Thrombotic Microangiopathy with Targeted Cancer Agents

John A. Blake-Haskins1,2, Robert J. Lechleider2, and Robert J. Kreitman3

Abstract

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are clinically similar disorders characterized by microvascular thrombosis, hemolysis, thrombocytopenia, and end-organ damage. Although they may present with overlapping symptoms, multiple etiologies have been proposed for these thrombotic microangiopathies (TMA). Chemotherapy-induced TMA, which has been described with the use of mitomycin, gemcitabine, and other drugs, has a poor prognosis. Recently, reports of TMA associated with targeted cancer agents have surfaced in the literature. We discuss the clinical presentation, outcome, and etiology of TMA reported with the use of immunotoxins, monoclonal antibodies, and tyrosine kinase inhibitors. A search of PubMed and meeting abstracts was conducted for cases of TMA with the use of targeted cancer agents. The defining symptoms, laboratory values, time to onset, and patient outcomes were compiled. Consistent definitions of TMA and grading of severity in these cases are lacking. However, presentation of TMA in these cases revealed the importance of monitoring for renal toxicity, hemolysis, and thrombocytopenia. Patient outcomes seem to differ from those seen in cases of chemotherapy-induced TMA and may reflect a different underlying etiology. Little is known about the pathogenesis of TMA with targeted cancer agents. In contrast to chemotherapy-induced TMA, partial to full reversibility may be a common outcome. However, further research is warranted into optimal management of patients diagnosed with TMA following treatment with targeted agents. Clin Cancer Res; 17(18): 5858–66. ©2011 AACR.

The thrombotic microangiopathies (TMA) are a group of disorders characterized by occlusive microvascular thrombosis, thrombocytopenia, and end-organ damage. The principal subtypes of TMA are thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). In turn, both TTP and HUS may each have several distinct subtypes caused by differing pathophysiologic mechanisms.

TTP is classically described by a pentad of clinical symptoms, including thrombocytopenia, microangiopathic hemolytic anemia (MAHA), neurologic abnormalities, renal failure, and fever. HUS may also present with many overlapping signs and symptoms (1). However, a diagnosis of TTP is usually reserved for cases in which neurologic abnormalities such as seizure and vision loss predominate, whereas HUS is diagnosed in cases in which renal failure is the most prominent sign.

The differentiation between TTP and HUS, their classification, etiologies, and prognoses have been the subject of much discussion. Recent research has focused on identifying the causes of TTP and HUS at the molecular level. Of particular interest is the relationship between severely low levels of the serum metalloprotease ADAMTS13 with TTP. Bianchi and colleagues reported that ADAMTS13 activity less than 5% of normal is specific for TTP (2). Note, however, that reduced ADAMTS13 levels have also been observed in patients diagnosed with HUS (3).

As extensively reviewed (4, 5), Von Willebrand factor (vWF) is initially secreted from Wiebel-Palade bodies as multimers tethered to the endothelial cell surface, where they provide glycoprotein Ibα receptor sites for platelet adhesion and thrombus formation. ADAMTS13 cleaves the multimeric vWF, regulating thrombus formation. However, a significant decrease in ADAMTS13 or ADAMTS13 activity allows pathogenic thrombus formation as seen in TTP (Fig. 1). Familial TTP is a rare and recurrent disorder caused by an inherited deficiency in ADAMTS13 (6). Development of autoantibodies against this same enzyme may lead to a more common form of acquired TTP.

The majority (~90%) of HUS cases appear in children infected with verocytotoxin-producing bacteria (7). Following infection of the colon, the Shiga-like toxins (Stx1 and Stx2) enter the bloodstream and bind to cells...
Thrombotic microangiopathies (TMA) are known to occur in malignancy and have been linked to cytotoxic chemotherapy, including mitomycin and gemcitabine. The pathogenesis of chemotherapy-induced TMA is likely due to direct endothelial cell injury. Recently, case reports of TMA related to the use of targeted cancer agents have also surfaced in the literature. Little is known about the etiology of TMA in these cases. Herein, we review the clinical presentation, outcome, and proposed mechanisms of TMA associated with the use of monoclonal antibodies, immunotoxins, and tyrosine kinase inhibitors.

Translational Relevance

Thrombotic microangiopathies (TMA) are known to occur in malignancy and have been linked to cytotoxic chemotherapy, including mitomycin and gemcitabine. The pathogenesis of chemotherapy-induced TMA is likely due to direct endothelial cell injury. Recently, case reports of TMA related to the use of targeted cancer agents have also surfaced in the literature. Little is known about the etiology of TMA in these cases. Herein, we review the clinical presentation, outcome, and proposed mechanisms of TMA associated with the use of monoclonal antibodies, immunotoxins, and tyrosine kinase inhibitors.

Thrombotic Microangiopathy Induced by Targeted Agents

Immunotoxins

Immunotoxins are proteins comprised of a cell-selective ligand chemically conjugated or genetically fused to a toxin (19, 20). The cell-selective portion of the immunotoxin is commonly a monoclonal antibody, antibody fragment, growth factor, or cytokine that binds to specific cell-surface receptors. Once bound to a surface antigen, immunotoxins enter the target cell through endocytosis and undergo processing to release the toxin into the cytosol (21). Several of these agents have shown promising activity in clinical trials; however, TMA has been reported with their use, and the mechanism behind this adverse effect is not completely understood.

CAT-3888, formerly called BL22, is an immunotoxin that targets CD22 and has been investigated for the treatment of hairy cell leukemia (HCL), non-Hodgkin lymphoma, and chronic lymphocytic leukemia (22–24). During phase 1 and II testing of CAT-3888, 9 cases of grade 1 to 4 HUS were reported in 8 of the 82 subjects treated (22–24). In addition, HUS was reported in 1 of 2 HCL patients treated by special exemption prior to the opening of the phase II trial (22). Subjects in the phase I study were treated with 6 to 12 days of plasmapheresis, whereas those on the phase II study were given only supportive care. HUS was completely reversible in 9 of the 10 cases, regardless of treatment, with up to 57 months of follow-up in the phase I study. Note that the first of the 10 cases was not evaluable for reversibility because the patient had an aggressive lymphoma and refused additional treatment for rapidly progressive disease. However, this patient, who became anuric with HUS, resumed normal urination prior to dying of progressive lymphoma. ADAMTS13 was reported to be adequate in all cases, suggesting that ultralarge multimers of vWF were not circulating in these patients.

Moxetumomab pasudotox, formerly known as CAT-8015 or HA22, is an affinity-matured recombinant anti-CD22 immunotoxin that offers enhanced binding affinity compared with CAT-3888 (25). A preliminary report of an ongoing phase I trial in HCL suggests that HUS may occur with lower frequency in patients treated with moxetumomab pasudotox compared with CAT-3888 (26). Two of 28 subjects treated had experienced reversible, grade 2 HUS following moxetumomab pasudotox administration. The clinical presentation of HUS seems to be similar to that seen in subjects treated with CAT-3888. However, in both of these cases, the peak creatinine was <2.0 mg/dL and the nadir platelet count was >100,000/μL.

Combotox is an investigational combination of 2 deglycosylated ricin A chain (dgA) immunotoxins directed against CD19 and CD22 (27). This combination has been evaluated in a phase I study (N = 22) in which 2 cases of HUS were observed (27). Both subjects were reported to have followed a similar clinical course of vascular leak, respiratory insufficiency, HUS, and eventual death. The severe morbidity and mortality in these subjects may have
been related to complicating vascular leak syndrome (VLS) rather than HUS. In fact, VLS has proved to be a prominent toxicity for large, conjugated immunotoxins because of either their long plasma half-lives or residues that bind to endothelial cells (28). The authors of this study suggest that HUS in these patients may be associated with low or absent levels of circulating tumor cells and possibly with high serum concentrations of the immunotoxins.

DAB486IL-2 is a recombinant immunotoxin targeting IL-2 with the membrane translocation and ADP-ribosylation domains of diphtheria toxin, and it is the precursor of DAB389IL-2 (denileukin diftitox; ref. 29). During a phase I trial (N = 23) of DAB486IL-2, the maximum tolerated dose was determined by HUS toxicity in 2 patients (30). Similar toxicity was noted in 1 patient in a later phase II study (N = 14; ref. 31). The HUS experienced by this patient was
Immunotherapy

Apolizumab (Hu1D10) is a humanized monoclonal antibody against an antigen on the β chain of HLA-DR called 1D10. HUS was observed to be a dose-limiting toxicity in 1 subject during a phase I and II trial (N = 23) of apolizumab (34). No schistocytes were observed by peripheral blood smear.

HUS has also been reported in a phase I study of apolizumab plus rituximab (35). In this study, subjects were administered weekly doses of 375 mg/m² rituximab followed by increasing doses of apolizumab. The dose-limiting toxicity was reported to be HUS, but the number of subjects experiencing HUS and their clinical presentation were not described. The authors reported that 1D10 was found on endothelial cells and might have played a role in the pathogenesis of HUS in these patients.

Alemtuzumab is a monoclonal antibody that targets CD52 on the surface of B and T lymphocytes. During an experimental study (N = 41) in rheumatoid arthritis, a 49-year-old female developed HUS after receiving her second dose of alemtuzumab (36). Following 2 days of plasmapheresis, renal function returned to normal and the patient made a complete recovery. Alemtuzumab has been noted to cause elevation of serum TNF-α and IL-6. Similar to one of the proposed mechanisms behind TMA following treatment with cytotoxic chemotherapy, cytokine release may be partly responsible for HUS in this subject.

**Anti-VEGF therapy**

Among the targeted oncology drugs associated with TMA, those that inhibit the VEGF pathway are the most widely studied. A series of 21 case reports of TMA associated with VEGF inhibition have been published in the past 5 years (37–44). These cases have been observed with the use of multiple anti-VEGF agents, suggesting a potential class-wide effect.

Eremina and colleagues describe 6 biopsy-documented cases of TMA resembling HUS following bevacizumab administration (39). All of the patients described in this series developed proteinuria or increased serum creatinine following the initiation of bevacizumab, ultimately leading to renal biopsy. However, beyond these indications of renal injury, the clinical presentation was not uniform across all patients. Following discontinuation of bevacizumab, improvement in renal function was noted, suggesting at least partial reversibility.

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**Table 1. Characteristics of TMA associated with mitomycin and gemcitabine**

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Incidence</th>
<th>Clinical presentation</th>
<th>Onset</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomycin</td>
<td>2–15%</td>
<td>Severe MAHA; thrombocytopenia; renal dysfunction; elevated lactate dehydrogenase; elevated bilirubin; pulmonary edema</td>
<td>Cumulative doses &gt;30 mg/m² and &gt;1 year of treatment</td>
<td>Mortality ~75% related to renal failure</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>0.25–0.4%</td>
<td>2,000–48,000 mg/m² and 5–8 months of treatment</td>
<td>Mortality ~60%; renal failure 34–69%; reversal of anemia and thrombocytopenia may be common</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Data summarized in this table are from references 13–16.
The clinical presentation of TMA seems to have some common characteristics for the cases described in this review (Table 3). Renal dysfunction was almost universal as manifested by elevations in creatinine and/or worsening proteinuria. Similarly, evidence of hemolysis was frequent, and laboratory values for hemoglobin, reticulocyte count, lactate dehydrogenase (LDH), haptoglobin, and bilirubin were outside of normal limits. Interestingly, identification of fragmented erythrocytes by peripheral smear was not documented in all TMA cases described. In fact, even in cases of biopsy-proven TMA...
### Table 3. Comparison of thrombotic microangiopathy associated with targeted cancer agents

<table>
<thead>
<tr>
<th>Targeted therapy</th>
<th>CAT-3888</th>
<th>Moxetumomab pasudotox</th>
<th>Combotox DAB486IL-2</th>
<th>Apolizumab</th>
<th>Alemtuzumab</th>
<th>Bevacizumab</th>
<th>Sunitinib</th>
<th>Aflibercept</th>
<th>Bevacizumab Imatinib and sunitinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Time to onset</td>
<td>2–3 cycles</td>
<td>3–5 cycles</td>
<td>3–5 days</td>
<td>NR</td>
<td>11 doses</td>
<td>2 doses</td>
<td>4–29 doses</td>
<td>2 weeks to 7 months</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Decreased Hgb</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hemolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Elevated reticulocyte count</td>
<td>+</td>
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<td></td>
<td>+</td>
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<tr>
<td>Elevated bilirubin</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Decreased haptoglobin</td>
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<td></td>
<td>+</td>
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<tr>
<td>Schistocytes</td>
<td>+</td>
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<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>Elevated LDH</td>
<td>+</td>
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<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>Elevated creatinine</td>
<td></td>
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<td>+</td>
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<tr>
<td>Proteinuria</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Decreased urine output</td>
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<td></td>
<td>+</td>
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<tr>
<td>Decreased albumin</td>
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<td>+</td>
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<tr>
<td>Pulmonary</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Edema</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Hypertension</td>
<td>+</td>
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<tr>
<td>Neurologic</td>
<td>+</td>
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<tr>
<td>Biopsy confirmation</td>
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<td></td>
<td>+</td>
</tr>
<tr>
<td>Outcome</td>
<td>9 of 10 fully reversible, 1 death due to progressive disease</td>
<td>2 of 2 fully reversible deaths</td>
<td>2 of 2 fully reversible (others NR)</td>
<td>1 death (others NR)</td>
<td>Fully reversible</td>
<td>1 fully reversible, 7 partially reversible with persistent proteinuria, 1 death due to progressive disease</td>
<td>3 of 3 fully reversible</td>
<td>Partially reversible, 5 NR</td>
<td>Partially reversible (hematologic recovery; dialysis dependent)</td>
</tr>
</tbody>
</table>

Abbreviations: NR, not reported; Hgb, hemoglobin.
following anti-VEGF therapy, schistocytes were only noted in 4 of 10 cases. However, doing biopsies to evaluate TMA is not appropriate for all patients. Thrombocytopenia was noted in most cases of TMA, but with varying severity. In the cases reviewed, consequences of thrombocytopenia, such as petechiae and hemorrhage, were not discussed. Strict monitoring of renal function, platelet count, and for signs of nonautoimmune hemolytic anemia is recommended for patients receiving these targeted therapies.

### Treatment and outcome

Many interventions for TMA have been described (Table 4). Optimal treatment of drug-induced TMA, and especially TMA induced by targeted agents, has yet to be proved. In the cases summarized in this review, plasma exchange and plasmapheresis were both used, but they may not be necessary if ADAMTS13 activity is unaltered in these patients. As noted for the cases of HUS associated with CAT-3888 and moxetumomab pasudotox, supportive care alone resulted in full recovery for several subjects. An advantage of these molecules compared with larger immunotoxins is their smaller size (~62 kDa), leading to relatively rapid (2–3 hours) half-life and fewer complications because of vascular leak syndrome. Until more is known about the mechanism of TMA following the use of targeted agents, immediate discontinuation of the offending drug may be the most important step in treatment. For patients with aHUS refractory to plasma exchange, eculizumab has resulted in clinical remission in selected cases (48–50). Likewise, rituximab has shown a high response rate without severe toxicity in a trial of refractory TTP (N = 24) and should be considered for these patients (51).

Reintroducing the drug at lower dose levels may be a strategy to avoid recurrent TMA, while allowing for continued treatment. Retreatment following TMA has been safely carried out with CAT-3888, DAB486IL-2, and bevacizumab (24, 31, 39). However, it may be difficult to choose an appropriate dose for reintroduction, and careful consideration of the potential risks and benefits to the patient should take place.

TMA with several of the targeted cancer agents seems to be reversible. HUS was fully reversible in 11 of the 12 subjects administered the immunotoxins CAT-3888 and moxetumomab pasudotox, at least 1 of the subjects given DAB486IL-2, and in the 1 case report involving alemtuzumab. Partial to full reversibility was also noted for anti-VEGF–treated patients; however, persistent proteinuria was common. Deaths were reported for both HUS cases seen with Combotox and in 1 of the cases of apolizumab treatment.

Unlike TMA induced by mitomycin and gemcitabine, there may not be a correlation between dose, cumulative dose, or time to onset and the use of targeted therapy. Perhaps any relationship between dose and time on therapy will become clearer as additional case reports are compiled.

### Table 4. Managing thrombotic microangiopathies

<table>
<thead>
<tr>
<th>TMA</th>
<th>Intervention</th>
<th>Proposed mechanism of action</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial TTP</td>
<td>Infusions of fresh-frozen plasma</td>
<td>Source of exogenous ADAMTS13</td>
<td>Reverses or prevents episodes of TTP in these cases</td>
</tr>
<tr>
<td>Acute, acquired TTP</td>
<td>Plasma exchange</td>
<td>Removal of ultralarge multimers of vWF, immune complexes, and autoantibodies to ADAMTS13</td>
<td>Survival improved from ~10% to 70–90%</td>
</tr>
<tr>
<td>Acute, acquired TTP</td>
<td>Glucocorticoids or splenectomy</td>
<td>Decreased formation of immune complexes</td>
<td>Has been used in combination with plasma exchange in severe cases</td>
</tr>
<tr>
<td>Acute, acquired TTP</td>
<td>Rituximab</td>
<td>Depletion of B cells responsible for production of anti-ADAMTS13 antibodies</td>
<td>Has been successfully used in TTP refractory to plasma exchange and in relapsed TTP</td>
</tr>
<tr>
<td>aHUS</td>
<td>Eculizumab</td>
<td>Binds complement protein C5, preventing formation of C5α and C5β-9 (the membrane-attack complex)</td>
<td>Has been used in aHUS refractory to plasma exchange and following renal transplant to prevent recurrent aHUS</td>
</tr>
<tr>
<td>“Typical” HUS</td>
<td>Dialysis</td>
<td>Renal replacement therapy</td>
<td>Dialysis may not be necessary in mild cases</td>
</tr>
</tbody>
</table>

NOTE: Data summarized in this table are from references 1, 15, and 48–51.
Conclusions

As strategies to treat cancer begin to incorporate more targeted therapeutics, such as monoclonal antibodies, immunotoxins, and small molecule inhibitors, it will be important to recognize the unique toxicities that accompany their use. TMA is one such toxicity that has been associated with these targeted agents, and research into its pathogenesis is in its infancy. Detailed evaluation of biopsy results, laboratory values, and ADAMTS13 levels of future cases may be warranted. In addition, animal models such as those used by Eremina and colleagues may help to further define the mechanisms of these disorders (39). Preliminary analysis seems to show a reversible course of TMA in the majority of patients described in this review.

References


Disclosure of Potential Conflicts of Interest

J.A. Blake-Haskins: postdoctoral fellow sponsored by MedImmune, LLC. R. Lechleider employee, MedImmune, LLC. However, the majority of the agents reviewed have no relation to MedImmune. R.J. Kreitman is a convener on the government-owned patent for anti-CD22 recombinant immunotoxins, although these agents constitute a minority of those reviewed.

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