# Deconstructing evolution:

## can number crunchers find the answer to resistance?

MARC BEISHON

Is there a logic, a pattern, a system behind the way cancer cells adapt to develop resistance to agents designed to kill them? Cancer research is calling on systems biologists to see if they can make sense of it all.

Imagine you are building a house from bricks. You can see how one brick works with another but you still have to work out how to build the house. Then imagine you have found a ruin – maybe an ancient Roman one, with bricks scattered about. How would you fit the pieces together to work out how people lived in those days? You can't reinvent the original, but you can use the number and location of the bricks to build models using a range of data sources that could give new insights into those ways of life.

That's an analogy Gordon Mills, chair of the systems biology department at the MD Anderson Cancer Center in Houston, uses to describe where we are with systems biology today. "We know an incredible amount about the pieces in cancer – all those molecules and receptors that people have been studying in exquisite depth for years. But none of them function in isolation and if you push on one the system will push right back and try and come into homeostasis.

"We have a very good idea of the wiring diagram of a basic cell – how all the pieces and pathways fit together so the cell functions as it should. In cancer we know we have hundreds of genetic aberrations in every cell that change the wiring diagram. It's that aberrant diagram, and the ability of the wiring to push back against the therapy, we are trying to tackle as we treat a cancer patient, particularly with targeted therapy."

The human body, he says, has robust mechanisms that have evolved from billions of years of life on earth to rewire itself to protect it from 'perturbations' caused by things like toxins in the environment. The problem is that this robust rewiring also comes into play when therapy is given say to hit a target such as EGFR in cancer, so that becomes a 'therapeutic liability', says Mills. It is at least one of the reasons why resistance can quickly develop to initially effective drugs, and only small and disappointing gains are seen with most new targeted therapies.

Further, as Mills describes, the traditional way of looking at single targets in a linear way – by drawing diagrams showing links between molecules and other entities – does not capture the feedback loops and regulatory processes at work in the system as whole. "The linear diagrams are qualitative in nature, whereas the systems biology approach is to put a mathematical and quantitative interpretation on what you have seen, because nothing happens in isolation and nothing is unidirectional, as high-school students learn with Michaelis-Menten kinetics [a famous enzyme reaction model]. The main point of systems is you can't look at a single piece but rather require a holistic view of the cell and the human body."

Systems biology, he adds, is about the thousands of things that happen in the steps required to generate a cancer and how they integrate with each other (and the term 'integrative biology', or indeed 'integrative systems biology', are also used to describe essentially the same field). "But the basic underlying step of why say DNA repair went wrong is not strictly systems biology – it is the many things that went wrong because of that step we are looking at," he says. It is about deciphering both the complexity of developing tumours and also their variability, or heterogeneity, which has dogged much traditional research.

What researchers are doing in cancer systems biology is taking huge amounts of data to build models that allow predictions to be made about what happens when a system is 'perturbed' by cancer or a drug, because it is only through building these models that interactions between parts can be uncovered and tested in experiments. "What happens when you build models is that 'emergent' properties arise – properties that you can't 'intuit' from the pieces alone," says Mills. "The challenge is building and testing a model that is robust enough to predict how a system will respond to perturbations, and this is why systems biology is an iterative process. We keep on using enormous and improved datasets to test concepts in experiments that arise from models. But the aim is that once you understand the system well enough, you can predict things like the bypass mechanisms and target them with therapies."

This means, he adds, that researchers could come up with new combinations of therapies, and their timing and dosing, which hit multiple targets and which could not have been tested in a conventional way as

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there may be no rationale for doing so, while resources for such trials are in any case severely limited. "We believe these rational combinations will be the next step in going from a transient response to targeted therapy, to durable response that will be equivalent to cures."

An example of where system biology approaches are making progress is in PI3K overactivity, associated with a number of cancers, such as triple negative breast cancer. Here, says Mills, modelling has shown that knocking out only say 60% of the activity of the pathway won't have an impact. "Instead you may need a minimum of 90% inhibition, which totally changes how you would think about implementing and dosing drugs. So you don't say, 'I need an inhibitor,' but 'I need a quantitative inhibitor.'" Further, the system has not one but at least two feedback loops, and probably more, that have to be hit, and again this is deduced from models.

Cancer system biology is furthest developed in how multiple therapies can target EGFR family members, according to Mills. "It's working out why a drug doesn't work where we will make leaps with systems biology," he adds, noting for example that in the HER2 receptor system, while a lot is known about why trastuzumab (Herceptin) works, very little is known about why it fails.

### An emerging field

As a field, cancer systems biology started in earnest within molecular oncology in the past decade, with the National Cancer Institute in the US establishing the Integrative Cancer Biology Program, which "encourages the emergence of systems biology as a distinct field" and which now has 12 associated centres, and with EU programmes emphasising the importance of systems biology in collaborative efforts. Mills set up one of the first cancer departments to use the systems biology name, at MD Anderson. Since then, he says, progress has been marked by finally having the technology needed to deliver the high-quality quantitative data needed to build the models required for systems biology, and vastly improved algorithms that can deal with the large datasets.

Awareness of the need to look at cancer as a system has certainly gathered pace, as there are now dozens of systems or integrative biology departments in Europe and the US, not just looking at cancer of course, although there has been a particular focus on the problem of drug resistance and targeted therapies in cancer.

"Most important of all we are now training the next generation of people who can handle the massive amounts of information and apply it to the systems approach," says Mills. "It's a different culture – I was brought up to look at one molecule at a time in classic biochemistry programmes – this is beyond what we thought about then. Being honest, despite knowing about systems I'm nowhere near having the skill sets of some of the people we now have in training, particularly those recruited from engineering and mathematics, who we are now prioritising more than biologists."

There are any number of analogies that can be applied to systems biology, as systems behaviour is a discipline that has been applied in many other areas, such as aircraft control systems, factory production lines, city traffic systems, and other human biological systems besides cancer. But the data and modelling techniques needed in cancer has meant that it has become critical to bring in people from other disciplines, in particular physics, engineering and mathematics, to help develop the systems thinking that can work in this complex disease.

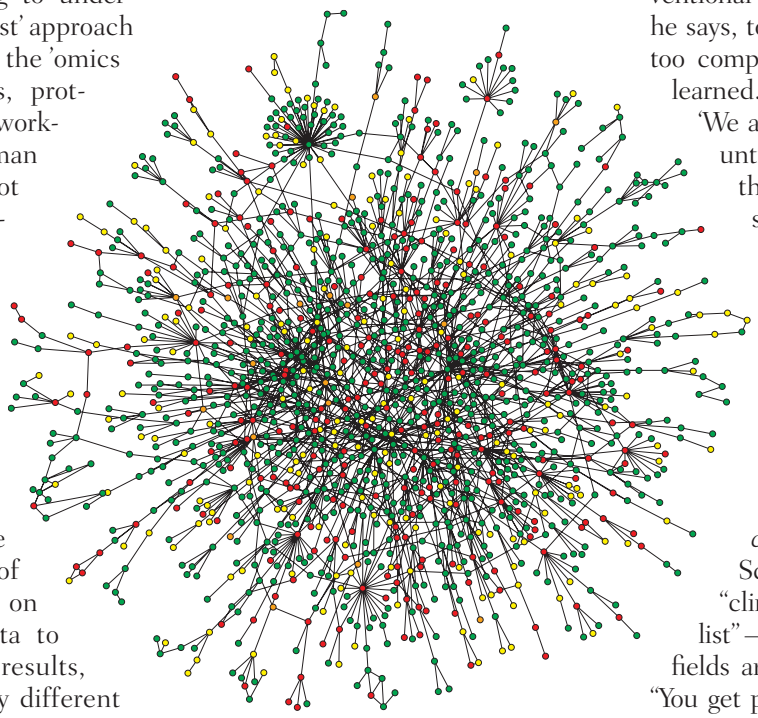
### The world of big data

Jacob Scott is a good example of this new band of researchers, who are adopting a very different scientific and cultural mindset, networking with a diverse community that is now applying radical systems thinking to cancer. A practising radiation oncologist at the Moffit Cancer Center in Florida, Scott is in the middle of a PhD in mathematical oncology – a related field where systems thinkers operate – which he is doing at the University of Oxford's Wolfson Centre for Mathematical Biology. "Biologists are not typically trained in the new world of 'big data' and systems, and need to work with people who are used to this sort of data – just as people trained in big data need to work with biologists," he says.

"Systems biology is a bit of a catch-all term, but what is clear is that

more people are coming to understand that the 'reductionist' approach of progressing through all the 'omics – genomics, epigenetics, proteomics and so on – isn't working in cancer. The human genome in itself has not provided the enlightenment once thought, and the 'whack a mole' way we now keep giving lines of therapy by looking for the next mutation isn't based on a deep understanding of the systems nature of the biology."

Scott adds that there are brilliant teams of researchers working on genomics and other data to see if they can predict results, but it is a fundamentally different approach – a 'top down' one, compared with the 'bottom up' systems approach, which is to build models that explain the data. Mills concurs, saying that the prediction modelling approaches that are combining data such as genomics are mainly qualitative – again, the crucial difference in systems biology is the quantitative approach that may use the same data but in a conceptual way. "Unfortunately, though, many types of data we have today are not of sufficient quality that they will work in systems biology, which is why we, for example, have built our own proteomics platform that so far has analysed 90,000 samples just to feed our programme."



The systems approach. This network diagram shows protein–protein interactions in a yeast cell; the biology of cancer is infinitely more complex and modelling needs to take account of quantitative aspects and feedback loops

Currently the researchers in these two camps barely know how to communicate with each other, says Scott. "The papers we modellers write are often impenetrable to, say, people in the predictive genomics camp, and vice versa," he says. That may not be surprising as systems people are bringing in all sorts of models based on fields such as competition and game theory, evolution, spatial processes, patterns and much more, together with conventional biology. It's also important, he says, to create models that are not too complex, otherwise little can be learned. "We have sayings such as, 'We are never done with a model until we can no longer take anything more away,' or as Einstein said, 'A model should be as simple as possible, but no simpler.' And there is also going to be some luck involved as we try and get a balance between adding and taking things away – there is art as well as science here."

In a short article in *Lancet Oncology* (vol 13, p 236), Scott described a new type of "clinician" – the "phase i triallist" – as people coming from other fields are "turned loose" on cancer. "You get people who dream that biology can be explained by first principles – that we can build models on a chalkboard or a computer chip that can predict how a tumour will grow and evolve, how a person may live or die.

"Why not try the tools that our conservation ecologists use to manage invasive species? That macroeconomists use to understand predatory business strategists? That agronomists use to manage pest infestations?" asks Scott. "Well, these phase i triallists have, and continue to. They have hijacked the beautiful differential equation system proposed by Lotka and Volterra to understand predator–prey systems, to try to understand how the dynamic interplay between healthy and normal is

IMAGE BY HAWOONG JEONG, KAIST, KOREA

**"Why not try the tools that our conservation ecologists use to manage invasive species?"**

## Integrating data from all the different platforms is a major challenge for enabling biological interpretation

affected by various traits or strategies. They have used Maynard Smith's evolutionary game theory to tease out the relationship between the shift to aerobic glycolysis (the Warburg shift) and cancer invasion. They have studied the prisoner's dilemma to understand cooperation between tumour cells of disparate lineage."

### Collaborating across boundaries

Scott's blog, Connecting the Dots at cancerconnector.blogspot.co.uk, is a good place to experience the eclectic nature of this new community and its experimental thinking and networking events. One of the big events is scheduled for this November, organised by the European Molecular Biology Organization in Heidelberg, Germany, under the title 'From functional genomics to systems biology', which will bring together the wide spectrum of researchers who need to collaborate to make progress in systems biology. As the organisers put it: "To gain a systems level understanding of a given process, cell or organism, the current challenge is to convert these static qualitative maps [from genomics] into dynamic quantitative models of cellular processes. This rather daunting task can only be achieved through a multidisciplinary approach, which requires intensive integration of technology and thinking from basic biology, genomics, computational biology, mathematics, engineering and physics."

Simply managing a group of diverse professionals is a big challenge in itself, says Mills. He insists that everyone in his group – which comprises clinicians and nurses as well as biologists and engineers – interact with others as much as possible. There's even a designated "interaction room", but he laments that too many people lapse into emails, whereas face to face meetings – or at least video or audio calls – are essential to communication when people are from different fields and conceptual cultures, he feels.

He adds that he considers Europe to be ahead of the US in cancer systems biology, owing to centres such as Heidelberg, Oxford and others, and to projects funded by the European Commission, including the European Systems Biology Community site (community.isbe.eu), and Infrastructure for Systems Biology Europe (project.isbe.eu), and a raft of framework projects such as MODHEP (on liver cancer) and Epigenesys, described as an "ambitious EC-funded research on epigenetics advancing towards systems biology".

Mills and colleagues describe in detail the resources and approaches that are coming together in a paper, 'Cancer systems biology: a peek into the future?' (*Nat Rev Clin Oncol* 2014, 11:167–176). They note that integrating data from all the different platforms – such as molecular profiles of tumour samples and patient data, and projects that characterise responses to perturbing cell lines – is a major challenge for enabling biological interpretation. 'Crowdsourcing' data analysis and 'big data' projects are among the advances.

They divide cancer systems biology into several approaches. For tissue complexity, they note that understanding the diverse mechanisms at work between tumour cells and the "microenvironment" may only be solved with systems biology. Then there is heterogeneity of cells in tumours, which they suggest "may represent the greatest challenge to deliver effective personalised therapy." Again, modelling is providing insights.

Targeted therapy, in particular for breast cancer, is an area of "intense research" for systems biology, and of course also for approaches for tackling drug resistance, which Mills and colleagues see as the current greatest opportunity, provided due attention is also paid to side-effects and toxicities.

That the paper poses a question – Is it the future of patient care? – does imply there is a good deal more work to do to prove that system biology models will make major contributions in cancer. "I am worried that there may be so many perturbations or changes that happen in a cancer that each may be a unique universe in itself," says Mills. "There may be sufficient heterogeneity that we cannot developed unified models. But that doesn't mean I'm not going to try.

"For now, I can comfortably say we don't need perfect data for some of the models currently in trials that could make progress in combinations of agents that target what is really going on cancer, and how cells are likely to adapt to a drug and what we can do about targeting mechanisms of resistance."

JASON HARRIS

**Communicating across the divide. Jake Scott argued the case for taking a systems modelling approach in a discussion on accelerating progress towards a cure, held at the World Oncology Forum, Lugano, 2012**

Asked to mention work he considers furthest advanced, Mills modestly doesn't mention his own lab but includes Merrimack, a biotech company near Boston, US, which is developing drugs based on a systems biology approach, such as an EGFR inhibitor combining three monoclonal antibodies that was modelled to block EGFR more completely than the 95% blockage achieved by other drugs, as the "remaining 5% of activity has the potential to still provide sufficient survival signals to allow the tumour to continue to grow and propagate," the firm says. The company has resources on its website to explain systems biology, including a video from Linda Griffiths, a professor of biological and mechanical engineering at MIT, talking about her own experience with breast cancer and how insight into personalised HER2 expression led her to opt against treatment with trastuzumab.

An academic group noted by Mills is at New York University, where they are building a systems model of the brain tumour, glioblastoma, to select likely therapies. Other groups, he mentions, are assembling concepts at a molecular level that could align patients with seemingly very different diseases such as leukaemia and breast cancer, but who may benefit from similar treatments.

At Moffit, Scott, apart from practising as a radiation oncologist, is a member of the pioneering Department of Integrated Mathematical Oncology, which is led by Alexander Anderson and Robert Gatenby and in March this year was profiled in a *Newsweek* cover story, 'You can't cure what you don't understand'. He is currently working on models of metastasis, and is particularly interested in helping bring people together in systems biology; he would like to have his own lab at some point.

This field could well develop into the kind of stage seen for brilliant young researchers in 'pure' mathematics and physics, and a benchmark has been set by Franziska Michor, an Austrian who studied molecular biology and mathematics at university, gained a PhD at Harvard in evolutionary biology, and at 32 already has her own lab, which focuses on the evolutionary dynamics of cancer, at the Dana-Farber Cancer Institute. At the age of 25, she was featured as the "Isaac Newton of biology" in *Esquire*, the men's magazine.

As *Cancer World* reported recently, Larry Norton, the breast expert at Sloan Kettering in New York, and a major mathematical modeller himself, said at the Advanced Breast Cancer conference in Lisbon that the answer to cancer may well already be in the data we have, and that ramping up data sharing is now critical. Mills agrees about data sharing and says there should be little tolerance now of people sitting on resources, but he is more cautious, saying, "We have the beginning of an answer." This is a field where both quantity and quality – from many respects – are needed in equal measure. ■