compared with obtaining metallicity measurements of diffuse gas. Stars emit enormous amounts of light and can be seen with large telescopes, whereas faint diffuse gas barely emits any light and is therefore almost impossible to view.

A clever technique to probe the gas metallicity over the age of the universe is to use bright quasars as background light beacons, whose light passes through and is selectively absorbed by gas on its earthward journey. Imprinted on the quasar spectrum are the motions, chemical content, ionization balance, density, and temperature of the gas. Decoding the absorption fingerprints—spectral lines—provides details that are otherwise unobtainable using any other method of observation.

Using this technique, the chemical evolution of the universe from the present time to 1.3 billion years after the Big Bang can be mapped out (*7*, *8*) (see the figure). Many models have also predicted the metallicity evolution since the birth of the first stars (*9*–*12*). A large variance in the metallicity measurements and in the models indicates that the distribution of metals is indeed patchy and not homogeneous. However, there are no gaseous systems found to have zero metals and, in fact, no diffuse gas has been found to have a metallicity below a "floor" of 1/700th solar (*8*).

Fumagalli *et al*. have discovered two gaseous regions ~2 billion years after the Big Bang that have zero observable metals. Calculated metallicity upper limits show that the true value must be less than 1/6000th (LLS0956B) and 1/16,000th (LLS1134a) of the solar metallicity—at least three orders of magnitude lower than the mean metallicity of the universe at that epoch and certainly well below the metallicity floor. These gaseous regions consist of virtually pristine gas at an epoch where none is expected to exist. Their discovery shows that the universe is not well mixed, and although we do not expect to see Population III stars today, it is possible that they could form in these massive reservoirs of pristine gas.

The BBN and measurements of the cosmic microwave background constrain the primordial deuterium-to-hydrogen (D/H) abundance ratio. Deuterium can only be created in BBN conditions and is easily destroyed by rapidly combining into helium. Thus, it exists only because of the rapid expansion and cooling of the universe, cutting short its conversion into helium. Therefore, the deuterium abundance is very sensitive to the initial conditions of the universe. The region LLS1134a has a measured D/H ratio consistent with the primordial value, providing additional evidence that the gas is pristine.

The results of Fumagalli *et al*. show that virtually pristine gas can exist at later times in the universe than is expected due to the inhomogeneous distribution of metals. These regions, LLS1134a and LLS0956B, are the first pockets of near-to-pristine gas ever discovered. Although these systems are likely quite rare, they do provide the fuel for future formation of nearly metal-free stars as seen around our galaxy, or even Population III stars, implying that these stars do not need to form at early epochs as predicted by current models. These new findings add an exciting twist on the possible formation epoch of metal-free stars.

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MEDICINE

Personalized Cancer Diagnostics

Christopher L. Corless

A pilot study marshals sequencing resources and broad expertise to analyze patients' tumors in a cost-effective and clinically relevant time frame.

More than a decade into the age of molecularly targeted cancer therapeutics, most clincal laboratories, which are required to operate under standards established by the U.S. Food and Drug Administration called the Clinical Laboratory Improvement Amendments (CLIA), are still using a one gene–one test approach to molecular diagnostics. For example, such tests are routinely used to screen for mutations in the gene encoding the signaling protein KRAS in colorectal carcinomas, and in the gene encoding the epidermal growth factor receptor in non–small cell carcinomas of the lung. There is a growing need, however, for broader approaches that can identify more rare mutations (e.g., mutations in the *ERBB2* and *BRAF* genes in lung carcinomas) that could have an impact on clinical care. Several CLIA labs have introduced multiplexed screens that cover as many as several hundred mutations across dozens of cancer genes (*1*, *2*). But even these approaches are limited to mutation "hotspots" and, for technical reasons, necessarily favor oncogenes over tumor suppressors. Larger panels of genes based on next-generation sequencing will be introduced by a number of labs in the immediate future; even so, some are asking: Why not sequence the entire genome of each patient's tumor?

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Whole-genome sequencing can be used to devise unique tests to detect the recurrence of an individual patient's tumor (*3*). Sequencing the entire genome of a leukemia uncovered a cryptic fusion gene that prompted a major change in the clinical management of the patient (*4*). Roychowdhury *et al*. (*5*) have now taken the approach one step further, sequencing not only the whole genome, but also the whole exome (the coding regions of the genome) and the whole transcriptome (the transcribed RNAs) of individual tumors in an effort to identify all potentially important anomalies. They show that this "sequence everything" approach can be done in a cost-effective and timely manner, delivering the ultimate in personalized cancer diagnostics and further opening the door to the new era of clinical cancer genomics.

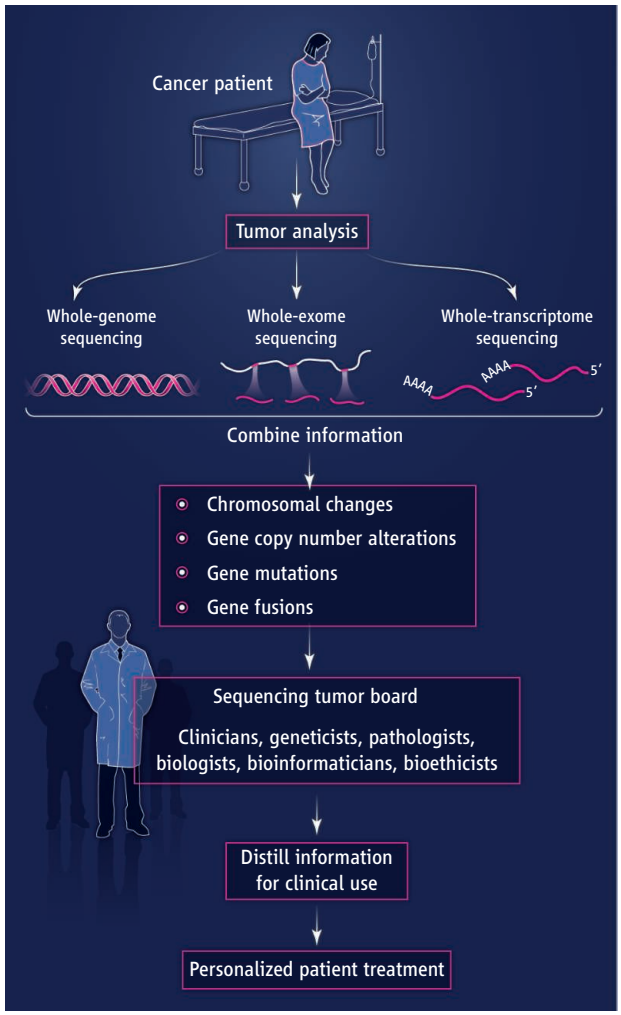
The approach of Roychowdhury *et al*. focuses on cancer patients with advanced disease and uses a consent process that includes upfront genetic counseling and the option to accept or decline information on incidental genetic findings. Fresh biopsies were collected for whole-genome sequencing of the tumor DNA (5× to 15× coverage), whole-exome sequencing of tumor and matched normal DNA (70× to 100×), and whole-transcriptome sequencing. This combination of approaches allows orthogonal confirmation of the findings. For example, of the four cases presented, one was a metastatic colorectal carcinoma in which both genomic

and exomic sequencing data predicted amplification of a region in chromosome 13q that includes the gene encoding cyclin-dependent kinase 8 (CDK8); overexpression of *CDK8* was confirmed by the transcriptome sequence. The same tumor harbored a mutation in the *NRAS* gene that was evident by all three modalities.

The time from biopsy to initial results was streamlined to just 24 days, which is within the time period often required for all of an administered drug to be eliminated from the body in patients transitioning to a clinical trial. The 72 hours of computer processing time needed to assemble and analyze the data were included in this total. With the data being generated so quickly, the issue becomes how best to distill it all for clinical use. Here, Roychowdhury *et al*. introduce an innovative concept: a multidisciplinary "sequencing tumor board" that includes clinicians, geneticists, pathologists, biologists, bioinformaticians, and bioethicists. The board discusses the findings of each tumor and determines what should be reported (see the figure). Members focus on a list of genes derived from the Sanger Institute's Cancer Gene Census, complemented by the Catalog of Somatic Mutations in Cancer (COSMIC), the genes of the human kinome (the protein kinase genes), and genes known to be targeted in current oncology trials. The results are categorized into three groups: those that may have a direct impact on care of the current cancer, those that may be biologically important but not currently actionable, and those that are of unknown importance. Because the sequencing data are generated in a research lab, mutations deemed important to clinical care require confirmation in a CLIA lab.

Roychowdhury *et al*. calculated the costs of sequencing and analysis to be $5400 per patient during the study, but indicated that they have since dropped to $3600, which approaches that of some of the multiplexed tests currently offered by CLIA labs and is below the monthly cost for some of the new targeted therapeutics. However, this figure does not include charges for the image-directed biopsy needed to obtain fresh tumor, nor does it reflect the very substantial investment in equipment and personnel needed to establish a high-quality data pipeline suitable for directing patient care.

It was not the goal of the study to prove that massively parallel sequencing of tumors can improve and extend the lives of cancer patients. Nevertheless, it is interesting to look at the cases that were presented. The colorectal carcinoma that was examined had been obtained after the patient was treated with an Aurora kinase (AURKA) inhibitor; interestingly, the tumor showed dual–copy number gain of the gene encoding AURKA, as well as a point mutation. The *NRAS* mutation found in this tumor would make the patient eligible for ongoing trials of MEK (mitogen-activated protein kinase kinase) inhibitors. Two samples of mouse xenografts derived from metastatic prostate cancers were used in the pilot phase of the project. Both showed the presence of a gene fusion (*TMPRSS2-ERG*) [making the tumor potentially sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors], deletion of the *PTEN* gene (making the tumor potentially sensitive to phosphatidylinositol 3-kinase inhibitors), and mutation of *TP53*, the gene encoding the tumor suppressor p53. One sample also showed amplification of the gene encoding the androgen receptor (*AR*), for which new antiandrogen compounds might be effective; the other showed elevated expression of PLK1, theoretically targetable with a Polo kinase inhibitor. The fourth tumor described was a melanoma with a *HRAS* mutation (new in this tumor type) that, again, might be targeted through MEK inhibitors. There was also a rearrangement of *CDKN2C* for which use of a CDK inhibitor might be invoked.



A "sequence everything" approach. A tumor analysis approach (*5*) combines whole-genome, whole-exome, and whole-transcriptome sequencing, thereby maximizing information on alterations in gene structure, copy number, and expression within a tumor. Evaluation of the findings by a multidisciplinary tumor board ensures that any resulting treatment recommendations are based on all available biological and clinical data as well as ethical considerations.

Treatment outcomes were not provided by the study, and the effectiveness of the "sequence everything" approach remains to be established. One could argue that the actionable alterations identified by the sequencing tumor board in the four presented cases (*HRAS* and *NRAS* mutations; loss of *PTEN*; amplification of *AR*) could have been detected through assays that are already clinically available, and that the massively parallel sequencing approach is akin to driving a pin with a sledgehammer. But this view goes against the genuine hope that identification of new gene mutations, fusions, and other alterations will lead to more appropriate and effective use of targeted therapeutics. As next-generation sequencing is introduced into the clinical arena, it is important that this be done by groups that have extensive experience in data generation and analysis, and that the results be examined in a multidisciplinary manner so that patients can be advised appropriately. Roychowdhury *et al*. have shown that it is technically feasible to perform deep sequencing in a clinically relevant time frame. The next critical step is to prove that this approach not only improves patient care, but also makes the most efficient use of available healthcare resources.

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