

with the possibility that they evolved as part of this process.

A second and quite unexpected property of antibodies specific for GP49-66 is their ability to induce oxidative platelet fragmentation *in vitro* (and presumably *in vivo*). GPIIb/IIIa is by far the most immunogenic of platelet proteins and a large number of auto-, allo-, and drug-dependent antibodies reactive with this target have been studied. Yet, no others have been shown to evoke this type of platelet response. Peterson et al previously studied a group of IgG antibodies from patients with quinine-induced immune thrombocytopenia that recognize the very peptide sequence (GPIIIa49-66) identified by Zhang et al as a target for HCV and HIV autoantibodies.<sup>10,11</sup> Yet the quinine-dependent antibodies appear not to be capable of inducing platelet fragmentation. Why the autoantibodies identified by Zhang et al have this unusual property is not fully understood.<sup>12</sup> Further studies of this process at a molecular level could lead to characterization of a new mechanism for immune-mediated cytopenia.

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## CLINICAL TRIALS

Comment on Adès et al, page 3947

# REvelation (del: 5q)

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Nowhere has the notion of personalized medicine been more realized than in oncology therapeutics. Within MDS, the use of lenalidomide for lower-risk patients with the del (5q) cytogenetic abnormality is emblematic of this approach. But is the drug personalized enough to be used in any patient whose cancer expresses del (5q)?

**T**herapy for myelodysplastic syndromes (MDS) has undergone a revolution of sorts in the past 5 years, with the approval by the US Food and Drug Administration of 3 drugs specifically for this collection of diseases: azacitidine in 2004,<sup>1</sup> lenalidomide in 2005,<sup>2</sup> and decitabine in 2006.<sup>3</sup> Only 1 of these, lenalidomide, is approved for an MDS subgroup: patients with lower-risk (International Prognostic Scoring System [IPSS] scores  $\leq 1.0$ ), transfusion-dependent disease who

harbor the del (5q) cytogenetic abnormality. In this narrowly defined group, who comprise approximately 8% to 9% of all MDS patients, lenalidomide is a wonder drug, yielding transfusion independence responses in 67% of patients, complete cytogenetic remissions in 44%, and a duration of transfusion independence lasting a median of 2.2 years.<sup>4</sup> One question that arises frequently is whether the drug can be used with equal efficacy, and as front-line therapy, for those MDS patients with

higher-risk (IPSS scores  $\geq 1.5$ ) disease and a del (5q) abnormality.

In this issue of *Blood*, Adès and colleagues report the results from a phase 2 study, exploring the safety and efficacy of lenalidomide in 29 del (5q) patients with higher risk MDS and 18 del (5q) patients with acute myeloid leukemia (AML) and 20% to 30% myeloblasts.<sup>5</sup> Among the MDS patients, 6 (21%) achieved a complete remission (CR), 2 (7%) a marrow CR, and 4 (14%) hematologic improvement in erythropoiesis. Among AML patients, 1 (6%) achieved a CR. Median response duration was 6.5 months for all patients, 11.5 months for those with an isolated del (5q) abnormality, and median overall survival was 272 days ( $\sim 9$  months). CR was more likely to occur in those with an isolated del (5q) lesion (in 6 of 9 patients, or 67%) compared with those with additional cytogenetic abnormalities ( $P < .001$ ). It was also more likely to occur in those with an initial platelet count greater than 100 000/mm<sup>3</sup> (in 6 of 13 patients, or 46%), compared with those with lower platelet counts ( $P = .001$ ). A majority of patients experienced severe neutropenia and/or thrombocytopenia, and 30 patients (64%) required hospitalization during their treatment course.

Compare these results to those presented by Fenaux and colleagues of a phase 3 study of azacitidine versus conventional care in patients with higher-risk MDS (AZA-001) at the American Society of Hematology Annual Meeting 1 year ago.<sup>6</sup> Median survival in the azacitidine arm was 24.4 months (a full 15 months greater than the present study), though CR rates were similar (17%), and partial remission and hematologic improvements were higher (12% and 49%, respectively). It is unclear whether these advantages translated to AZA-001 patients with the del (5q) abnormality, either in isolation or with additional cytogenetic abnormalities, though a preliminary report suggests that a combination of MDS and AML patients with the del (5q) lesion had a median survival of 9 months, significantly lower than similar patients without this abnormality (15 months,  $P = .007$ ).<sup>7</sup>

Why the inferior responses to single-agent lenalidomide in the study by Adès et al, when presumably therapy is "targeted" more to a distinct, identifiable lesion for which this therapy has demonstrated presumed cytotoxicity (with cytogenetic response rates) in lower-risk disease? We may be witnessing a recapitulation of the FMS-like tyrosine kinase 3 (*flt3*) internal tandem

duplication story, in which *flt3* inhibitors demonstrated only a modicum of activity in *flt3* positive leukemias when used as single agents, likely because patients with more advanced disease (such as higher-risk MDS or frank AML) have additional genetic abnormalities that promote proliferation and impair differentiation.<sup>8</sup> This may bespeak the need to optimize dosing or to combine lenalidomide with additional therapy. Ongoing studies are investigating the use of lenalidomide as a single agent at higher doses for AML patients with the del (5q) abnormality (Southwest Oncology Group study S0605); lenalidomide in combination with azacitidine in higher-risk MDS<sup>9</sup>; and lenalidomide in combination with traditional cytotoxic chemotherapy for AML.

Returning to our question, what to do with the higher risk MDS patient with the del (5q) abnormality, either in isolation or in addition to other chromosome abnormalities? Given the superior (or, in the case of an isolated del (5q) lesion, lack of data indicating inferior) response rates and overall survival compared with single agent lenalidomide, the firstline treatment of choice still must be a hypomethylating agent or combination therapy that includes lenalidomide as part of a clinical trial. The study by Adès et al further illuminates lenalidomide's specificity for the del (5q) clone and provides unequivocal justification for exploring dosing modification or combination strategies.

## ● ● ● MYELOID NEOPLASIA

Comment on Breitenbuecher et al, page 4063, Zhou et al, page 4052, and Breitenbuecher et al, page 4074

# Fathoming Flt3

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Mutated Flt3 has emerged as a promising drug target in AML. In this issue of *Blood*, Breitenbuecher and colleagues describe a common novel type of Flt3 ITD mutations and, in 2 additional studies, Breitenbuecher and colleagues as well as Zhou and colleagues present mechanisms for resistance to Flt3 kinase inhibitors.

**F**lt3, a receptor tyrosine kinase belonging to the PDGFR family, can be mutated in acute myeloid leukemia (AML). The inhibition of mutated Flt3 with kinase inhibitors leads to apoptosis *in vitro* and, hence, mutated Flt3 constitutes a promising drug target.

Two principal types of Flt3 mutations have been described in AML. The first is an internal tandem duplication (ITD) in the

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intracellular domain of the receptor found in about 20% to 30% of AML patients.<sup>1,2</sup> The second, point mutations predominantly substitutions of aspartate at position 835, are located in the activation loop of the kinase domain and is present in about 10% of patients.<sup>3,4</sup>

In the first paper by Breitenbuecher et al, the authors perform a systematic analysis of

the ITD insertion sites in 753 AML cases positive for Flt3 ITD.<sup>5</sup> To date, all ITDs have been localized to the juxtamembrane region of Flt3, which is encoded by exon 14. Interestingly, the authors find ITD insertions not only in the juxtamembrane region but also in the adjacent first kinase domain, which is encoded by exon 15. In total, 28.7% of ITDs were nontypical ITDs.

The authors subsequently use one of the identified nontypical ITD mutations (Flt3 ITD627E) to study its biological activity. The mutated receptor demonstrated ligand independent autophosphorylation reflecting constitutive activation. Furthermore, Flt3 ITD627E was able to transform hematopoietic 32D cells, and it caused a fatal myeloproliferative disease in a mouse model. Therefore, the nontraditional insertion mutations appear to contribute to AML oncogenesis and constitute promising drug targets.

In their second paper, Breitenbuecher et al explore why the AML patient, in whom the Flt3 ITD627E was discovered, showed up-front resistance to PKC412. PKC412 is one of the Flt3 kinase inhibitors currently in clinical trials.<sup>6</sup> The authors show that the resistance was due to enhanced association of Grb2 with Flt3 ITD627E. As a result, the antiapoptotic Mcl-1 protein was significantly up-regulated. Down-regulation of Mcl-1 by RNA interference resensitized cells to PKC412.

In their paper, Zhou et al also investigate resistance mechanisms vis-à-vis Flt3 inhibitors.<sup>7</sup> The authors generate an AML cell line that was resistant to the Flt3 inhibitor ABT-869 by culturing the cells with the compound for an extended period of time. The basis for resistance was the up-regulation of Flt3 Ligand expression. This promoted the activation of STAT1, STAT3, and STAT5 and an increase in the antiapoptotic protein survivin. Cells could be resensitized to ABT-869 by down-regulation of survivin and by inhibiting STAT signaling with a small molecule named IDR E804.

Today, there are multiple Flt3 inhibitors in clinical trials.<sup>8</sup> The most advanced compound is PKC412 which is currently being tested in a phase 3 trial in AML patients with mutated Flt3. Clinical results with various compounds have been mixed so far. Although there are significant responses in some patients, in many other patients, there are no responses or the responses are limited and short-lived.