Fact or VIII brand and immunogenicity

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In this issue of Blood, Calvez et al and Collins et al, independently, add to the growing literature on the role of factor VIII brands and risk of hemophilia inhibitor formation.1,2 Ever since the observation that inhibitor titer waned after a fall in CD4 count <200/μL in hemophilia patients with HIV infection, inhibitor formation has been regarded as a T cell-dependent B-cell response directed against infused factor VIII.3 The inhibitor alloantibody binds to infused (foreign) factor VIII, neutralizes its activity, and disrupts normal hemostasis. For an affected patient, the burden of disease is significant: uncontrolled bleeding results in twice the hospitalizations4 and 10 times the cost.5 Current treatment is difficult, as bypass agents, ie, VIIa or IX complex, provide less effective hemostasis than standard factor VIII in noninhibitor patients. Eradication of the inhibitor by immune tolerance induction, ie, regular factor VIII infusions, provides less effective hemostasis than standard factor VIII in noninhibitor patients. Eradication of the inhibitor by immune tolerance induction, ie, regular factor VIII infusions, is costly, inconvenient, and, in 20%, ineffective. Thus, there is increasing interest in preventing inhibitors before they occur. A number of studies have shown that inhibitors may be reduced by avoiding high intensity factor exposure early in life or by initiating prophylaxis or regular factor infusions to prevent bleeds rather than on-demand or intermittent factor infusions to treat bleeds.6 The contribution of factor brand to inhibitor risk, however, remains controversial and is the focus of the papers by Calvez et al and by Collins et al.

Small retrospective studies have suggested inhibitor risk is greater with recombinant than plasma-derived products. A recent prospective observational study of 574 previously untreated patients (PUPs), the RODIN study (Research of Determinants of Inhibitor Development), however, found there was no difference in inhibitor risk between recombinant and plasma-derived products.7 Surprisingly, they further reported that among recombinant factor VIII brands, there was a 60% greater inhibitor risk with second-generation than third-generation products.7 These unexpected findings led to questions about their biological plausibility and validity, as well as concerns with the study’s nonrandomized design and small sample size, leaving decisions regarding product choice unclear.

In an attempt to assess the findings of the RODIN study, Calvez et al and Collins et al independently analyzed inhibitor risk associated with recombinant factor VIII brands in 2 separate PUP cohorts. Calvez et al evaluated a subset of 303 PUPs with severe hemophilia A from a French prospective cohort established 20 years earlier to monitor product safety. These children were born and treated between 2000 and 2009, similar to the time frame of the RODIN study. In a series of 10 sensitivity analyses, they confirmed that inhibitor risk was higher with second-generation than the third-generation factor VIII products and persisted after adjustment for other inhibitor risk factors (adjusted hazard ratio [HR], 1.58; 95% confidence interval [CI], 1.17-2.14; see table). Collins et al, evaluating a British prospective cohort of 407 PUPs with severe hemophilia A born and treated from 2000 to 2011, also observed greater inhibitor incidence with second- than third-generation factor VIII brands (adjusted HR, 1.70; 95% CI, 1.15-2.52). In each of these studies, the findings remained unchanged after excluding those who had participated in the RODIN study: 50 of 303 (16.5%) in the French cohort and 88 of 407 (21.6%) in the United Kingdom (UK) cohort.

Inhibitor risk and factor VIII brands in three hemophilia cohort studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>No. HTCs</th>
<th>No. PUPs</th>
<th>Follow-up (E.D.)</th>
<th>Timespan</th>
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<th>Adjusted HR for second- vs third-generation FVIII*</th>
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<tr>
<td>Calvez et al</td>
<td>FranceCoag PUP cohort</td>
<td>12</td>
<td>303</td>
<td>75</td>
<td>2000-2009</td>
<td>37.6</td>
<td>1.55 (0.97-2.49)</td>
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<td>Collins et al</td>
<td>UK National Haemophilia Database</td>
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<td>407</td>
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<td>29.5</td>
<td>2.17 (0.99-4.74)</td>
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<td>Gouw et al</td>
<td>Research of Determinants of Inhibitor Development (RODIN) study</td>
<td>29</td>
<td>547</td>
<td>50</td>
<td>2000-2010</td>
<td>32.4</td>
<td>1.60 (1.08-2.37)</td>
</tr>
</tbody>
</table>

E.D., exposure days; FVIII, factor VIII; HTC, hemophilia treatment center.

*Adjusted HRs did not change after excluding those participating in the RODIN study: 50 of 303 (16.5%) in the French cohort and 88 of 407 (21.6%) of the UK cohort.
What do these data mean and how should one approach product choice for patients? The findings from 3 large prospective studies now demonstrate greater immunogenicity with second-generation recombinant products. Although the findings represent grade 1C evidence based on observational studies, they may be the best we have, because a randomized trial is not likely to be feasible in this rare disease. For previously treated patients (PTPs), the likelihood of inhibitor formation is low, so these findings are unlikely to affect product choice. For PUPs, the decision regarding product choice, as some have suggested,8 may be as simple as taking the safest analgesic for a headache, or, in this case, simply using the least immunogenic factor VIII to manage hemophilia bleeds, especially during the most vulnerable first 10 to 20 exposure days. Treatment decisions, as always, should involve a discussion between parent/patient and provider regarding risk and benefit and also include consideration of patient choice.

Why the second-generation brands were found to be more immunogenic than the third-generation brands remains unknown. It has been suggested that the increased immunogenicity of second-generation recombinant products may relate to their higher FVIII protein aggregate content,8,9 although this has not been proven. It is worth noting that the new extended half-life factor VIII proteins may be less immunogenic than current factor VIII brands. In hemophilia A mice receiving a recombinant factor VIII Fc fusion protein, Eloctate, inhibitor formation was significantly lower than in mice receiving third-generation factor VIII (full-length and B domain-deleted),10 a finding attributed to the Fc sequence that induces expansion of T-regulatory cells and suppression of an antibody–specific immune response.11 Although no inhibitors have occurred in clinical trials, none were expected because the subjects were PTPs. Whether inhibitors will occur in PUPs receiving extended half-life proteins will require prospective study. Thus, the findings of Calvez et al and Collins et al will continue to inform the ongoing debate regarding factor VIII brands and immunogenicity, even as extended half-life proteins come to market.

Comment on Irving et al, page 3420

Risk of RAS in relapsed childhood ALL

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In this issue of Blood, Irving et al report a high frequency of rat sarcoma (RAS) signaling pathway mutations in relapsed childhood B-cell precursor acute lymphoblastic leukemia (BCP ALL) and present detailed data on the clonal evolution of RAS mutation–positive blasts from diagnosis to first, and, in some cases, second relapse.1 This study is important for 2 reasons; first because it adds to our understanding of the complex genetic mechanisms involved in chemoresistant ALL and second because the authors also present data from in vitro and in vivo experiments indicating that the mitogen–activated protein kinase (MEK) inhibitor selumetinib could be a novel treatment option in relapsed ALL with activation of the RAS pathway (see figure).

Although ALL usually displays 1 dominant clone—ie, identical somatic mutations in the majority of blast cells—at diagnosis, next-generation sequencing and xenografts have recently revealed that cases frequently also harbor mutations that are present in only a subset of the cells, indicating a previously unrecognized genetic heterogeneity.2,3 Evidence pointing in the same direction has come from comparisons of ALL at diagnosis and relapse, where microdeletions and mutations present only at relapse have been backtracked to minor subclones in the corresponding diagnostic sample.1,5 Genetic heterogeneity at diagnosis may be one of the underlying factors when the leukemia relapses, because small subclones may survive the treatment that eradicates the main diagnostic clone and subsequently lead to disease recurrence. Irving et al investigate mutations affecting the RAS signaling pathway in samples from 206 relapsed BCP ALLs. Mutations in this pathway are among the most common somatic changes in human malignancies and are found in a wide range of tumors. In pediatric ALL, mutations of the KRAS, NRAS, PTPN11, and FLT3 genes, all involved in the RAS pathway, are seen in ~35% of cases at diagnosis, with varying frequency in different genetic
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