MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

ADVANCES AT **MASS GENERAL CANCER CENTER** SUMMER 2015

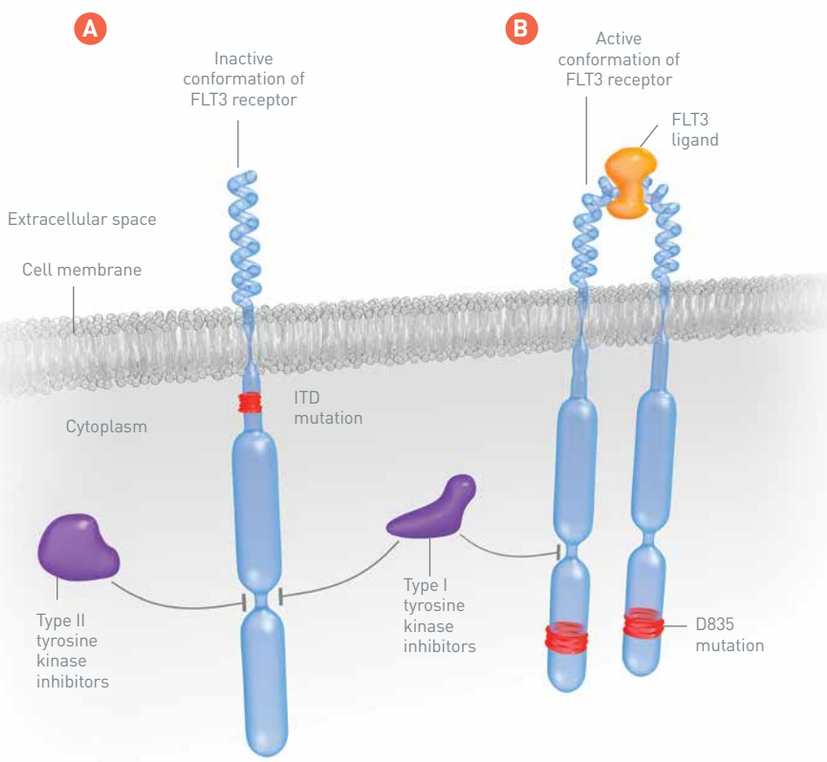
Using FLT3 Inhibitors to Treat Acute Myeloid Leukemia

Can FLT3 inhibitors yield a cure?

In a paper published in the April 2014 issue of *Therapeutic Advances in Hematology*, Amir T. Fathi, MD, medical oncologist and hematologist at the Massachusetts General Hospital Cancer Center, and his colleagues reviewed the results from preclinical studies and early clinical trials of first- and second-generation FLT3 inhibitors in acute myeloid leukemia (AML), which have been tested in combination with more traditional chemotherapy and hematopoietic stem cell transplants.

Dr. Fathi was joined by Seth A. Wander, MD, PhD, a senior resident in medicine at Mass General Cancer Center, and Mark J. Levis, MD, PhD, director of the leukemia program at Johns Hopkins' Sidney Kimmel Comprehensive Cancer Center. Together, Dr. Fathi and his colleagues examined numerous studies published between 1994 and 2013.

One of the more prominent DNA mutations in AML (the most common form of acute leukemia in adults) affects the FLT3 enzyme, a tyrosine kinase receptor at the surface of bone marrow cells *(continued on page 2)*



SOURCE: Amir T. Fathi, MD

ILLUSTRATION BY BRYAN CHRISTIE

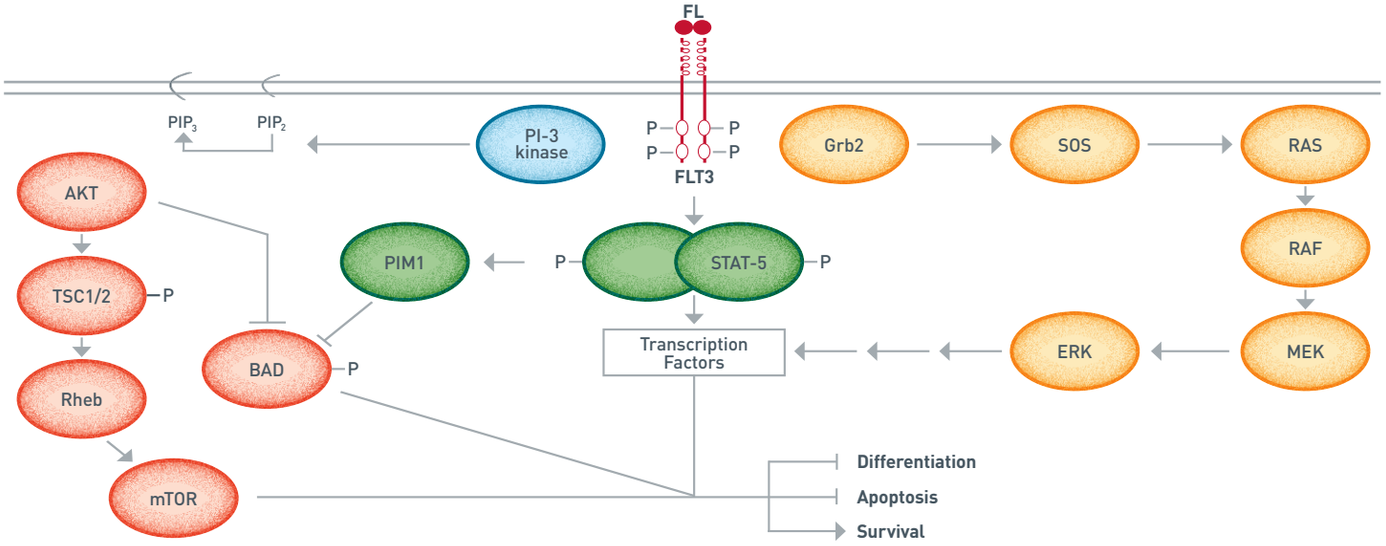
Binding of mutated FLT3 receptors by type I and type II TKIs

(A) Type II inhibitors are thought to bind the inactive conformation of the FLT3 tyrosine kinase receptor and in this manner potently inhibit the ITD-altered FLT3 tyrosine kinase. But they have limited activity against secondary point mutations such as D835, because (B) D835 point mutations are thought to destabilize the inactive conformation of the FLT3 kinase in favor of the active conformation. Therefore, type I inhibitors, which can also bind the active conformation of the enzyme, can effectively inhibit the FLT3 kinase altered by the D835 mutation.

INSIDE →

FLT3 structure and function

The FLT3 enzyme is a tyrosine kinase receptor involved in cell cycling whose mutations are commonly seen in acute myeloid leukemia. The schematic below illustrates the structure and function of FLT3, including the sites of the most common activating mutations.



SOURCE: Amir T. Fathi, MD

(continued from page 1) usually involved in cell cycling. The mutation results in unregulated activity of the FLT3 enzyme, which causes an uncontrollable replication of leukemic cells, and generally leads to an aggressive form of AML, with frequent relapses and poor prognosis. But Dr. Fathi and his colleagues across the country are studying drugs called FLT3 inhibitors that can effectively suppress the unregulated FLT3 activity in vitro and in vivo, with clinical response in some patients with advanced AML.[1]

ABERRANT FLT3 SIGNALING

FLT3 mutations come in two varieties. The more common is the ITD (internal tandem duplication) mutation, which consists of multiple repeats of DNA segments that are inappropriately inserted into the gene. A less common form is a tyrosine kinase domain (TKD) duplication, a point mutation. Both of them lead to abnormal unregulated activation of the FLT3 enzyme, which plays an established role in growth and differentiation of hematopoietic precursor cells. Normal FLT3 enzyme must bind to a FLT3 ligand to become activated, but the mutated FLT3 is less dependent on the ligand, and this can lead to an uncontrollable proliferation of cells.

Early FLT3 inhibitors, including sunitinib, midostaurin and lestaurtinib, were not initially designed to target FLT3. These drugs inhibit other key enzymes in cancer, but were later found to also inhibit FLT3. Sunitinib and lestaurtinib, as well as other early FLT3 inhibitors, ultimately were limited in their clinical impact, because of suboptimal pharmacokinetics and drug metabolism, non-sustained FLT3 inhibition or drug toxicity.[2] These early suboptimal results from clinical trials nevertheless were not wholly discouraging. The studies offered molecular insights that allowed researchers to better understand FLT3 pathobiology and design FLT3 inhibitors that more narrowly targeted the FLT3 enzyme with greater potency and duration and lower toxicity. In addition, some older, less selective FLT3 inhibitors, such as midostaurin and sorafenib, are still under clinical study and may have a future role in AML.

A newer, more selective agent, quizartinib, was identified as particularly effective in patients with *FLT3-ITD* mutations. In one study of patients over 60 years of age with relapsed or refractory AML, those with the *FLT3-ITD* mutation demonstrated 54 percent composite complete remission following treatment, a very high rate of single-agent remission for the test group. A significant number of these patients were able to successfully receive hematopoietic stem cell transplants. Similarly, a second cohort, consisting of patients aged 18 or older who had relapsed or were refractory to second-line treatment or transplants, and had the *FLT3-ITD* mutation, demonstrated a 44 percent composite complete remission rate. A separate compound, PLX3397, has also

[1] Wander, Seth A., Mark J. Levis and Amir T. Fathi, "The Evolving Role of FLT3 Inhibitors in Acute Myeloid Leukemia: Quizartinib and Beyond," *Therapeutic Advances in Hematology,* vol. 5, no. 3 (2014): 65.

[2] Fathi, Amir, and Mark Levis, "FLT3 Inhibitors: A Story of the Old and the New," *Current Opinion in Hematology,* vol. 18, no. 2 (2011): 71–76.

been identified as a potent targeted inhibitor of *FLT3-ITD* mutant AML and is under study.

OVERCOMING RESISTANCE

Additional trials assessing the efficacy of quizartinib and other FLT3 inhibitors are ongoing, even as new evidence suggests that many patients develop resistance to FLT3 inhibitors over time. Some research suggests that resistance to quizartinib and other FLT3 inhibitors develops because of new TKD mutations acquired after treatment has begun. Resistance has also been associated with upregulation of parallel and downstream signal transduction pathways. Other mechanisms may be implicated as well, including the tumor microenvironment, which may provide important pro-growth and anti-apoptotic cell signaling.

Some research indicates that combinatorial FLT3 inhibitor therapy may prevent the emergence of resistance. One highly potent FLT3 inhibitor, crenolanib, has been found to be effective in patients who have developed resistance to other FLT3 inhibitors. This is thought to be related to crenolanib being a type I tyrosine kinase inhibitor, and therefore can bind the active conformation of the TKD-altered FLT3 enzyme. Further study will be needed to determine whether this finding will be of clinical use. Individual pharmacokinetics, toxicities and drug interactions may be a significant challenge in combinatorial therapies. ■

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Patient-Derived Models of Resistant Cancers

Can cultured strains from patients promote individualized cancer therapy?

Medicines are increasingly tailored to the genetic and molecular signatures of individual cancers. But most tumors quickly develop resistance to drug therapies designed to inhibit target oncogenes, either through secondary resistance mutations in the oncogene or through the development of a "bypass track" that reactivates downstream proliferation and survival signals, which keeps the cell line alive even after the oncogene has turned off.[1] To identify personalized combinations of drugs that can overcome this resistance, a team of researchers from Massachusetts General Hospital Cancer Center helped to develop an innovative pharmacogenomic strategy, described in research published in the December 2014 issue of *Science*.

The study was a coordinated multi-disciplinary effort spearheaded by researchers from Mass General Cancer Center. The team was led by Mass General medical oncologist Adam S. Crystal, MD, PhD; Jeffrey A. Engelman, MD, PhD, director of the Center for Thoracic Cancers at Mass General Cancer Center; Cyril Benes, PhD, director of the Center for Molecular Therapeutics at Mass General; and Alice T. Shaw, MD, PhD, thoracic oncologist at the Mass General Cancer Center, among others.

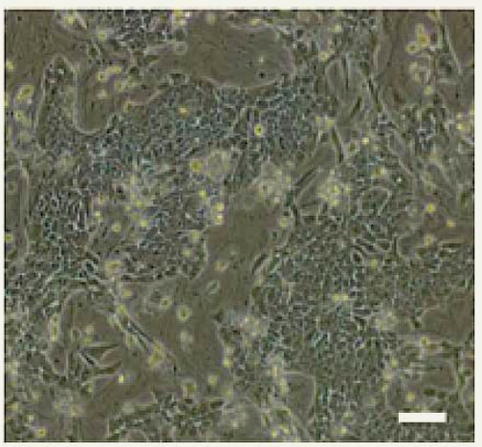
CULTURING RESISTANT CELL LINES FROM PATIENT BIOPSIES

Using irradiated feeder cells, the team was able to grow lab cultures of 60 resistant cancer cell lines directly from biopsies of cancer patients. This offered an advance on previous efforts to study resistance, which focused on two more problematic approaches: growing cell lines until drug resistance emerged, and analyzing biopsies of resistant cells to identify genetic anomalies.

Using these lab-grown cell lines, 201 different combinations of drugs were found to serve as effective therapies, or about 3.4 "hits" per cell line. The researchers genetically sequenced the cells and compared this against their pharmacological results to identify potential genetic causes of resistance. They were also able to detect previously unknown mechanisms of resistance that could not have been identified by genetic analysis alone. Such an approach can be helpful in the development of personalized therapies for resistant cancers that arise in clinical settings and guide future studies of cancer cell drug resistance. *(continued on page 4)*

Cell lines derived from patient biopsy

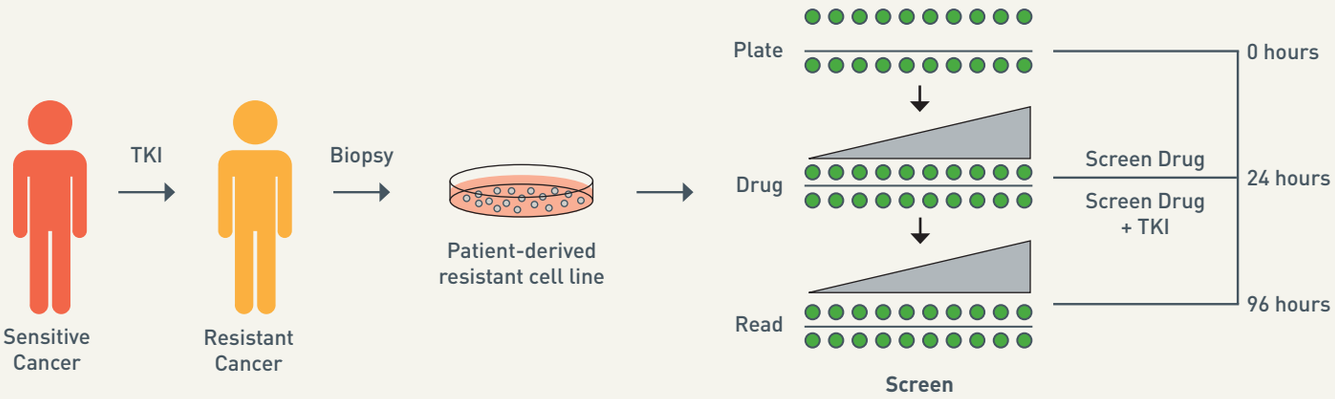
Phase contrast microscopy of a cell line derived from an EGFR-mutant lung cancer metastatic lesion with acquired resistance to EGFR inhibitors.



SOURCE: Jeffrey A. Engelman, MD, PhD. Reprinted with permission from AAAS.

Schematic of work flow for finding effective agents

Cell line models of acquired resistance were obtained directly from biopsies of patients after the development of acquired resistance to either an EGFR inhibitor or an ALK inhibitor in the clinic. Screen drugs were then tested as single agents and in the presence of a single fixed concentration of the primary tyrosine kinase inhibitor (TKI).



SOURCE: Jeffrey A. Engelman, MD, PhD. Reprinted with permission from AAAS.

(continued from page 3) The resistant biopsy cells were taken from patients with non-small cell lung cancers (NSCLCs) that had progressed in spite of treatment with common targeted oncogene therapies, such as EGFR or ALK tyrosine kinase inhibitors.[2] To grow the resistant cell lines, in many cases, the researchers established cell viability on growth-arrested but bioactive "feeder" cells, and then transitioned off those cells prior to screening. The team had a 50 percent success rate at growing cell lines in the lab out of effusions and biopsy samples taken from NSCLC patients. Cell lines established from biopsy samples had a 38 percent survival rate.

IDENTIFYING MECHANISMS OF RESISTANCE AND COMBINATION THERAPIES

To identify drug combinations that could effectively treat acquired resistance, the group then subjected these cells to 76 different pharmacological agents as single agents and in the presence of a single fixed concentration of the primary tyrosine kinase inhibitor (TKI) that inhibited the original oncogene. The 76 drugs were directed at a range of key regulators of cell proliferation and survival, including growth factor and development signaling pathways, apoptosis regulators, transcription and protein-folding machinery, and DNA damage sensors.

Their approach built on prior work that identified so-called bypass track mechanisms of acquired resistance, in which the original oncogene and bypass track both promote cell survival and proliferation through downstream signaling, such as PI3K (phosphatidylinositol 3-kinase) and MAPK (mitogen-activated protein kinase) pathways. To arrest growth and kill these cells requires simultaneous inhibition of the primary driver oncogene and the bypass track.

The soundness of their strategy was verified against five previously established models of acquired resistance developed in vitro with known bypass tracks. In these previously investigated models, unbiased screening of the 76 drug panels successfully identified known inhibitors. To identify effective combination treatments, the researchers then tested these same drug panels against 55 models of acquired resistance with unknown mechanisms of resistance.

Researchers are currently working to speed up testing so that effective drug combinations for individual patients can be identified as a routine diagnostic test within three to four weeks. ■

[1] Crystal, Adam S., Alice T. Shaw, Lecia V. Sequist, et al., "Patient-Derived Models of Acquired Resistance Can Identify Effective Drug Combinations for Cancer," *Science*, vol. 346, no. 6126 (Dec. 2014): 1480-1486.

[2] Niederst, Matthew J, and Jeffrey A. Engelman, "Bypass Mechanisms of Resistance to Receptor Tyrosine Kinase Inhibition in Lung Cancer," *Science Signaling*, vol. 6, no. 294 (Sept. 2013): re6.

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Inhibition of the Alternative Lengthening of Telomeres (ALT) Pathway

Can the ALT pathway be disrupted to treat cancer?

The telomere plays a key role in the continuous duplication of proliferating cells, and its erosion eventually leads to a cell's senescence. Cancer cells overcome this replicative senescence in one of two ways: through activating telomerase, an enzyme that extends telomeres, or using another process called the alternative lengthening of telomeres (ALT) pathway. In contrast to the RNA-directed DNA synthesis by telomerase, ALT relies on recombination and replication of telomere DNA to extend telomeres. But precisely how the ALT pathway is activated in cancer cells and how it works mechanistically remain largely unclear.

Roughly 5 percent of human cancers, and possibly more, utilize ALT; the process is prevalent in up to 60 percent of osteosarcomas and 40 percent to 60 percent of glioblastomas. Currently, no cancer therapies specifically disrupt ALT pathways.[1] Lee Zou, PhD, and his team at the Massachusetts General Hospital Cancer Center investigated the molecules and genes that drive the ALT pathway and how new treatments might disrupt this process.

TERRA CONTROLS SINGLE-STRANDED DNA BINDING PROTEIN RPA

In a paper published in *Science* in January 2015,[2] Dr. Zou and his team mapped a model of critical steps along the ALT pathway. The processes that maintain telomeres employ replication protein A (RPA), a single-stranded DNA binding protein. Dr. Zou and his team had previously investigated the role for RPA at telomeres[3] and found that it associated transiently with telomeres during S phase of DNA replication. This process is facilitated by a type of RNA called telomeric repeat-containing RNA (TERRA). Levels of TERRA fluctuate during the cell cycle as RPA binds and detaches from telomeres.

Some of the cell lines under investigation, however, did not show this fluctuation in TERRA throughout the cell cycle. Dr. Zou and his team postulated that such cancer cell lines were those without active telomerase, relying instead on the ALT pathway, which lengthens telomeres through recombination with telomeric DNA sequences from the same or other chromosomes.

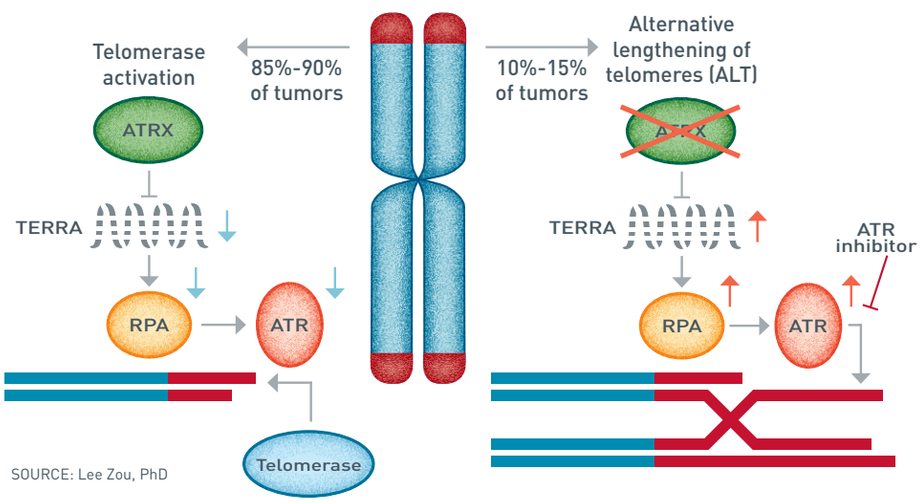
Dr. Zou and his team then looked to other known factors of ALT pathways that might intersect with TERRA fluctuation. Previously published research had shown that cancer cell lines using ALT commonly carry mutations in the ATRX gene, but researchers did not have a working model of its mechanisms. Knockdown of ATRX protein in ATRX-expressing cancer cells disrupted TERRA's fluctuation, recapitulating the situation in ALT-positive cancer cells. The study predicted that if TERRA levels did not change, RPA binding must be dysregulated as well. And indeed the team found that in cells that use the ALT pathway, RPA binds persistently to telomeres, not detaching after replication.

INHIBITING ATR DISRUPTS ALT

This insight into the role of RPA proved to be key *(continued on page 6)*

The difference between telomerase and ALT-mediated telomere elongation

In cells that use ALT, a mutation in ATRX seems to drive the ALT pathway by creating TERRA dysregulation, which in turn affects RPA, leading it to stay bound to the telomere and activate the kinase ATR. In Dr. Zou's experiment, inhibiting ATR led to cell death by stopping the ALT pathway.



SOURCE: Lee Zou, PhD

(continued from page 5) in mapping the ALT pathway. RPA functions as a DNA repair protein that promotes DNA recombination, and the ALT pathway is known to be a process that is dependent on recombination. Dr. Zou's team discovered that loss of ATRX leads to dysregulation of TERRA, which in turn causes RPA to bind persistently to telomeres. Then the persistent binding of RPA promotes recombination and the activation of the ALT pathway, allowing the cancer to continue to proliferate.

A further investigation, however, revealed that the ATRX mutation alone is not enough to activate this ALT pathway, and a fuller understanding of this mechanism is needed. Dr. Zou's team postulates that ALT is established via a multistep process that includes loss of ATRX, as well as additional genetic or epigenetic changes.

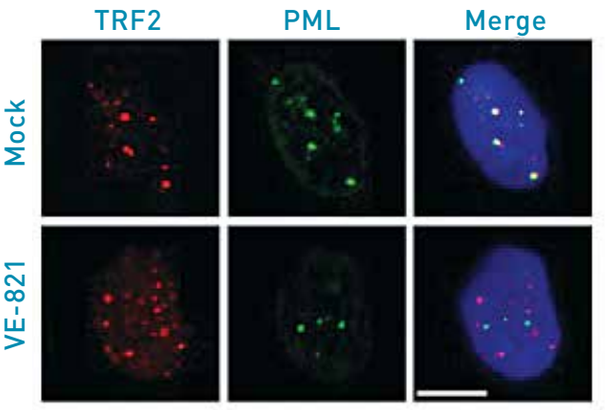
The model presented a novel avenue for treatment. Previous research by Dr. Zou and his colleagues had shown that RPA-bound single-stranded DNA activates the kinase ATR, which is known to be a master regulator of DNA repair and recombination. The implication of RPA in ALT suggests that this pathway may also be regulated by ATR and susceptible to ATR inhibitors.

Dr. Zou and his team tested the ATR inhibitors VE-821 and AZ20. These selectively eliminated ALT-positive osteosarcoma and glioblastoma cancer cells, leading to the rapid death of these cells. (The expectation was that cell death would happen more gradually.) While the mechanism is not yet fully understood, Dr. Zou speculates that cancer cells relying on the ALT pathway have rewired their DNA repair pathways to maintain telomeres. Thus, eliminating ALT would have a dramatic effect on cellular survival.

With further research and clinical trials, ATR inhibitors such as VE-821 and AZ20 could offer a promising treatment for those tumors dependent on the ALT pathway. ■

ATR inhibitor disrupts the ALT pathway

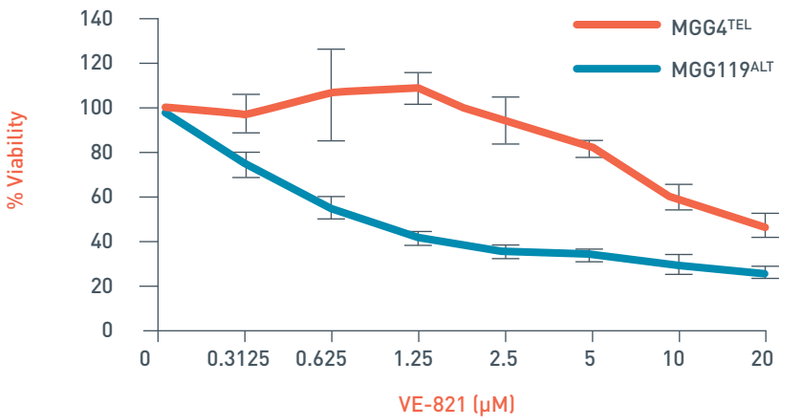
Application of an ATR inhibitor (VE-821) disrupts the colocalization of telomeres (marked by TRF2) and promyelocytic leukemia protein bodies (marked by PML) in ALT-positive U2OS cells. The colocalization of telomeres and PML bodies is a hallmark of the ALT pathway.



SOURCE: Lee Zou, PhD

Cell viability and the ALT pathway

MGG119, an ALT-positive glioma stem cell line, and MGG4, a telomerase-positive glioma stem cell line, were each treated with increasing concentrations of VE-821, an ATR inhibitor. MGG119^{ALT} cells showed significantly decreased cell viability as compared with MGG4^{TEL} cells, demonstrating that VE-821 is selectively effective in killing ALT glioma stem cells.



SOURCE: Hiroaki Wakimoto, MD, and Lee Zou, PhD

[1] Shay, Jerry W., Roger R. Reddel and Woodring E. Wright, "Cancer and Telomeres—An ALTernative to Telomerase," *Science*, vol. 336, no. 1687 (June 15, 2012): 1388–90.

[2] Flynn, Rachel L, Kelli E. Cox, Maya Jeitany, Hiroaki Wakimoto, et al., "Alternative Lengthening of Telomeres Renders Cancer Cells Hypersensitive to ATR Inhibitors," *Science*, vol. 347, no. 6219 (January 16, 2015): 273–277.

[3] Flynn, Rachel L., Richard C. Centore, Roderick J. O'Sullivan, Rekha Rai, et al., "TERRA and hnRNPA1 Orchestrate an RPA-to-POT1 Switch on Telomeric Single-Stranded DNA," *Nature*, vol. 471, no. 7339 (March 24, 2011): 532–6.

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13-239

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

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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

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13-542

An open-label, dose escalation trial of TH-302 in combination with gemcitabine and nab-paclitaxel in previously untreated subjects with metastatic or locally advanced unresectable pancreatic adenocarcinoma

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

GENITOURINARY CANCERS

13-068

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13-245

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14-344

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13-371

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14-096

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14-186

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14-049

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10-439

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PEDIATRIC CANCERS

09-361

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SARCOMA

13-115

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TARGETED THERAPIES

14-282

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THORACIC CANCERS

14-416

A biomarker-driven master protocol for second-line therapy of squamous cell lung cancer

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- **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**
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- **A user-friendly format designed to guide physicians through what can be an increasingly complex field with unique opportunities**

ANDREW ONORATO, FROM THE NOUN PROJECT

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