cancerous clones that might indicate insufficient treatment or herald imminent recurrence, also known as minimal residual disease (MRD).

MRD is a powerful prognostic indicator for disease-free survival. Currently, MRD is measured either by flow cytometry, which is difficult to standardize and only moderately sensitive, or allele-specific PCR assays, which must be custom-designed for each patient. With NGS-based techniques, sensitivity increases dramatically and—when applied to genomic DNA—can yield truly quantitative results.

The protocol for immune profiling begins by isolating the B- or T-cell subpopulations of interest, amplifying the CDR3 segment by PCR, and then conducting high-throughput sequencing and analysis to generate an immune 'census'. Many groups prefer to use cDNA libraries generated from mRNA transcripts, which are inherently more abundant than genomic DNA, and thus more likely to ensure broad representation of CDR3s in the final data set. This approach also enables use of PCR strategies that dramatically reduce the complexity of sample preparation. By comparison, genomic DNA analysis requires sophisticated multiplexed PCR assays with large sets of primers that can accurately target each individual V and J gene, raising the potential for amplification bias and error and making it a challenge to devise suitable reaction conditions where every primer can anneal efficiently.

Sequenta and Adaptive have devised sequencing-based approaches that address these limitations. Before treatment begins, they obtain a cancer fingerprint from the over-represented receptor sequences in blood samples. They later use these cancer signatures as red flags for potential MRD if they start to reappear after chemotherapy. To achieve this, both companies have had to devise reliable strategies that enable them to ensure that the sequences they obtain reflect the true diversity of the sample, and that rare sequence variants are actually present and not the result of errors introduced during the data-generation process.

PCR remains highly vulnerable to bias, and errors can accumulate from cycle to cycle. To help identify chimeras and artifacts some users include spike-in panels of control DNA and iterative algorithmic filters, but depth of analysis remains a critical countermeasure for achieving high confidence. "We have error models to correct for PCR errors, which is really tricky," says Robins. "But the only way to truly correct error is with redundancy." Given these challenges, he notes that MRD detection is a natural proving ground for optimizing the sensitivity and accuracy of clinical repertoire analysis.

With a Clinical Laboratory Improvements Amendments certification for their diagnostic laboratories issued in 2012, Sequenta and Adaptive Biotechnologies are preparing for commercial launch in 2013. In the meantime, they are extensively testing their respective platforms, ClonoSIGHT and clonoSEQ, in collaboration with clinical researchers and cancer centers nationwide. Both companies have also published research demonstrating that the sensitivity of repertoire sequencing greatly exceeds what can be achieved by flow cytometry, leading to early detection of MRD that might otherwise have been overlooked (*Sci. Transl. Med.* **4**, 134ra63, 2012; *Blood* **120**, 5173–5180, 2012). Such information can help doctors decide whether to extend or curtail maintenance therapy or proceed with stem cell transplantation.

Adaptive and Sequenta have initially focused on a service model, where clinical samples are processed and analyzed at a centralized laboratory, although both foresee the possibility of launching a user-friendly kit down the line. As steadily falling costs of NGS instruments and reagents continue to broaden access to such technology, this is likely to become an area of great opportunity. iRepertoire already offers its customers such an option, providing sets of reagents for the company's proprietary multiplexed PCR platform as well as data analysis and error-correction software, and it has proven to be an unexpected hit. "Probably more than half of our customers buy primers and do everything on their own," says Han. "We only have a very limited service business right now." He sees this early democratization of repertoire analysis as a means to broaden the technology's reach to researchers with limited or no genomics experience, accelerating the rate at which such tools can be applied to diseases with a prominent immune component.

To broaden the customer base to those with limited or no genomics experience, Han's group has set up Repertoire 10K, known as R10K. The focus for R10K is on identifying CDR3 sequences over-represented in patients relative to healthy controls. This project provides clinical researchers with funding, and reagents to test the technology on their own samples. "They get to be the lead author on any papers, and [keep] nonexclusive rights to do further research and development," says Han. "In exchange, we also get nonexclusive rights, and can then sell these data to pharmaceutical and diagnostic companies." R10K currently has active collaborations around 20 different diseases.

Applying immune repertoire sequencing to profile the B-cell antibody repertoires

IN brief

Another blow for ALS

In January the experimental drug dexpramipexole to treat amyotrophic lateral sclerosis (ALS) joined the junk heap of over 20 failed experimental drugs to treat the debilitating condition, also known as motor neuron disease. Biogen Idec of Weston, Massachusetts, discontinued clinical studies after a phase 3 trial of 943 patients showed no benefit. "We looked at function, survival, respiratory decline and even performed subgroup analyses," explains Douglas Kerr, director of neurodegeneration clinical research at Biogen Idec. "Quite simply the drug did not work, not on any measure." The results were a blow to the ALS research community, adds Steve Perrin, CEO of the nonprofit ALS Therapy Development Institute in Cambridge, Massachusetts, as phase 2 results had suggested a 39% slowing in disease progression rate compared to placebo. What has emerged from this and other failed ALS drug candidates is that the phase 2 studies were too underpowered to reliably gauge phase 3 outcomes, Perrin says. "We are trying to do too many things in the phase 2 trials. Either pick your dose so there are more patients in your group or power your studies a lot higher." Compounding the difficulties is the heterogeneity of ALS and the lack of biomarkers to track disease progression. Next in line are Cytokinetics' Tirasemtiv, BrainStorm Cell Therapeutics' NurOwn, Neuraltus Phamaceuticals' NP001, GlaxoSmithKline's Ozanezumab and Novartis' Gilenya, all currently in phase 2. "The failures show that these drugs should move through early trials cautiously," says Perrin. In February, the US Food and Drug Administration held a public hearing to garner input from the ALS community. Comments are due by March 25. *Gunjan Sinha*

IN their words

"A lot of companies got created with the thinking, 'If you build it they will come'." Corey Goodman, co-founder of venBio of San Francisco, now advises startups not to do what he did back in the early days of the biotech industry, when an initial public offering was a viable exit strategy. (*Xconomy*, 23 January 2013)

"It's not a foregone conclusion that payers are going to pay." Richard Pops, CEO of Alkermes of Dublin, on how insurers have replaced the FDA as biotech's 'new boogeyman'. (*Xconomy*, 7 January 2013)