evaluated for the treatment of hematologic malignancies are listed in the table. The ADC used in this study by Deckert et al comprises a humanized anti-CD37 antibody, K7153A, linked via a SMCC [N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate] linker to the maytansinoid DM1 payload. DM1 exerts its antimitotic effect by depolymerizing microtubules and arresting the cells in prometaphase/metaphase. The authors go on to dissect in detail the antitumor mechanism of the DM1-conjugated antibody (IMGN529), demonstrating that the conjugated compound retains the same strong immune effector activities as the unconjugated K7153A. When the naked K7153A and IMGN529 were compared in cytotoxicity assays, the cytotoxic potency induced by IMGN529 was far superior compared with the naked antibody, with induction of cell death in a dose-dependent manner in the picomolar range. Mice inoculated with a human B-cell lymphoma cell line receiving single doses of IMGN529 had a better tumor-free survival time than mice treated with the unconjugated antibody or with rituximab, confirming the additive effect of DM1 delivery. B-cell depletion was observed in the treated animal, and it was more profound than that induced by rituximab treatment.

Given these results, IMGN529 seems to be a promising drug, and certainly the planned first-in-man clinical trial is warranted. Overall, the success of trastuzumab-emtansine, as well as that of brentuximab vedotin, suggests that ADCs might take a spotlight in the current landscape of antitumor drugs.

### ADCs undergoing clinical evaluation for the treatment of hematologic malignancies

<table>
<thead>
<tr>
<th>Target</th>
<th>Conjugate</th>
<th>B-cell</th>
<th>Myeloid leukemia</th>
<th>Hodgkin lymphoma</th>
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<tr>
<td>CD3</td>
<td>Diphtheria toxin A</td>
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<td>X</td>
<td></td>
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<tr>
<td>CD19</td>
<td>DM4</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>CD22</td>
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<td>CD30</td>
<td>MMAE</td>
<td>FDA approved*</td>
<td>FDA approved*</td>
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<tr>
<td>CD33</td>
<td>DM4, gelonin</td>
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*At this time, only brentuximab vedotin is approved by the FDA.

### References


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**Comment on Willems et al, page 3521**

**Asparaginase unveils glutamine-addicted AML**

**Ismael Samudio** and **Marina Konopleva**

**Terry Fox Laboratory; The University of Texas MD Anderson Cancer Center**

In this issue of *Blood*, Willems et al describe the dependence of acute myeloid leukemia (AML) cells on glutamine for maintaining protein synthesis downstream of mammalian target of rapamycin (mTOR) and show that the enzyme asparaginase can be used to target this dependence. Using various AML cell lines, primary samples, and CD34+ stem cells from healthy donors, the authors support the notion that asparaginase may offer a therapeutic benefit in AML—not from its well-known enzymatic activity, but from its “off-target” effects on glutamine levels that result in inhibition of downstream mTOR signaling, inhibition of protein synthesis, and ultimately loss of viability.

Although not without side effects, both clinically available forms of asparaginase (Kyndrolase and Erwinase) have demonstrated good pharmacokinetics and documented success for the treatment of pediatric and adult acute lymphocytic leukemia (ALL) as well as some pediatric AML. Traditionally, it has been assumed that the therapeutic effects of the enzyme are mediated by reduction of asparagine plasma levels via deamination, although glutamine deamination is also observed clinically. Notably, some of the significant side effects of asparaginase therapy such as acute pancreatitis, thrombotic complications, and immunosuppression have been attributed (pp40-52). Structure and subcellular expression of an extensively glycosylated glycoprotein. *J Immunol.* 1988;140(3):905-914.

to its glutaminase activity, leading to the pharmaceutical efforts to generate an enzyme that does not affect the metabolism of glutamine. Hence, one of the important implications of the study by Willems et al is that, at least in AML cells, glutaminase activity of asparaginase is necessary for its antitumor activity, substantiating the importance to characterize its molecular mechanisms of action.

In light of the reported importance of glutamine for fueling solid tumor growth via its anaplerotic contribution to Krebs cycle oxoglutarate (reviewed in Hensley et al), the findings of Willems et al add valuable insight into another possible mechanism by which glutamine promotes malignant cell survival, namely the regulation of mTOR downstream signaling and protein translation. Although asparaginase has long been reported to inhibit protein synthesis, the mechanism was assumed to depend strictly on asparagine deamination—and to a lesser extent, on its glutaminase activity—which would in turn inhibit protein synthesis because of limiting amounts of asparaginyl transfer RNA. The work presented in this issue of Blood supports the intriguing notion that before substrate limitation could take place, the machinery for cap-messenger RNA (mRNA) translation is inactivated via inhibition of the amino acid/mTOR targeting machinery at the lysosomal surface and dephosphorylation of p70S6 kinase (see schematic below).

Although it is unclear if glutamine is an ipso facto signal on the amino acid sensing machinery, convincing evidence is provided that reduced intracellular glutamine severely limits leucine uptake, which would in turn hinder Rheb-mediated mTOR activation at the lysosomal surface. It is tempting to speculate that mTOR inhibition is triggered to prevent the futile translation of mRNA into polypeptide fragments that would otherwise accumulate and lead to toxicity. In support of this, Willems et al demonstrate that protective autophagy is activated in response to glutamine depletion, and that genetic interference with key autophagic proteins (beclin and ATG5) results in the rapid onset of apoptosis. This key finding may lay the groundwork for future combination studies of asparaginase with inhibitors of autophagy such as the antimalarial agent chloroquine.

Willems et al also identified the glutamine transporter SLC1A5 as the main transporter for glutamine uptake in most AML cell lines and primary samples. It is unclear if SLC1A5 is the main transporter for glutamine uptake in AML blasts in vivo and if glutamine synthetase will also be upregulated in AML patients that may have received asparaginase therapy. Nonetheless, recent evidence suggests that the bone marrow microenvironment—in particular adipocytes—may contribute to asparaginase resistance via, in part, asparagine and glutamine release, and that this resistance mechanism may be more evident in obese patients (at least it is in obese mice). This observation may support the notion that patient selection via body mass index could be an important consideration for future selection of the patients who might benefit from introduction of asparaginase into current treatment paradigms for AML.

In conclusion, the results presented by Willems et al in this issue of Blood uncover a novel, anaplerotic-independent role of glutamine in leukemic cells, and support the development of asparaginase—or agents that target glutamine transport into leukemic cells—as a novel therapeutic strategy to inhibit mTOR and downstream cap-dependent mRNA translation in AML. Given the satisfactory clinical track record of Kydrolase and Erwinase in ALL and pediatric AML, these results warrant an evaluation of their clinical efficacy and pharmacodynamic action on mTOR signaling in the context of adult AML. Further preclinical studies are warranted to characterize most potent and safe combinations of asparaginase with chemotherapeutic and targeted clinical modalities in AML to assure its success in this difficult-to-treat disease with depressingly low cure rates.

Conflict-of interest disclosure: The authors declare no competing financial interests.

REFERENCES
Dengue platelets meet Sir Arthur Conan Doyle

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In this issue of Blood, Hottz et al provide compelling evidence that dengue virus (DV) induces (1) platelet synthesis of interleukin-1β (IL-1β); (2) platelet-derived IL-1β-containing microvesicles (MV); and (3) DV-triggered inflammasome activation in platelets.1

Dengue is a viral disease spread by mosquitoes. Although most commonly occurring in the tropics, there has been a worldwide increasing geographic expansion and the World Health Organization considers half the world at risk for infection (http://www.who.int/mediacentre/factsheets/fs117/en/). Severe dengue (previously known as dengue hemorrhagic fever) is relatively common (~500 cases each year), and is characterized by severe bleeding, thrombocytopenia, vascular permeability with plasma leakage and shock, and severe organ impairment. It is the leading cause of death among children in some Asian and Latin American countries. The responsible molecular mechanisms are not well understood, but the proinflammatory cytokine IL-1β is increased in the plasma of patients and levels are associated with disease severity. The authors have previously shown that DV causes platelet activation and mitochondrial dysfunction. This manuscript tests the hypothesis that DV-mediated platelet activation induces platelet synthesis and processing of IL-1β, which is capable of disrupting the endothelial cell barrier function.

Using flow cytometry, the authors found that compared with platelets from healthy age- and gender-matched controls, a higher percentage of platelets from patients with dengue were positive for IL-1β. Importantly, when leukocyte-depleted platelets from normal individuals were incubated with DV in vitro, IL-1β levels increased in both platelets and the platelet supernatant. The authors found DV also induced platelet release of IL-1β-containing MV. The simplest interpretation of these data are that DV induces both platelet translation of IL-1β mRNA into protein and platelet release of IL-1β. The authors collected 36 patients with serologically/molecularly confirmed dengue. Among these, the 16 with clinical signs of vascular permeability had significantly higher IL-1β levels in platelet-derived MV. When the authors exposed cultured human microvascular endothelial cells to MV recovered from DV-exposed platelets, they observed a significant increase in permeability to albumin, and this increase was inhibited by IL-1 receptor blockade.

IL-1β is synthesized as an inactive precursor that is cleaved to an active form by caspase 1, but little is known about caspase 1 in platelets. The authors show that compared with control platelets, the platelets from dengue patients have enhanced caspase 1 activation and DV induces caspase 1 processing and activation in normal platelets. How might DV do this? Caspase 1 is activated by inflammasomes, which are multimolecular

Model of dengue-mediated platelet IL-1β synthesis and release. (A) Model proposes that platelets in healthy state have relatively modest levels of the components of the inflammasome and caspase 1 activity, and limited messenger RNA (mRNA) translation into protein. The low level of translation may be due to microRNA (miRNA) inhibition of translation and/or low mRNA levels. (B) DV induces in vivo platelet translation of mRNA into IL-1β. DV mediates inflammasome-mediated caspase 1 activation, enabling processing into active IL-1β with subsequent release into MVs for systemic transport. Activated platelets also release RNA in vesicles. Because DV replication involves silencing host mRNA production, megakaryocytes may deliver less miRNA for inhibiting platelet mRNA translation, or DV could compete with endogenous miRNAs. In addition, DV may induce increased megakaryocyte delivery of IL-1β mRNA to the platelet. Dashed lines indicate the uncertainty of the early events in the model.
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Ismael Samudio and Marina Konopleva

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