# NEW HOPE IN ACUTE MYELOID LEUKEMIA TREATMENT: AT LAST, A PROVEN THERAPEUTIC TARGET

A collaboration led by UCSF and involving scientists at Mt. Sinai, Pacific Biosciences, UC Berkeley, Johns Hopkins University, the University of Pennsylvania, and Ambit Biosciences demonstrates the therapeutic validity of targeting the tyrosine kinase FLT3, bringing potential for new treatments

Background

Translational research, which aims to go "from the bench to the bedside and back", has the potential to impact clinical care as well as our understanding of human disease biology. An MD/PhD and Associate Professor in the Department of Medicine at the University of California, San Francisco, Neil Shah has in the past performed groundbreaking translational studies of chronic myeloid leukemia (CML). Eventually, he took his work in understanding why certain patients developed resistance to imatinib, the first active tyrosine kinase inhibitor (sold as Gleevec®), and shepherded a new compound to treat imatinib-resistant CML through clinical trials. That compound, now known as dasatinib (Sprycel®), was approved by the FDA in 2006.
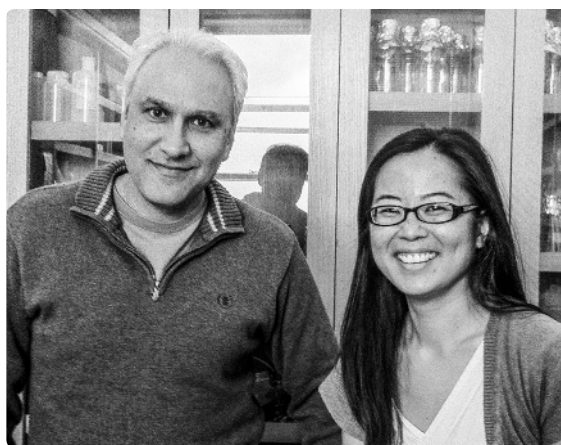
Dr. Neil Shah, Associate Professor, with Dr. Catherine Smith, Assistant Adjunct Professor Department of Medicine, UCSF

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With imatinib and other compounds like dasatinib on the market, outcomes for CML patients suddenly improved considerably — and left the oncology expert looking for another type of cancer where he could help make the same impact on patients' lives. Acute myeloid leukemia (AML) seemed to be the perfect fit. "Despite a substantial amount of research that's been done to identify potential disease-causing mutations," Shah says, "there have been no highly effective new therapies approved for years." According to the National Cancer Institute, 1 in 256 Americans will be diagnosed with acute myeloid leukemia in their lifetime. Treatment outcomes for patients diagnosed with AML, a particularly aggressive cancer that usually runs its course in a few months to a couple of years in the majority of patients, have seen no notable improvement "over the past several decades," Shah notes.

In a paper published in the April 15, 2012, edition of *Nature*, Shah and collaborators from institutions including the Mt. Sinai School of Medicine, Pacific Biosciences, University of California, Berkeley, Johns Hopkins University, the University of Pennsylvania, and Ambit Biosciences present evidence to show that FLT3, a tyrosine kinase that had fallen out of favor in pharmaceutical research, is in fact a valid therapeutic target. With the right inhibitors, as well as next-generation compounds to treat inhibitor-resistant cases, Shah says, "We anticipate that a higher proportion of patients would respond to FLT3 inhibitors and that the durability of the responses would hopefully be substantially better than what has been achieved so far."

> "We believe our findings represent a significant step toward improving patient outcomes with this disease."

The FLT3 Challenge

Previous studies in AML patients had identified activating mutations in FLT3 in 25 percent to 30 percent of cases. "Because it's an activated tyrosine kinase there were a lot of efforts to develop inhibitors," Shah says. Long before this collaboration began, pharmaceutical companies had brought several FLT3 inhibitors to clinical trials; but of more than 125 patients treated in phase II monotherapy clinical trials, Shah adds, none achieved deep remission in the bone marrow. After that, many companies and academic scientists lost interest in FLT3, assuming that the mutations it harbored were not causative to the onset of AML or critical for leukemic cell survival.

But when Ambit Biosciences came up with a potent new FLT3 inhibitor, Shah and his then-postdoctoral fellow Catherine Smith, MD, now an Assistant Adjunct Professor at UCSF, realized this might be a good way to determine once and for all whether this tyrosine kinase had any value in AML treatment. "One possibility for the lackluster responses observed with the first generation of drugs was that they were just not very effective at hitting the target at the doses they were administered at," Shah says. In the first human study that was done with Ambit's AC220 compound, some of the AML patients "had very deep remissions, including in the bone marrow. This really had never been seen to any appreciable extent in the previous FLT3 inhibitor trials,"Shah adds.

This led Shah and his team to their most recent study, which aimed to look at several patients who initially responded well to AC220 but subsequently relapsed. Had the compound shut down a different target that was more important for the leukemia cells than FLT3? Or was it hitting FLT3, and the AML patients were simply becoming resistant to the drug somehow? Shah's research plan had a problem, though: in order to study the tyrosine kinase associated with AML patients who had the poorest prognosis, he would have to look at alleles harboring activating internal tandem duplications (ITDs) in FLT3 — and the ITD mutation of the gene was about 800 bases away from the mutations in the kinase domain that Shah had identified in a laboratory screen as likely to cause drug resistance. Next-generation DNA sequencing technologies generated reads far too short to span the region, and that data would not have allowed Shah to determine whether any resistant genes found were from the same strand as the FLT3-ITD.

SMRT® Sequencing

When Smith and Shah connected with the team at Pacific Biosciences, they found that the single molecule, real-time (SMRT) sequencing technology produced reads long enough to enable the study. "We needed to sequence larger regions," Shah says. "We estimated that we would need to look at a region nearly 1,000 nucleotides in length in order to determine if we were looking at drug-resistant mutations in

the context of the pre-existing activating mutations"

Using circular consensus sequencing, the PacBio® *RS* generated reads with an average length of 1.4kb. To maintain very high accuracy, the team then analyzed only the reads that had covered each molecule at least three times in succession.

Another technical hurdle that Shah had faced in study design was detecting the signal of low-frequency mutations among the noise of both normal cells and leukemic cells that were heterozygous for activating FLT3-ITD mutations, and thus contained a normal FLT3 allele. "Standard sequencing methods have an approximate sensitivity of about 20 percent," he says. "If your drug-resistant mutant gene is present in the population at less than 20 percent frequency, it will not be readily identified." That sensitivity limit threatened the ability to get to the bottom of the FLT3 question: Shah needed to be able to detect much weaker signals.

In the *Nature* paper, the PacBio *RS* platform delivered on this challenge as well: the scientists report detecting mutations present at a level as low as 2.7 percent of the population. Shah notes that that number is, if anything, an underestimate of the system's sensitivity. "What we were able to do with the Pacific Biosciences platform is focus our interrogation on those alleles that had the activating mutation.

In fact, that 2.7 percent represents 2.7 percent of all of the alleles that have the activating mutation" — not 2.7 percent of all alleles fed into the sequencer. "It's hard to put a number on what the sensitivity will be, but a conservative estimate would be 1-3 percent," Shah says.

Analysis of the PacBio sequence data "gave us a much more precise idea of the frequency and complexity of mutations, and also identified more mutations, including substitutions at lower frequency, than what we had seen before," Shah says. "It really opened our eyes to the extent to which there was molecular heterogeneity within these relapse samples in patients."

Tracking Drug Resistance

In the study reported in *Nature*, the team sequenced a region spanning the FLT3 gene from the juxtamembrane domain, which harbors the majority of activating mutations found, through the kinase domain, where drug-resistant mutations would be expected, in samples from eight AML patients, taken both before AC220 treatment and after they stopped responding to the treatment. Comparing those sequences against each other, "what we found in all eight cases was evolution of one or more of the drug-resistant mutants that we had identified previously in a laboratory screen," Shah says. A powerful FLT3 inhibitor like AC220, in other words, was hitting the precise target it was designed for — the loss of response in patients was due to selection for drug-resistant mutants

that may possibly have been present prior to treatment. "We suspect that these drug-resistant cells are probably present in minuscule amounts pretreatment, just given how quickly some patients relapsed," Shah says.

That means a next-generation compound targeting AC220-resistant FLT3 could do for AML patients what dasatinib has done for imatinib-resistant CML patients. Indeed, Shah's team has already moved forward in identifying some promising candidate compounds that may target these particular drug-resistant mutations and one is currently in a clinical trial led by Smith at UCSF. "We believe our findings represent a significant step toward improving patient outcomes with this disease," Shah says.

But the results of this study are not just a potential boon for his own lab. "I think without question this is going to rekindle interest in not only the pharmaceutical but in the academic circles to develop not only more potent FLT3 inhibitors but also inhibitors that take into account these drug-resistant mutations," Shah says. "A number of factors took the spotlight off of FLT3 as a therapeutic target, and I think our studies unequivocally demonstrate that it is a valid therapeutic target in acute myeloid leukemia."

Looking Ahead

While Shah's team — and probably many pharmaceutical researchers as well — dive into evaluating

compounds targeting FLT3, he and Smith already have ideas for using the PacBio sequencing platform for new studies. "We are in the process of using it to address several other potentially interesting questions, such as disease evolution over time," Shah says. "Patients with CML, for instance, are treated with drugs sequentially, and patients who sequentially relapse frequently develop more than one mutation. We'd like to determine how commonly those mutations occur on the same strand of DNA versus how commonly they're in separate populations, which could influence the next choice of therapy. Additionally, we'd like to learn about clonal evolution in this setting."

We think that the Pacific Biosciences platform offers an ability to evaluate these issues in a manner that has not been possible previously. We also think that this platform has the potential to be adapted for the growing number of cancers that have activating mutations in one part of the gene and drug resistant mutations some distance away," Shah says. Examples include non-small cell lung cancer and gastrointestinal stromal tumors associated with activating mutations in EGFR and KIT, respectively. "The ability to more accurately and sensitively detect drug-resistant disease may one day enable clinicians to choose the most appropriate therapy in a more timely manner than is possible today."

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