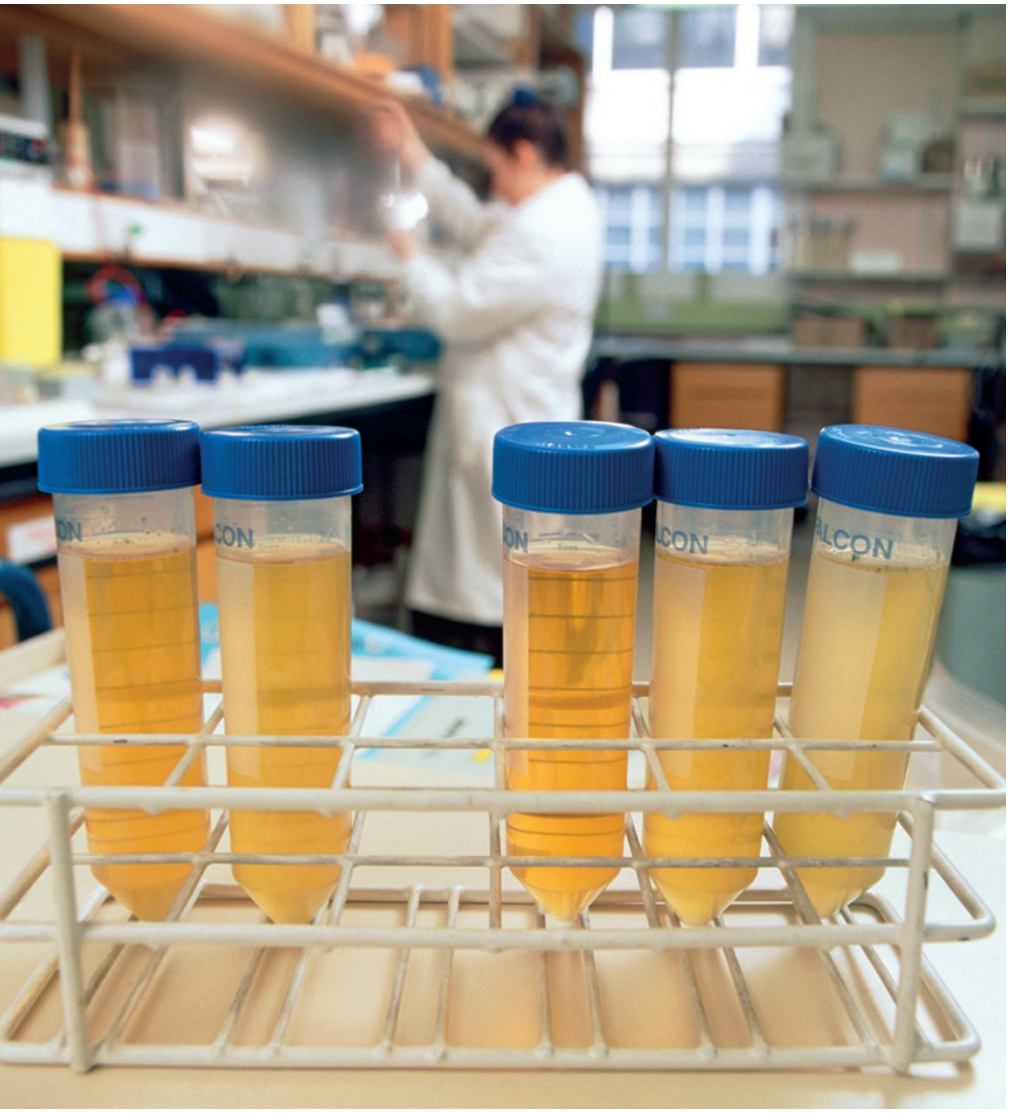


JAMES KING-HOLMES/SPL

Faecal samples contain cells that can be tested for genetic mutations associated with colorectal cancer.

DRUG DEVELOPMENT

Mix and match

Doctors face a maze of drug options and genetic markers to find the right treatment for people with advanced colorectal cancer.

BY MEGAN SCUDELLARI

First, the good news: since 2004, the arsenal of approved drugs to fight colorectal cancer has more than doubled. Five targeted therapies newly approved by the US Food and Drug Administration (FDA) have joined four established chemotherapeutic agents that were already on pharmacy shelves. Together, these drugs have extended average overall survival for advanced colorectal cancer from 15 months to 30 months.

And the bad news? That figure for average survival has not climbed above 30 months. The new drugs have been of "marginal, incremental benefit", says Scott Kopetz, a colorectal-cancer specialist at the University of Texas MD Anderson Cancer Center in Houston. "They are not home runs." And a home run is badly needed, because colorectal cancer is the fourth leading cause of cancer-related death globally (third in the United States).

NATURE.COM
You can see an animation about colorectal cancer here: go.nature.com/wgiqvp

Surgery is used when colorectal cancer is diagnosed early and achieves an impressive 90% cure rate. But the other 10% of patients, and those for whom the disease was not detected early, develop advanced disease, marked by metastatic spread of tumour cells to the lymph nodes and other sites in the body. The 5-year survival rate plummets to just 11% for those patients whose disease has spread to distant organs.

Late-stage colorectal cancer is treated with drugs, usually a combination of general chemotherapy and a molecularly targeted therapy — a drug that interferes with a specific gene or molecule in a cancer cell. "In stage-4 disease, it takes an all-hands-on-deck approach," says Adam Snook, who develops treatments for colorectal cancer at Thomas Jefferson University in Philadelphia, Pennsylvania.

Targeted therapies give doctors more options for treating advanced colorectal cancer, but that very pool of choices provides the field's current challenge — figuring out which drugs to give to which patients. No two tumours are the same, and patients can have very different responses to the same drug, so clinicians find themselves involved in an elaborate guessing game to keep their patients alive. If scientists were able to link tumour characteristics, such as DNA mutations and gene-expression profiles, to positive drug responses, that would lead to diagnostic tests to select the best treatment for a given patient.

"We can now target virtually any kind of pathway and have the drugs available," says Thomas Seufferlein, director of internal medicine at Ulm University in Germany, who runs clinical trials for colorectal-cancer therapies. "Now we really need to match the treatment to the tumour."

Researchers are taking a three-pronged approach to try and overcome the 30-month plateau for colorectal-cancer survival. First, they are testing various combinations of approved therapies in clinical trials. Second, the recent identification of four molecular subtypes of colorectal cancer means that new biomarkers might help to identify which drugs are appropriate for individual patients. Finally, the effort to discover new drugs is continuing, with strategies that include targeting biochemical pathways in tumours that have so far resisted attack, as well as activating the immune system against the cancer.

DRUG ROULETTE

The first drug proven to work against colorectal cancer — 5-fluorouracil (5-FU) — arrived on the scene in 1962. For decades, it was the only effective colorectal-cancer drug. The late 1990s and early 2000s saw the development of three other chemotherapeutic agents, oxaliplatin, capecitabine and irinotecan, which all

inhibit DNA synthesis and stop cancer cells dividing (see 'Drug options'). When combined with 5-FU, these drugs made up the first chemotherapy combinations for colorectal cancer, with names that read like a strange game of Scrabble: FOLFOX, FOLFIRI and FOLFOXIRI.

Targeted therapies first appeared in 2004, starting with the monoclonal antibody bevacizumab, which inhibits the growth of new blood vessels. Bevacizumab was followed by a string of kinase inhibitors, which block the chemical messages that encourage cell division. "Each agent on its own has maybe not been that impressive, but combinations have greatly improved survival," says Snook.

"But," he quickly adds, "there's still a long way to go." That's because no single combination has successfully treated the wide diversity of tumours. DNA and RNA sequencing of cancer-cell genomes reveals that colorectal cancer is not a single disease, but rather many types of cancer that are instigated and propelled by different mutations in different people. There is no one-drug-fits-all solution. For now, combination therapy is the best option.

Part of the reason why combinations are necessary is the sheer amount of redundancy of cancerous pathways. Colorectal cancers, like many other cancers, can compensate for loss. If you target one pathway with an inhibitor, the tumour will become resistant by mutating and upregulating another pathway to perform a similar function as the blocked pathway. Unfortunately, using combinations of three or four drugs to block numerous pathways at once can become overwhelmingly toxic for patients. A phase III trial in Sweden, for example, found that general chemotherapy followed by a combination of two targeted therapies caused serious side effects in more than half of the patients, including bowel bleeding and holes in the walls of the intestine, with no difference in overall survival[1].

"Each agent on its own has maybe not been that impressive, but combinations have greatly improved survival."

So clinicians walk a fine line between trying to prevent tumour resistance and avoiding toxicity. The best way to navigate that line in the future, most agree, is to identify which patients will respond best to which combinations. That ability requires some sort of biological marker, typically a genetic mutation or a specific pattern of gene expression.

But colorectal cancer is too complex to have just one biomarker — researchers expect to find dozens. So far, though, only three biomarkers have been identified for advanced-colorectal-cancer drug treatment, and all are negative biomarkers, which means they indicate that a patient will not respond to a particular therapy. Patients with mutated *KRAS*, *NRAS* or *BRAF* genes do not respond to two of the available targeted therapies, panitumumab and cetuximab.

And that is the whole list. No other biomarkers exist to guide care for patients with advanced colorectal cancer.

DRUG OPTIONS

After decades of stagnation, the US Food and Drug Administration has approved several agents for metastatic colorectal cancer in recent years. The table shows all approved agents as of December 2013.

Class	Agent	Year	Characteristics
Cytoxic chemotherapy	5-fluorouracil	1962	Inhibits the action of an enzyme that synthesizes a nucleoside required for DNA replication; listed as one of the World Health Organization's (WHO's) 'essential medicines'.
	Capecitabine	2001	This 'prodrug' is administered in an inactive form; enzymes in the body convert it to 5-fluorouracil.
	Irinotecan	2000	Derived from the Asian 'happy tree', a deciduous tree native to southern China.
	Oxaliplatin	2004	A platinum-based drug that causes DNA to crosslink, preventing DNA synthesis.
Inhibits growth of new blood vessels	Bevacizumab	2004	A monoclonal antibody used to treat a variety of cancers; one of the WHO's essential medicines.
	Aflibercept	2012	First approved for the treatment of macular degeneration.
EGFR antibody	Cetuximab	2004	An antibody binds the EGFR receptor; it does not work if a downstream protein of EGFR, called KRAS, is mutated.
	Panitumumab	2006	Very similar in activity to cetuximab; a 2014 comparison found the two drugs to have similar overall survival benefit and toxicity.
Kinase inhibitor	Regorafenib	2013	The most recently approved drug for colorectal cancer, it is believed to inhibit at least eight different kinases.

Source: Sridharan, M. *et al. Oncology* **28,** 110–118 (2014).

FINDING SUBTYPES

Oncologists would love to find positive biomarkers, indicating that a patient will benefit from a particular therapy. If they could use molecular information to predict positive responses to treatment, they could turn the chaotic landscape of colorectal-cancer treatment into a smooth flowchart of expected outcomes. And they have a great deal of molecular information, thanks to the availability of next-generation sequencing technologies.

"It's like we stepped out of the age where you judge the weather by watching the birds," says Seufferlein. "This is what we did for a long time — judge the tumour by looking at X-rays or CT scans. Now, we just need to translate the molecular data into therapeutic strategies."

One of the first steps towards making this translation is to better understand the diversity of the tumours. That is why, three years ago, researchers around the globe began categorizing colorectal cancer into distinct subtypes based on gene-expression patterns. The first results came from The Cancer Genome Atlas Network in 2012, based on the analysis of 276 human colorectal-cancer tumours[2]. The study proposed three classes of colorectal-cancer tumour. Other teams subsequently proposed two, four, five, even six tumour subdivisions.

The jockeying to find the best classification was reaching a frenzy when, in a remarkable display of collaboration, more than 15 competing institutions joined forces to agree on the number of subtypes. Kopetz remembers being sceptical at the idea that so many differing groups, that had previously competing conclusions, might find consensus. But they did.

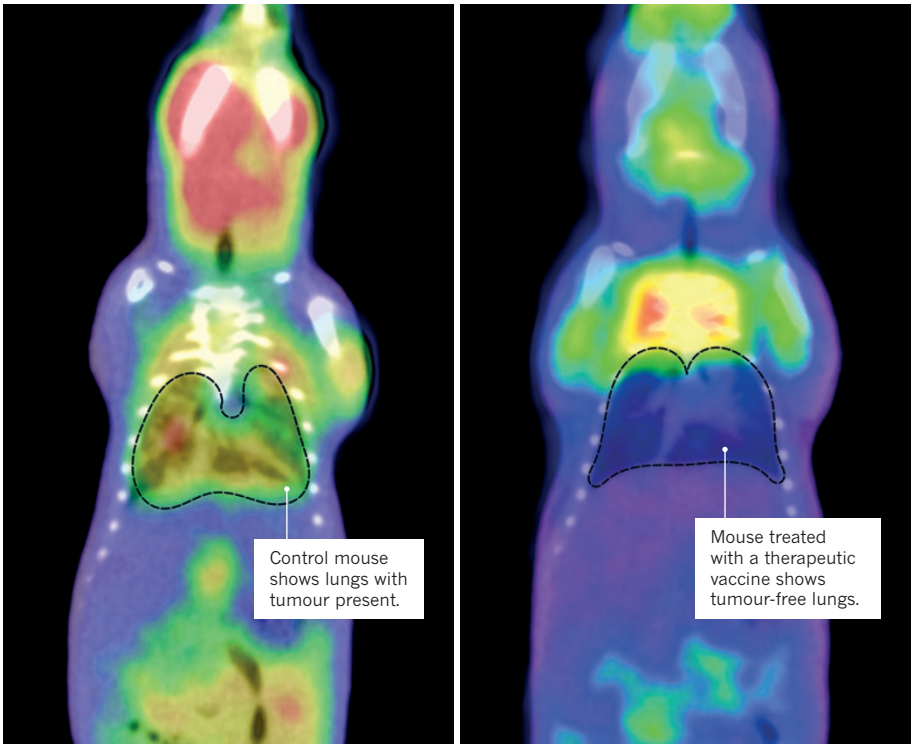
Organized by Sage Bionetworks, a non-profit company in Seattle, Washington, that specializes in collaborative data analysis, the Colorectal Cancer Subtyping Consortium used data from more than 5,000 tumour samples to identify four molecular subtypes[3]. "Everyone is setting aside their own classifications and saying this unified classification is what we should all use to move forward," says Kopetz. "It is tremendous."

So far, the consortium has identified some subtypes in which particular drug combinations clearly do not work, but many of their results are yet to be published. The goals now are to identify the best approved treatments for each subtype, and to use the subtypes to stratify patients in clinical trials.

DRUGGING THE 'UNDRUGGABLE'

There is still a huge need for new drugs to treat colorectal cancer, particularly therapies that are more effective and have fewer side effects than the current ones. But much of the pharmaceutical pipeline is made up of drugs that work in the same way as existing therapies. "There are a number of 'me-too' drugs, but we're not really tackling the root of colorectal cancer," says Federica Di Nicolantonio of the University of Turin in Italy, who specializes in preclinical drug research for colorectal cancer.

That root may be one, or both, of two types of mutation that pop up in every colorectal-cancer



ADAM SNOOK/SCOTT WALDMAN/THOMAS JEFFERSON UNIVERSITY

Immunotherapy has eliminated colorectal-cancer metastases from the lungs in mice (outlined area).

screen: *RAS* genes and the Wnt pathway. Neither has been successfully targeted with a drug, earning them the label 'undruggable'.

RAS mutations drive one-third of all human cancers, including 45% of colorectal cancers, by causing uncontrollable cell growth. For more than 30 years, scientists have tried to target the three human *RAS* genes — *KRAS*, *HRAS* and *NRAS* — but they keep hitting a brick wall. The RAS enzymes lack an active site that would normally be the target for a small-molecule drug, says Frank McCormick, who works on RAS at the University of California, San Francisco. And the rest of the protein's surface has no deep pockets that could make alternative targets. "It's just not a traditional enzyme," he says.

"We're trying to bring RAS back into focus as a drug target."

Because *RAS* mutations play such a large part in so many cancers, in 2013 the US National Cancer Institute began the RAS Initiative, a collaborative effort to better understand how *RAS* mutations drive cancer and to find new ways to silence the proteins. "We're trying to bring RAS back into focus as a drug target," says McCormick. "New technologies being developed are enabling us to target the protein in ways that were not possible when it was first attacked 15–20 years ago."

Academic teams are making progress with two small molecules that use chemical tricks to block RAS function. Both molecules are actively moving towards clinical trials in partnership with biopharmaceutical companies, says McCormick.

Less progress is being made in attempts to drug the Wnt pathway, a cascade of proteins that pass signals from the outside of a cell to the inside. This pathway is normally inactive in healthy adult cells but is activated by mutations in cancer cells. A 2012 analysis by the Cancer Genome Atlas Network found the Wnt pathway to be altered in 93% of all colorectal tumours[2]. Yet, like RAS proteins, the pathway has evaded all attempts to silence it.

A few Wnt inhibitors are now in early clinical trials, however. A team at the Icahn School of Medicine at Mount Sinai in New York, for example, is testing the addition of a soya-derived compound called genistein, which seems to inhibit a protein in the Wnt pathway, to a standard chemotherapy regimen in a phase I/II clinical trial. And drugmaker Novartis Pharmaceuticals is conducting a phase I trial of another potential small-molecule Wnt inhibitor. So far, there have been no late-stage clinical trials for Wnt inhibitors in colorectal cancer.

ACHIEVING IMMUNITY

As well as sending in molecules to kill tumour cells, scientists are also trying to activate the immune system to fight cancer cells. Immunotherapy has shown great promise in the treatment of other cancers, but has so far been unsuccessful against colorectal cancer.

One immunotherapy approach that showed remarkable results when treating melanoma and leukaemia, for example, proved disastrous against colorectal cancer. This 'adoptive cell therapy' technique involves removing immune T cells from a patient's blood, multiplying them in a dish (often after genetically engineering the cells to make them specifically attack tumour cells), and injecting them back into the patient. In a phase I trial that tested the procedure on three people with advanced colorectal cancer, all three experienced severe inflammation of the colon and had only temporary reductions in their tumours[4]. In another case, a person with advanced colorectal cancer treated with engineered T cells had a severe immune reaction and died[5].

Another type of immunotherapy deploys antibodies to shut down molecules that normally keep the immune system in check, unleashing the immune system to attack a tumour. But these 'checkpoint inhibitor' treatments have failed in colorectal cancer; in some studies, not one patient responded. "It's been a major disappointment," says Di Nicolantonio.

But not everyone has given up hope of using immunotherapy to treat colorectal cancer. Maybe drug developers have been using the wrong antigens — proteins on the surface of tumour cells that activate the immune system — says Snook, who is leading a clinical trial for a therapeutic vaccine.

Two antigens — CEA and MUC1 — have been widely studied in colorectal cancer and tested in therapeutic vaccines for more than 30 years, with mixed results. Snook's team recently turned to an antigen called GCC, which is found in 95% of colorectal-cancer tumours. The researchers are testing the safety and activity of a GCC therapeutic vaccine, and Snook hopes to have results by autumn 2015.

Immunotherapy may work on its own, or it could be combined with other treatments. Snook's team recently showed that radiation therapy followed by a therapeutic vaccine significantly amplified the immune response and shrank tumours in a mouse model of colorectal cancer[6]. "Neither therapy alone was particularly effective, but when we combined the two together, we saw a great benefit," says Snook.

Perhaps the future of colorectal-cancer therapy lies not only in combinations of drugs, but in combinations of different treatment types.

Until then, researchers continue to try to incorporate the newly defined colorectal-cancer subtypes and other biomarkers into clinical trials to improve the personalized treatment of advanced colorectal cancer. "We have all the molecular data available," says Seufferlein. "Now we need to translate it into therapeutic strategies." ■

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