followed by 80 mg of the FLT3 inhibitor lestaurtinib twice daily. By intention-to-treat analysis, there was no statistically significant improvement in remission rate or overall survival for patients receiving lestaurtinib. Obviously, this is a discouraging result for the field of therapeutic FLT3 inhibition in AML. Do results obtained challenge the concept of therapeutic FLT3 inhibition?

A major strength of the study by Levis and colleagues is that bioactivity of lestaurtinib was carefully monitored using a plasma inhibitory activity (PIA) assay. In addition, lestaurtinib plasma concentrations, FLT3 ligand levels, and AGP levels were also determined. From previous in vitro and in vivo studies the authors had estimated that FLT3 needed to be suppressed to less than 15% of its baseline activity to induce a cytotoxic effect. However, at aplasia assessment, only 46 of 79 patients (58%) analyzed actually achieved this goal and only 21 of these achieved this degree of inhibition later on at the day of outcome assessment. Why did lestaurtinib fail to induce meaningful bioactivity? The authors provide some important suggestions: (1) Decreasing plasma levels of lestaurtinib over the course of treatment were observed and could have contributed. (2) Elevated FL levels after chemotherapy as described by Sato et al were observed and thus may have also impeded bioactivity of lestaurtinib. (3) In addition to FL, α-1 acid glycoprotein (AGP) rose from baseline by day 15 of induction chemotherapy, and remained elevated by day 42. In human plasma, lestaurtinib binds with high affinity to AGP. Thus, higher levels of AGP in plasma result in lower levels of free lestaurtinib. (4) In some patients, very high levels of lestaurtinib had been noted that appeared to predict toxicity and yet did not predict in vivo FLT3 inhibition. Thus, the complexities of the pharmacokinetics resulting in toxic off-target effects may have also contributed to limited efficacy of lestaurtinib in this trial.

Another reason that lestaurtinib failed to provide benefit to patients may relate to the population of AML patients being studied. It is conceivable that a number of resistance mechanisms are already operating in leukemic cells of relapsed patients. This may include activation of alternate pathways, resistance mutations, and FLT3 up-regulation. The latter mechanisms would not have been detected by the PIA assay used. The accompanying figure summarizes molecular mechanisms potentially impeding bioactivity of FLT3 tyrosine kinase inhibitors (FLT3-TKI) as suggested by Sato et al and by the results of the trial presented by Levis et al.

One encouraging aspect of the study by Levis et al, however, was that in vivo FLT3 inhibition correlated very highly with remission rate. This suggests that FLT3 inhibition as a therapeutic modality is still very promising once we are able to apply inhibitors with improved pharmacokinetic and pharmacodynamic properties and once we are able to overcome bioactivity issues identified in the 2 papers.

Conflict of interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Sato T, Yang X, Knapper S, et al. FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. *Blood*. 2011;117(12):3286-3293.
2. Levis M, Ravandi F, Wang ES, et al. Results from a randomized trial of salvage chemotherapy followed by lestaurtinib for patients with FLT3 mutant AML in first relapse. *Blood*. 2011;117(12):3294-3301.
3. Frohling S, Schlenk RF, Breitruck J, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood*. 2002;100(13):4372-4380.
4. Schnittger S, Schoch C, Dugas M, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood*. 2002; 100(1):59-66.
5. Kindler T, Lipka DB, Fischer T. FLT3 as a therapeutic target in AML: still challenging after all these years. *Blood*. 2010;116(24):5089-5102.
6. Qiu C, Tarrant MK, Boronina T, et al. In vitro enzymatic characterization of near full length EGFR in activated and inhibited states. *Biochemistry*. 2009;48(28): 6624-6632.

● ● ● CLINICAL TRIALS

Comment on Avery et al, page 3277

What's up with 2 cord transplantation?

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Transplantation of 2 cord blood units to a single patient has been enthusiastically adopted as a strategy to increase access to unrelated donor cord blood transplantation (UCBT) for larger pediatric and adult patients. With this strategy, 1 cord blood unit ultimately dominates and confers durable engraftment, but prospective identification of the engrafting unit has not been possible.

The article by Avery and colleagues in this issue of the *Blood* sheds light on this biologic mystery in double umbilical CBT by demonstrating, in a series of 84 patients, that indicators of cord blood unit potency, for example, total nucleated cell count (TNC), colony-forming unit (CFU), CD3, and viable CD34 cell content, predict the dominating unit.[1] Surprising to some, human leukocyte antigen (HLA) matching does not appear to play a role in unit dominance.

Since the demonstration that unrelated donor, banked umbilical cord blood provides a source of cells for hematopoietic reconstitution after myeloablative therapy, more than 500 000 publicly donated units have been banked and more than 25 000 UCBTs have been performed worldwide. The fact that cord blood could be transplanted without full HLA matching also emerged as a major advantage providing access to transplantation to patients unable to find a fully matched adult donor. Early on, the importance of transplanting an adequate cell dose was established with generally accepted thresholds of 25-30 million nucleated cells per kg required for engraftment.[2-3] Given the limited volumes of blood available from typical term placentas, less than 10% of collected units will contain sufficient cell doses for patients weighing > 70 kg. In 2005, a group at the University of Minnesota reported enhancement of engraftment in adults with hematologic malignancies receiving 2, sequentially transplanted, partially HLA-matched unrelated donor cord blood units.[4] Interestingly, even though engraftment rates were higher than those seen after transplantation of a single cord blood unit, only 1 of the 2 units conferred durable engraftment. Furthermore, the dominating unit was generally declared within the first month after transplantation. However, despite rapid adoption of this strategy by adult transplant physicians, the mechanisms underlying the success of this approach are unknown. Furthermore, the ability to predict the engrafting unit was not previously possible. In early series, obvious candidates such as total

nucleated cell dose, HLA match, and CD3 dose did not predict the engrafting unit. Some investigators questioned whether the infusion of 2 cords was really necessary and hypothesized that the engraftment rates were higher because using 2 units gave the patient a better chance of receiving at least 1 unit with high potency. Others thought that 1 of the 2 units facilitated engraftment of the dominating unit. Others thought that concomitant changes in the preparative regimen (substitution of fludarabine for antithymocyte globulin) and/or graft-versus-host disease (GVHD) prophylaxis (substitution of mycophenolic acid for methylprednisonlone) were the reason(s) for success. The latter questions remain unanswered but are under study through the Blood and Marrow Transplant Clinical Trials Network 0501 study of double versus single cord blood transplantation in children with hematologic malignancies.

While traditional selection of cord blood units uses TNC and HLA matching, recent studies in single cord blood transplantation have shown that dosing of CD34 cells and CFUs are better predictors of unit potency as measured by engraftment potential.[5-7] The current study supports this concept; in addition, units with higher viable CD34 cells and higher CFUs tended to be associated with higher sustained engraftment and more rapid neutrophil recovery.

The conclusions of the current article are intriguing, but do not prove that 2 cord blood units are better than 1 or, in fact, necessary for engraftment in a larger patient. One must question whether the observation that the more potent unit engrafts proves that there is a benefit to infusing 2 cords for 1 transplant. This is important because some series have shown increased GVHD with double cord transplantation and also because the costs to procure 2 cord blood units per patient are expensive and even prohibitive in some health care models. This question could be addressed in a clinical trial that assessed cord blood potency at the time of unit selection from an attached segment and then randomized patients to receive 1 or 2 potent units. If it is shown that, despite potency, 2 are better than 1, strategic decisions about which unit's engraftment might be favored (eg, the better HLA match) may be possible ranking potency of the preferred unit above the helper unit. We still have a lot to learn about the biology of CBT. These authors have taken us 1 more step along the journey, but we still have a lot more work to do before we master the field and optimize clinical practice.

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REFERENCES

1. Avery S, Weiji S, Lubin M, et al. Influence of infused cell dose and HLA-match on engraftment after double-unit cord blood allografts. *Blood*. 2011;117(12):3277-3285.

2. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from related and unrelated donors. *N Engl J Med*. 1997;337:373-381.

3. Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood*. 2010;115(9):1843-1849.

4. Barker JN, Weisdorf DJ, DeFor TE, et al. Transplantation of two partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood*. 2005;105(3):1343-1347.

5. Migliaccio AR, Adamson JW, Stevens CE, Dobrila NL, Carrier CM, Rubinstein P. Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. *Blood*. 2000;96(8):2717-2722.

6. Yoo KH, Lee SH, Kim HJ, et al. The impact of post-thaw colony-forming units-granulocyte/macrophage on engraftment following unrelated cord blood transplantation in pediatric recipients. *Bone Marrow Transplant*. 2007;39(9):515-521.

7. Alonso JM III, Regan DM, Johnson CE, et al. A simple and reliable procedure for cord blood banking, processing, and freezing: St Louis and Ohio Cord Blood Bank experiences. *Cytotherapy*. 2001;3(6):429-433.

GENE THERAPY

Comment on Li et al, page 3311

Quest for safety at AAValon

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In this issue of *Blood*, Li and colleagues show an exhaustive, long-term safety assessment of adeno-associated virus (AAV) vector integration in mouse liver.[1] Massive amounts of histopathologic, vector integration, and transcriptomic data provide a highly detailed view of the genomic impact of AAV vector integration in vivo. AAV vector integrations are not associated with tumor formation in these settings.

AAV-based vectors are promising tools for the treatment of several genetic diseases. Indeed, depending on the AAV serotype, a single systemic administration allows massive gene transfer, enough for entire organs to obtain therapeutic levels of transgene expression from AAV genomes in episomal form.[2] Despite the lack of a professional integrase, AAV vectors are still able to integrate into the host genome at measurable levels[3] and, in a mouse model of lysosomal storage disease, these levels were enough to induce a significantly higher incidence of hepatocellular carcinoma (HCC).[4] Indeed, vector integration analysis showed that 4 HCCs harbored integrations targeting *Rian*, a nonprotein-coding gene within the complex imprinted *Dlk1-Dio3* region, implicated in HCC[5,6] and other cancers.[7] The finding of a Common Insertion Site (CIS) in different tumors is an important indication that HCCs were triggered by AAV vector–mediated insertional mutagenesis, similar to the mechanisms found int retrovirus and transposon-based oncogene-tagging screenings in mice[8] and γ-retroviral vector (γRV)–based clinical trials.[9]

Thus the risk of insertional mutagenesis, one of the major hurdles of gene therapy with integrating vectors, also applies to AAV vectors. This has implications for the AAV vector–mediated gene therapy field and mandates a thorough assessment of the safety of AAV vector integration.

In the article from Li et al in this issue, 2 groups well known in AAV gene therapy and retroviral integration combined their expertise to perform a very detailed and long-term in vivo safety assessment of an AAV vector proposed for the therapy of hemophilia B.[10] Therapeutic or high AAV vector doses did not increase the incidence of HCC when given systemically to a large number of wild-type mice. Molecular analysis on HCCs and surrounding healthy liver tissue provide a vector integration landscape composed of > 1000 integrations, as demonstrated by vector copy number measurements. A whole transcriptome analysis was preformed in HCC samples. The massive amount of data obtainedwas analyzed by powerful and sophisticated approaches developed and optimized to study vector integration profiles at specific