Single-cell RNA Sequencing Charts Heterogeneity in Glioblastomas

Could this heterogeneity help focus future research?

Glioblastoma is one of the most lethal malignancies. Human cancers are complex ecosystems comprised of multiple populations of cells with distinct phenotypes, and glioblastoma is no exception. In fact, it is an archetypal example of a heterogeneous cancer. A study led by a multidisciplinary team at Massachusetts General Hospital and the Broad Institute and published in the June 2014 issue of *Science* provides a deep analysis of the nature of intratumoral heterogeneity in primary glioblastoma, yielding valuable insights as to why this cancer is so lethal.¹

Using single-cell RNA sequencing to analyze five freshly resected primary glioblastoma tumors, the team generated full-length transcriptomes for 430 individual tumor cells that enabled them to characterize the cellular diversity of the tumors. The researchers identified heterogeneity in major cellular regulatory programs, highlighted mosaic expression of signaling receptors that drive proliferation, and characterized chromosomal aberrations in individual cells. Although glioblastoma researchers have long been aware that there were multiple types of cells within a glioblastoma, it had been difficult to quantify the full extent and nature of the diversity.²

These five tumors are also the first whole tumors to be characterized through single-cell RNA sequencing. This analysis has provided the highest level of detail on glioblastoma tumors to date, allowing researchers to refine their understanding of this type of cancer and guiding future investigations into potential therapies for the disease.

The study required close collaboration among the members of a team made up of surgeons and researchers from the Mass General Department of Neurosurgery, Department of Pathology, the Center for Cancer Research, and molecular and computational biologists at the Broad Institute. The team included Mass General pathologists Bradley Bernstein, MD, PhD, and Mario Suvà, MD, PhD; Aviv Regev, PhD, core member of the Broad Institute and director of the Broad’s Klarman Cell Observatory; (continued on page 2)

**Glioblastoma tumor cells.** These immunofluorescent images illustrate a strikingly heterogeneous population of cells present in tumors.

**NEW THERAPY FOR TREATMENT-RESISTANT NSCLC**
Clinical trials on CO-1686 in patients show promise.

**TRACING IDH MUTATIONS IN ICC LIVER CANCERS**
Scientists probe the mechanisms by which IDH mutations lead to tumor formation.

**OPEN TRIALS**
A selection of clinical trials currently enrolling new cancer patients.

SOURCE: Anoop Patel, MD
Deriving Single-Cell Transcriptional Programs in Glioblastoma

This chart shows the methodology by which researchers conducted single-cell RNA sequencing of glioblastoma tumors. The process required collaboration among neurosurgeons, neuropathologists, experimental pathologists, and molecular biologists.

Primary GBM

1. Neurosurgery (1, 2)
   Neurosurgeon removes the tumor from the patient and performs tumor resection.

2. Dissociation
   T=0

3. Experimental Pathology (3)
   The neuropathologist evaluates the tumor specimen for quality, and takes samples of excess material for the next step.

4. Experimental Pathology (4)
   The pathologist performs mechanical and enzymatic tissue dissociation, then removes debris, red blood cells, dead cells, and inflammatory cells. Next, a single-cell suspension is created and enriched for living tumor cells.

5. Remove RBC/Debris
   CD45 Depletion

6. Single Tumor Cells

7. Experimental Pathology (5)
   The pathologist sorts single cells into 96-well plates and stores them, frozen, at -80 degrees Celsius. A piece of bulk tumor is also banked and stored frozen at -80 degrees Celsius. Then the pathologist derives short-term primary cultures from the single-cell suspension.

8. Serum Culture

9. Glioma Spheres

10. Single Cell Sort

11. T=2–3 hrs

12. Single Cell cDNA Library

13. POPULATION cDNA Library

14. BROAD INSTITUTE (6)
   Molecular biologists perform the single-cell RNA extraction and single-cell library sequencing steps. Computational biologists perform computational analysis on the resulting data.

15. Source: Bradley Bernstein, MD, PhD, and Maria Suva, MD, PhD

Patient-to-patient differences, with distinct signature genetic events affecting patients of different ages and at different locations in the brain. These tumors also contain cellular niches enriched for specific phenotypic features, most importantly those related to glioblastoma stem cell programs, subpopulations that represent a reservoir for recurrences. No current model recapitulates the complexity of these tumors; thus, the analysis sought to map the extent and pattern of cellular heterogeneity directly in tumors. The information was also used to determine whether characteristics observed in different models were seen in vivo tumors.

The epidermal growth factor receptor (EGFR) is a drug target that is amplified in some tumors and is often considered to be the driver of tumor growth. Yet the team’s single-cell analysis showed that the mutations affecting EGFR can vary from cell to cell and that not all tumor cells express EGFR. Thus, proliferation can be driven by different EGFR variants or, alternatively, by other pathways. That explains why a drug targeting a specific EGFR mutation probably would not be sufficient on its own to eradicate a glioblastoma tumor.

In addition, the results of the analysis showed that some cells in each tumor were closer to a stem cell state than others were. This confirmed the importance of these alternate “epigenetic” cell states in human tumors. However, their analysis also showed that there was not a single distinct population of cells in the tumors that had stem-cell-like properties, but rather a continuum of cells with stem-cell-like properties.
signatures. These stem-like programs may contribute to tumor regrowth after therapy.

SUBTYPES IN A SINGLE TUMOR
Glioblastomas have been classified according to a subtype scheme that analyzes the tumor by averaging the expression of genes across millions of tumor cells in a single sample to determine the dominant subtype. However, this more granular analysis enabled researchers to show a mixed population of subtypes within each tumor. It now appears that tumors in different patients contain most or all of the same states, with only the proportion of each cell type varying. In addition, the team found a correlation between increased intratumoral heterogeneity and decreased survival.

This suggests that research into possible drug therapies should take into account heterogeneity within tumors as well as the limitation of classifying tumors according to subtype. For example, future research might identify therapies that will be more effective against a larger portion of the tumor, or a combination of therapies that together would eradicate most or all of the tumor cells.

A New Drug for Treatment-Resistant Non-Small Cell Lung Cancer

Does CO-1686 hold promise for treating EGFR-mutation NSCLC?

Lung cancer is the most common cancer worldwide, with 1.7 million new cases diagnosed annually. Some 85 percent of those diagnoses involve non-small cell lung cancer (NSCLC). Ten years ago, a Massachusetts General Hospital Cancer Center team that included Daniel Haber, MD, PhD, and Thomas Lynch, MD, discovered that a subset of NSCLCs (about 10 to 15 percent of total NSCLCs in Caucasian patients and 30 to 35 percent in East Asian patients) carried a mutation in the epidermal growth factor receptor (EGFR). Tyrosine kinase inhibitors (TKIs), including erlotinib (Tarceva) and gefitinib (Iressa), were developed to treat the cancer by targeting the EGFR mutation. Approximately 60 percent of EGFR-mutation cancers eventually become resistant to tyrosine kinase inhibitors (TKIs) because of the presence of a second, “gatekeeper” mutation: T790M. And while a new category of drugs—second-generation EGFR inhibitors—seemed to dissolve those tumors in vitro, they were unsuccessful in treating patients because, at the required dosage, the side effects—rash and diarrhea—proved too severe.

Now a third-generation EGFR inhibitor, CO-1686, shows promising results for patients whose tumors have become resistant to TKIs. The results of a phase I/II clinical trial of the drug, conducted by lead investigator Lecia Sequist, MD, medical oncologist at Mass General Cancer Center, have yielded very positive data. In addition, CO-1686 does not cause the skin rash and diarrhea seen with second-generation EGFR inhibitors. (continued on page 4)

Combating Multiple Mutations

MRI imaging of an NSCLC patient at baseline (left) and after six weeks of therapy (right). Early clinical studies of CO-1686 have shown promising results for individuals with both EGFR and T790M mutations, with a reduced side-effect profile compared with previous EGFR inhibitors.

Contributors

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SOURCE: Lecia Sequist, MD
The early findings of the phase II trial were reported in a clinical science symposium at the 2014 American Society of Clinical Oncology (ASCO) Annual Meeting in Chicago in May 2014.

Most patients with NSCLC and an EGFR mutation who initially respond to TKIs develop resistance after about one year, because they develop a second mutation called T790M that prevents the TKIs from binding to the cancer cells and thus allows the cancer to continue to grow.

To participate in the phase I trial, patients had to have been treated with an EGFR-targeted therapy. They were not required to have the T790M mutation, although all patients were biopsied to determine whether they did have that mutation. Sequist says that by the time the phase II trial was launched, it had become clear that the greatest effects of the drug were for patients who did have the T790M mutation, and so only those patients were enrolled in phase II.

The preliminary results of the phase II trial showed a response rate of 58 percent among patients with the T790M mutation who were treated with CO-1686. And thus far, the progression-free survival time for those patients is at or above one year.

WILD-TYPE EGFR SPARED TO REDUCE SIDE EFFECTS

CO-1686 seems to inhibit both active forms of EGFR mutation: the original mutation as well as the T790M mutation. And while other EGFR drugs target wild-type, or non-mutated, EGFR as well as the cancerous mutations, CO-1686 spares wild-type EGFR, which is found in many places in the body, particularly in the skin and the lining of the gut. Destruction of wild-type EGFR is the cause of TKIs’ significant toxic effects.

Because it spares the wild-type EGFR, CO-1686 caused far fewer side effects for patients in the trial. There were almost none of the grade 3 or grade 4 toxicities that occur at fairly high rates with most cancer drugs, and the skin rashes and diarrhea that typically plague patients on TKIs have not been a problem for those taking CO-1686.

Sequist did find an unexpected side effect: hyperglycemia. A substantial minority of patients experienced this side effect, which did not occur for those taking other EGFR-targeted drugs. While seeking to determine why it happens with CO-1686, researchers are trying to treat hyperglycemia prophylactically by having patients monitor their blood sugar and by prescribing medications to reduce it as soon as it rises.

BREAKTHROUGH THERAPY DESIGNATION FOR CO-1686

In May 2014, the U.S. Food and Drug Administration granted Breakthrough Therapy Designation to CO-1686, and a New Drug Application is expected to be filed by mid-2015 to make the drug available as quickly as possible to treating physicians and the patients who may benefit from it.

(continued from page 4)
What role do key mutations play in causing this lethal liver cancer?

Intrahepatic cholangiocarcinoma (ICC) is a type of liver cancer with a poor prognosis and rising incidence. Three years ago, Massachusetts General Hospital researchers found mutations in genes encoding the enzymes IDH1 and IDH2 in about 25 percent of cases of ICC. These mutations are also seen in brain tumors and in certain types of leukemia and bone cancer.

The mutant forms of IDH1 and IDH2 are thought to cause cancer through an unusual mechanism: by producing, at very high levels, a rare metabolite, 2-hydroxyglutarate (2HG), which is not normally present in the cell. Non-mutated IDH produces α-ketoglutarate (αKG), a molecule that is used to generate energy and to produce fats and other building blocks of the cell as well as serving as a cofactor used by many other enzymes. Mutant IDH, on the other hand, converts αKG to 2HG, which interferes with enzymes that require αKG.

However, the mechanisms by which mutated IDH led to tumor formation were still unclear. So the researchers developed a genetically engineered mouse model of IDH and used it to discern the mechanism of action of mutated IDH. Their study, published in the September 2014 issue of Nature, showed that the IDH mutation prevents cellular differentiation and creates a vulnerable cell state primed for transformation by other oncogenes.

Their work also created a viable mouse model system for continued study of ICC with mutated IDH.

A MUTATION THAT BLOCKS HEPATOCYTE DIFFERENTIATION

Supriya Saha, MD, PhD, Christine Parachoniak, PhD, and Nabeel Bardeesy, PhD, researchers at the Mass General Cancer Center’s Center for Cancer Research, led these studies with the help of a team of collaborating scientists. They hypothesized that mutated IDH might block liver cells in an immature or undifferentiated state. To address this, they first employed an in vitro model system of liver cell differentiation using a type of liver stem cell called a hepatoblast.

The team found that mutated IDH completely blocked the ability of stem cells to mature into differentiated hepatocytes. They discovered that the mutation did so by suppressing the production of HNF-4α, a master regulator of hepatocyte differentiation. To prove that 2HG is the mechanism that blocks differentiation, the researchers added 2HG to a normal, non-mutated stem cell, and indeed, differentiation was blocked. By contrast, a drug that inhibited mutant IDH from producing 2HG completely restored the ability of cells with this mutation to become hepatocytes.

IDH1 & IDH2 Mutations and Cancer Incidence

This chart displays the frequency of each tumor type exhibiting a mutation in either IDH1 or IDH2, present in more than 20 percent of ICC but rare or absent in other GI cancers, as well as the frequency of IDH mutations in other tissues, such as the CNS and bone.

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(continued on page 6)
**Proposed Model of IDH Mutations**

The left panel shows expression of mutant IDH in a liver hepatoblast (HB) leading to production of 2HG, blocking hepatocyte differentiation through suppression of HNF4α. On the right, IDH acts in the adult liver to block oval cell differentiation. These cells are sensitized to transformation by additional oncogenic hits, and can progress through graded premalignant biliary lesions, ultimately leading to ICC.

**IN VITRO MODEL**

- HB
- Cholangiocyte
- Quiescent Hepatocyte
- Progenitor / Oval Cell
- 
- Mutant IDH → HNF4α → 2HG → Hepatocyte

**IN VIVO MODEL**

- Cell Turnover / Injury
- + hits
- Oval Cell Expansion
- BIIIN → ICC


(continued from page 5)

**CREATING A MOUSE MODEL TO TEST THE IMPACT OF MUTATED IDH**

To further understand the process by which mutated IDH leads to ICC, the team developed a mouse genetically engineered to express mutated IDH in the liver. Yet while this produced large amounts of 2HG, it had no effect on the biology of the healthy adult liver. The team concluded that this was because the liver consists of fully mature cells unless the liver is injured, so they altered the mouse model by feeding it a chemical that would slightly injure hepatocytes. Based on prior work, this chemical was known to activate an oval cell, a type of undifferentiated cell resembling the embryonic hepatoblasts used in the *in vitro* model.

In the control mice, the liver was slightly damaged by the introduction of the chemical, but recovered and returned to normal after one month. Notably, in the livers of mice with mutated IDH, the HNF-4α gene was turned off and the oval cells continued to divide, losing their ability to differentiate into mature liver cells.

**COMBINED EFFECT OF MUTANT IDH AND KRAS ACCELERATE CELL DIVISION**

Still, this injury alone did not lead to development of tumors. The researchers hypothesized that while mutant IDH alone is not sufficient to cause cancer, its ability to block liver cells from differentiating may result in a state vulnerable to the impact of additional oncogenic mutations. In this regard, like IDH, KRAS is often mutated in human ICC tumors, but genetically engineered mice with KRAS oncogene by itself are not highly prone to ICC. Next the research team bred the mutant IDH mouse with the KRAS model. In the offspring, they found that the combined effect of mutant IDH and KRAS was accelerated division of the oval progenitor cells and the rapid development of ICC that closely modeled the multistage tumor progression that characterizes the human disease.

**A NEW MODEL FOR ICC**

The team is exploring the potential of using the newly developed mouse model to study the effects of drugs that can block mutated IDH, which they believe may restore the ability of the tumor cells to differentiate and therefore lose their malignant properties. The results of such studies will be vital to finding a treatment for ICC caused by this mutation.
Selected Open Clinical Trials

The Massachusetts General Hospital Cancer Center conducts nearly 400 clinical trials. These trials are conducted through Dana-Farber/Harvard Cancer Center, an NCI-designated Comprehensive Cancer Center, and may be open at other member institutions.

For a complete list, go to massgeneral.org/cancer/trials. To receive a monthly email about select open clinical trials at the Cancer Center, please send your contact information to MGHAdvancesInCancer@partners.org.

**Bone Marrow Transplant**

13-239

Trial of brentuximab vedotin for refractory chronic graft vs. host disease (GVHD)

Phase I | Yi-Bin Chen, MD | 617-726-1124

**Breast Cancer**

13-043

An open-label study of ARN-810 in postmenopausal women with locally advanced or metastatic estrogen receptor positive breast cancer

Phase I | Aditya Bardia, MD, MPH | 617-723-2208

**Gastrointestinal Cancers**

10-462

A first-in-human study evaluating the safety, tolerability and pharmacokinetics of AMG 337 in adult subjects with advanced solid tumors

Phase I | Eunice Kwak, MD, PhD | 617-726-8478

**Genitourinary Cancers**

13-068

A multicenter, randomized, double-blind, placebo-controlled study of ARN-509 in men with non-metastatic (MO) castration-resistant prostate cancer

Phase III | Matthew Smith, MD, PhD | 617-724-5257

**Head and Neck Cancers**

13-021

A randomized study of single agent GSK2118436 (BRAFi) vs. combination regimen GSK2118436 (BRAFi) and GSK1120212 (MEKi) in patients with BRAF mutated thyroid carcinoma

Phase II | Leri Wirth, MD | 617-726-4000

**Hematology**

2012P002359

Tissue repository for the study of idiopathic/immune thrombocytopenic purpura and autoimmune hemolytic anemia

David Kuter, MD, DPhil | 617-726-8743

**Leukemia**

13-371

A multicenter, open-label, dose-escalation, safety, pharmacokinetic, pharmacodynamic and clinical activity study of orally administered AG-221 in subjects with advanced hematologic malignancies with an IDH2 mutation

Phase I | Amir Fathi, MD | 617-726-6799

**Lymphoma**

13-348

A study of CPI-0610, a small molecule inhibitor of BET proteins, in patients with progressive lymphoma

Phase I | Jeremy S. Abramson, MD | 617-726-4000

**Melanoma**

13-424

A study of dabrafenib, trametinib, and navitoclaxin in BRAF mutant melanoma and other solid tumors

Phase I/II | Ryan Sullivan, MD | 617-643-3614

**Multiple Myeloma**

13-557

A multicenter, open-label, dose-escalation study to determine the maximum tolerated dose, safety, and efficacy of ACY-1215 in combination with pomalidomide and low-dose dexamethasone in patients with relapsed- and refractory multiple myeloma

Phase Ib/II | Noopur Raje, MD | 617-724-4000

**Neuro-Oncology**

12-466

An open-label study of bevacizumab in children and young adults with neurofibromatosis 2 and progressive vestibular schwannomas that are poor candidates for standard treatment with surgery or radiation

Phase II | Scott Plotkin, MD, MPH | 617-643-1938

**Pediatric Cancers**

09-361

A study of proton beam radiotherapy for medulloblastoma and pineoblastoma: an assessment of acute toxicity and long-term neurocognitive, neuroendocrine and ototoxicity outcomes

Phase II | Torunn Yock, MD | 617-726-6876

**Sarcoma**

13-115

A study of olaparib and temozolomide in adult patients with recurrent/metastatic Ewing’s sarcoma following failure of prior chemotherapy

Phase I | Edwin Choy, MD | 617-643-0230

**Targeted Therapies**

14-301

A study of the safety and pharmacology of MPDL3280A administered with cobimetinib in patients with locally advanced or metastatic solid tumors

Phase Ib | Keith Flaherty, MD | 617-643-5817

**Thoracic Cancers**

12-504

Randomized study comparing concise (3 months) versus prolonged (2 years) afatinib as adjuvant therapy for patients with resected stage I-II NSCLC with EGFR mutation

Phase II | Lecia Sequist, MD, MPH | 617-724-4000

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A New Way to Access Targeted Clinical Trials: TargetedCancerCare.org

At the Mass General Cancer Center, we are closing the gap between groundbreaking gene-based cancer research and life-changing treatments. We developed and launched an industry-leading website, TargetedCancerCare.org, that connects users with the Cancer Center’s innovative clinical research in the field of targeted cancer therapies.

TargetedCancerCare.org features:

- The latest information related to tumor-specific genetic and molecular biomarkers.
- An interactive tool that enables users to search for targeted cancer therapy clinical trials. Users can input as much information as they have available, searching by disease type, cancer gene or specific mutation.
- Customized search results.
- A user-friendly format designed to guide physicians through what can be an increasingly complex field with unique opportunities.
New Physician Appointments

Massachusetts General Hospital is pleased to announce new physician appointments in the areas of Hematology/Oncology, Pathology, and Surgery. For more information about the Mass General Cancer Center or our new physicians, please visit massgeneral.org/cancer.

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Kerry Reynolds, MD
Leukemia
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Controversial Cases in Hematologic Malignancies and Hematology 2015

March 27–28, 2015 • The Colonnade Hotel, Boston, MA
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Course Description: This interactive, case-focused course is designed to address challenges faced by community specialists in hematology/oncology, answer your questions, and provide a focused update on current best practices in hematologic malignancies and hematology. Presentations will be case-focused, supported by a short didactic presentation with time allotted for discussion. The case studies will utilize an audience response system to facilitate learning and encourage interaction. There will be opportunities for audience members to submit cases during panel discussions. Faculty from the Massachusetts General Hospital Cancer Center and other academic institutions will be available for small group discussion during breaks, reception, and lunch. This will be our second year offering the Hematology ABIM Maintenance of Certification (MOC) course. Participation in this session will prepare attendees for ABIM’s online Hematology module to earn 10 MOC credits.

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