on the conformation of the adjacent wild-type band 3 polypeptides.1

In this issue of Blood, Perrotta and colleagues report a novel band 3 mutation (Neapolis) that they identified in a child affected by severe transfusion-dependent microspherocytic hemolytic anemia and that was improved by splenectomy. Both parents had mild HS. Band 3 Neapolis is characterized by absence of the first 11 amino acids (aa) at the N-terminal portion of the wild-type polypeptide. This mutation results in complete loss of the red cell membrane’s ability to bind the key glycolytic enzyme, aldolase, likely due to the loss of its binding site on the band 3 N-terminal peptide missing from band 3 Neapolis. There is almost no phosphorylation of band 3 Neapolis in intact red cells, due to the loss of a critical tyrosine in position 8. In addition, the ability of Plasmodium falciparum to invade and mature in band 3 Neapolis erythrocytes is markedly diminished.

As with other band 3 mutations, the relative role of cytoskeletal abnormalities and functional/regulatory abnormalities in producing premature destruction of red cells is still not entirely clear. The severely reduced level of band 3 Neapolis in the red cell membrane of the homozygous child (only ~12% of the normal number) is the most likely determinant of the cytoskeletal disease and associated spherocytic hemolytic anemia. This is supported by the substantial clinical improvement seen in this patient after splenectomy. It will be more difficult to tease out the effects on red cell function specifically attributable to the altered binding of aldolase to band 3. However, this patient may offer the possibility of testing definitively the physiologic relevance of these protein-binding interactions often debated in the past. The functional consequences of the inability to phosphorylate band 3 Neapolis by treatment of intact cells with the sulphydryl oxidizing agent, diamide, remain uncertain, as is the relationship of this altered phosphorylation to the relative resistance of these cells to malaria infection. However, this exciting report demonstrates one more time the incredible multifunctional complexity of protein band 3, as key an ingredient for producing a normal red cell as tomatoes are to a perfect Neapolitan pizza.2

REFERENCES

Comment on David et al, page 4322 and Kardosh et al, page 4330

Not all that glitters is gold

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Multiple myeloma (MM) is a difficult disease to treat because of confounding factors such as genetic heterogeneity and the multiplicity and redundancy of survival pathways constitutively activated in this disease.

Some of the confounding factors in multiple myeloma (MM) are intrinsic and are manifested in the myeloma cell, while some are extrinsic and involve cells in the microenvironment. The latter secrete chemokines and growth factors such as interleukin-6 (IL-6), oncostatin M, macrophage inflammatory protein 1α (MIP-1α), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF), which constitute the “autocrine/paracrine” loops that support myeloma cell survival; proliferation; and acceleration of bone disease, the hallmark of this disease. Multiple myeloma is also “blessed” with genome instability, resulting in ubiquitous uniploidy and translocations involving up-regulation of c-myc; dysregulation of cyclin D, Rb, and p53; activation of Ras; deletions of p16 and p18; and overexpression of antiapoptotic molecules associated with evolving drug resistance. This is manifested in the fact that even the recently developed drugs such as bortezomib and lenalidomide are effective in only 20% to 30% of patients.1 Hence, targeting of multiple survival pathways by a combination of target-specific drugs is critical for future development of effective treatment for MM.

One of the hallmarks of MM is the constitutive activation of nuclear factor kappa B (NF-κB) in myeloma cells2 leading to overproduction of IL-6, resulting in activation of Akt and the Ras–mitogen-activated protein (MAP) kinase cascade (Figure 1). The following commentary deals with 2 preclinical papers describing the successful targeting of these pathways in vitro in myeloma cells.

David and colleagues have reported the effect of the farnesyl transferase inhibitor, lonafarnib, in combination with the proteasome inhibitor, bortezomib, in inducing cell death in myeloma cell lines and primary myeloma cells. The authors report synergy between these 2 drugs in killing myeloma cells, enhancement of the activation of caspases-8 and -9, and enhanced dephosphorylation and down-regulation of Akt compared with each drug alone. Furthermore, as was shown before for bortezomib,3 IL-6 or IGF-1 could not reverse the antiproliferative effect of the combination of the 2 drugs. The authors also show that transient transfection with wild-type (WT) Akt, activated Akt, or Bcl-2 expression vectors did not reverse the combined apoptotic effect of the 2 drugs, although increased expression of the target protein was shown by Western immunoblotting. This finding is somewhat unexpected since overexpression of active Akt, by and large, protects against apoptosis involving this pathway. This discrepancy could be due to the fact that the authors gated on the green fluorescent protein–positive (GFP+) cell population to determine annexin V+ apoptotic cells. However, GFP+ cells undergoing apoptosis become GFP−, as dead cells lose GFP expression rapidly.

As for the clinical applicability of this drug combination, at this point it is not clear whether lonafarnib could indeed be combined with bortezomib for the treatment of MM. This is because of the high dose of lonafarnib of 4 to 5 µM used in this in vitro study to achieve synergy with bortezomib. This conclusion is based on a phase 1 study of lonafarnib in patients with solid tumors, reported by Eskens et al.1 In this study, the dose-limiting toxicity (DLT) for lonafarnib was 400 mg twice a day and the maximum tolerated dose (MTD) was 300 mg twice a day. At these doses, the concentration maximum (Cmax) of lonafarnib in plasma was 2 to 5 µM. However,
due to multiple toxicities at this dose range, the recommended dose for phase 2 was 200 mg twice a day, at which dose the plasma Cmax was less than 1 μM. Furthermore, the pattern of grades 3 to 4 toxicity from lonafarnib was similar to that of bortezomib, especially with regard to neutropenia and neurocortical toxicity, which are dose limiting for bortezomib. Of note, in the paper by David and colleagues, a range of 1 to 3 μM lonafarnib alone was ineffective and did not result in any synergy when combined with bortezomib.

Kardosh and colleagues have reported the antiproliferative and apoptotic effects of the cyclooxygenase-2 (COX-2) inhibitor, celecoxib, and the non–COX-2 inhibitor, 2,5-dimethyl-celecoxib (DMC), in a variety of drug-resistant myeloma cell lines. In agreement with previous work in other cancer cell types, both drugs, which chemically differ by only one methyl group, induced myeloma cell kill in a similar fashion, suggesting antitumor activity outside inhibition of COX-2. Kardosh and colleagues demonstrated time/dose-dependent apoptosis involving down-regulation of p21, p27, and cyclins A and B; activation of caspase-3 and poly(adenosine diphosphate-ribose) polymerase (PARP); degradation of survivin; and decrease in phosphorylated MAP kinase kinase 1/2 (pMEK1/2), but with marginal effect on IκB kinase and no effect on Akt or phosphorylated Akt (pAkt) levels. This lack of effect on the NFκB/Akt pathway is in contradiction to other reports, clearly demonstrating an effect of celecoxib and DMC on these pathways in various types of cancer cells.

Finally, the results from this study merit a phase 1 clinical trial in MM patients with DMC; however, the results from such a trial are not clear at this point, since the median inhibitory concentration (IC50) of 40 μM and 50 μM reported here for DMC and celecoxib, respectively, is 2– to 3-fold higher than the effective Cmax reported for these drugs by Kulp et al in a mouse xenograft model of prostate cancer. In those studies, doses of celecoxib and DMC of 200 mg/kg per day were effective in reducing tumor size by 50% and 75%, respectively; however, at these doses the plasma Cmax for celecoxib and DMC was lower, 20 μM and 14 μM, respectively.

REFERENCES
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