

# ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

Section Editor: Mark J. Ratain, MD

## Heritable Resistance to Tyrosine Kinase Inhibitors



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**H&O** When did you first become interested in the existence of heritable resistance to tyrosine kinase inhibitors?

**STO** I had been working with others in the field of drug resistance in chronic myeloid leukemia (CML) for several years. One of the mysteries I had become interested in was why some patients with chronic-phase CML were resistant to tyrosine kinase inhibitors (TKIs) but did not have kinase domain mutations. Clinical studies had shown that a significant proportion of patients were resistant to nilotinib (Tasigna, Novartis), for example, but did not have kinase domain mutations, presumed to be the predominant mechanism of resistance initially. Interestingly, when cells were removed from patients and treated in vitro with a TKI, the BCR/ABL kinase was inhibited. And yet these cells would not die in vivo, and thus appeared to be intrinsically resistant to TKIs.

**H&O** How did you begin studying alternative causes of resistance?

**STO** To investigate this mystery, we obtained patient samples that had the clinical phenotype of TKI resistance but did not have kinase domain mutations, and subjected these samples to genome-wide interrogation using high-throughput sequencing.

In part by luck, we identified a polymorphic deletion in the *BIM* gene. We discovered that patients with this variant were more likely to have a suboptimal response to

TKIs. In addition, it turned out that this genetic variant was heritable. We published these findings in *Nature Medicine* in 2012, with Ng as the first author.

**H&O** Were you surprised to find a heritable genetic variant associated with TKI resistance?

**STO** Yes, it was very surprising. In our initial results, the deletion in our patient samples was exactly 2903 base pairs. At first we were disappointed because we had not anticipated discovering a germline variant capable of conferring resistance to targeted therapies. We thought we had stumbled across just another polymorphism that would not have any significance. But then we realized that the polymorphic deletion was in the middle of a gene called *BIM*, whose proapoptotic function other groups had described as being critical to TKI sensitivity. We then became very interested in pursuing this phenomenon further, in large part because the discovery of a germline contribution to targeted therapies might address the initial mystery we were looking to solve, that of intrinsic drug resistance.

**H&O** What was your next step in the research?

**STO** We wanted to investigate how the *BIM* deletion might cause TKI resistance, and whether the presence of the polymorphism could predict resistance to TKIs not only in CML but also in other cancer types where treatment depends on *BIM* expression for sensitivity. Our

vision of the work became enlarged, and as a result, a lot more exciting.

### H&O Had other examples of heritable resistance been identified prior to this finding?

**STO** Yes. Prior to our work, groups working in other cancers, including acute lymphoblastic leukemia (ALL) at St. Jude Children's Research Hospital, had reported germline variants associated with inferior outcomes following standard therapy. Yang and colleagues published their research in 2009 in the *Journal of the American Medical Association*, and Perez-Andreu and colleagues published their findings in 2013 in *Nature Genetics*.

### H&O Were there other clues that germline variation could contribute to treatment response?

**STO** The heterogeneity in responses that we observe among patients with CML was itself a clue that there could be heritable variants. Normal variants in the human genome could account for much of the variation in response.

But even in light of this presumed genetic variation, the finding about *BIM* provided a relatively clean and simple model to study because it was a single gene variant in a disease with a single oncogenic driver, treated with a single drug. Also, we were able to provide substantial *in vitro* data specifying the precise mechanism by which this variant induced TKI resistance. With these studies, we were able to provide a crucial mechanistic link between the polymorphism, alterations in protein function, and the mechanism of resistance.

### H&O Have other studies revealed more about BIM variants?

**STO** Yes. Since the publication of our research, 2 other groups have described single nucleotide polymorphisms (SNPs) in *BIM* associated with different responses to treatment. One 2013 study by Augis and colleagues in *PLoS One* found a SNP in *BIM* associated with a slower major molecular response among a French population of patients with CML treated with imatinib. This group had sequenced the coding sequences of *BIM* and discovered a SNP that coded for a T allele. This SNP did not change the amino acid sequence, but the study authors showed that it was associated with a longer time to achieving a major molecular response. Although the mechanism was not fully described, the authors suggested that the polymorphic variant was associated with lower levels of *BIM-EL*, a proapoptotic form of *BIM*.

Another group, also from France, found a polymorphism that appears to be important for treatment

response among children with ALL. This research was published in 2013 in *Clinical Cancer Research*, with Gagne as the lead author.

### H&O How did you identify the mechanism of TKI resistance associated with the BIM variant you identified?

**STO** The mechanism we eventually homed in on stemmed from the fact that the deletion we found was an intronic sequence. Because the deletion was not in a coding region, we knew that the variant was not impacting the amino acid sequence. But how could an intronic deletion cause changes in *BIM* function?

Our first consideration was that the deleted region might contain an enhancer. If the variant lacked an enhancer for *BIM* expression, then there might be lower levels of proapoptotic *BIM* in cells, leading to resistance. However, we could not find any evidence at the molecular level to support this hypothesis.

Next we considered the possibility that the deletion causes changes in splicing, and indeed this turned out to be the actual mechanism. The *BIM* protein contains a domain known as BH3, which is critical for its proapoptotic function. The variant of *BIM* that we identified biases splicing to isoforms of *BIM* that do not contain the exon encoding the BH3 domain. In other words, the deletion was changing the splicing of the *BIM* gene, and generating splice isoforms of *BIM* that were ineffective at causing cell death. This research was published in *PLoS One* in 2014, with Juan as the first author.

There may also be other mechanisms by which other polymorphic variants in *BIM* might contribute to TKI resistance. It is easy to imagine scenarios where other variants might affect *BIM* transcription, translation, or protein stability, for example. Additionally, genes other than *BIM*—but with equally important roles in cell survival and cell death pathways—could also confer similar modes of TKI resistance.

### H&O Most patients diagnosed with CML will opt for treatment with a TKI. Why is it important to identify heritable mechanisms of resistance? How will this finding impact patients in the clinic?

**STO** To answer this question, it is important to understand what this variant does *in vitro*. In our cells lines, we found that *BIM* deletion was sufficient to confer resistance in cells. But *BIM* deletion resistance is not absolute; the resistance to TKIs is relative. We found that if we engineered a cell line to be resistant by inserting this deletion into it, and then exposed this cell line to TKIs either at higher concentrations or for longer durations, then the cells would eventually

become sensitive to the drug. We expect that the same may be true for patients who have the TKI-resistant *BIM* variation: perhaps if the dose is increased or we use more potent TKIs, the CML cells will eventually be killed.

Current clinical guidelines for CML treatment emphasize the achievement of depth of response benchmarks at specific interval following treatment initiation—3 months, 6 months, 9 months, 1 year, etc. If a patient has a *BIM* deletion and achieves these benchmarks, then there is probably no need for concern. But some patients with this genetic variant will not respond as well as other patients to treatment. These patients should be monitored very closely, and adherence with therapy should be emphasized. If there is evidence of a poorer response and the patient does not achieve the therapeutic benchmarks, then they should be switched to a more potent TKI without delay, an approach that matches what we found in vitro, as described above.

**H&O** Does the *BIM* deletion have any association with other mutations that could impact drug response?

**STO** Our in vitro work, which my colleague Dr Tun Kiat Ko presented at the 2013 meeting of the American Society of Hematology, did show that the *BIM* deletion polymorphism is permissive for the acquisition of acquired mutations that confer resistance. Here, we exposed cell lines with the *BIM* deletion to suboptimal concentrations of a TKI. These cells grew more vigorously compared with cells without the deletion, and also acquired somatic mutations that independently confer resistance. We think this same phenomenon may well happen in patients with the *BIM* deletion in whom BCR-ABL1, the oncogenic driver, is incompletely inhibited.

**H&O** Have you found the *BIM* deletion in other cancer types?

**STO** Yes. We found that among patients with non-small cell lung cancer (NSCLC) with mutated epidermal growth factor receptor (EGFR), the *BIM* deletion was associated with a progression-free survival (PFS) that was half as long as that seen among patients without the deletion (approximately 6 months vs 12 months, respectively). However, there was no significant difference in overall survival.

Two recent publications verified these findings. Writing in the *Journal of Thoracic Oncology* in 2014, Isobe and colleagues found a PFS of 227 days vs 533 days for Japanese NSCLC patients with vs without the *BIM* deletion. Zhao and associates, who published their work in *Cancer* in 2014, found a PFS of 4.7 months vs 11 months in Chinese populations with vs without the *BIM* deletion, respectively. However, in both of these studies, there was

no clear difference in overall survival. I should also mention that there was a retrospective study from Korea that could not find a significant difference in PFS, although the numbers were small. This study was published in 2013 in the *Annals of Oncology*.

**H&O** Why do you think overall survival did not differ between the 2 groups?

**STO** It is hard to say for sure of course, but most likely the subsequent treatment was able to overcome the resistance conferred by *BIM*.

**H&O** For a disease other than CML, what treatments might work for patients with a *BIM* deletion?

**STO** My prediction here would be chemotherapy, an assessment based on recent data in another disease. In a study conducted with Dr Allen Yeoh at the National University of Hospital Singapore, my coworkers and I studied more than 400 children with ALL, all treated uniformly (Ms Sheila Soh presented this work as a poster at the 2013 annual meeting of the American Society of Hematology). In general, patients with ALL receive several different drugs, including corticosteroids, which are dependent on *BIM* for inducing apoptotic cell death. To gain an insight into the molecular biology, we created ALL cell lines with the *BIM* deletion, and found that the deletion was sufficient to confer resistance to corticosteroids. We then showed that 3 different chemotherapy agents—L-asparaginase, methotrexate, and vincristine—could each independently induce cell death in ALL cells harboring the *BIM* variant. This study showed us that although *BIM* may confer resistance to a single agent, other drugs that do not depend on *BIM* expression to cause cell death can overcome that resistance. Therefore, the *BIM* deletion matters in some cancers and not in others.

**H&O** What are your next steps with this research?

**STO** We are planning a clinical trial, led by Dr Darren Lim at the National Cancer Centre Singapore, for patients with EGFR-mutated NSCLC. This cancer subtype is much more common in Asia than it is in the West—in our lung cancer clinics in Singapore, about 50% of NSCLC patients have EGFR mutations (vs about 10% in the West), and are therefore candidates for treatment with EGFR inhibitors.

In this proposed trial, patients with the *BIM* deletion polymorphism will be treated with an EGFR inhibitor plus a BH3 mimetic to see if this approach might extend their PFS to that of patients without the *BIM* deletion.

## H&O Is heritable resistance likely to be tied to particular regions of the world?

**STO** This work has highlighted the importance of studying different ethnic populations. A group of 3 papers published in *Science* in 2012 (with Casals, Nelson, and Tennesen as the first authors) described an explosion in the number of rare polymorphic variants (<0.5% frequency) that have occurred in modern populations that have not been subjected to purifying selection. There are more SNPs in relatively modern populations that affect gene function than we had realized, and that includes genes encoding drug targets, as discussed in a 2012 article in *Science* with Nelson as the lead author. It also seems that many of these modern variants are private to different ethnic populations. So we do need to study a wide variety of ethnic and geographic populations in clinical studies, and—given their low frequency—in large numbers.

### Suggested Reading

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