Anti-CD20 Antibodies for Idiopathic Nephrotic Syndrome in Children

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Abstract
Rituximab, a chimeric anti-CD20 monoclonal antibody originally licensed for lymphoma, is emerging as a novel steroid-sparing agent for idiopathic nephrotic syndrome in children. The potential use of anti-CD20 monoclonal antibodies in idiopathic nephrotic syndrome has contributed to shifting the view of podocytopathies from T cell–mediated to more complex immunemediated disorders that can benefit from targeting B cells and other mediators of the early immune response. Clinical data on the use of rituximab also have implications on disease management and classification. In this review, we present results of clinical studies that support rituximab as an effective steroid-sparing agent in steroid-dependent idiopathic nephrotic syndrome. Recent randomized controlled trials suggest that potential benefits of rituximab therapy in steroid-dependent forms of idiopathic nephrotic syndrome vary depending on whether children are dependent on steroids alone or on both steroids and calcineurin inhibitors, with greater probabilities to achieve drug-free remission in the former group. Multiple-drug dependence may identify a different disease state with different prognosis and treatment options. Insufficient data are available on optimal use of rituximab as a maintenance steroid-sparing agent in these steroid-sensitive forms of the disease, including how often and for how long rituximab infusions should be repeated to maximize expected benefits and minimize potential harms. Finally, one randomized controlled trial in children with steroid-resistant idiopathic nephrotic syndrome yielded negative results. New anti-CD20 antibodies are under study in this patient population.


Idiopathic Nephrotic Syndrome
Idiopathic nephrotic syndrome is characterized by episodes of severe proteinuria and hypoalbuminemia (serum albumin <2.5 g/dl), often associated with dyslipidemia and hypercoagulability. Although mechanisms are poorly defined, idiopathic nephrotic syndrome includes some pathologic variants with polymorphic podocyte injury as a unifying feature (1). Diffuse foot-process effacement of podocytes is pathognomonic of the two most common glomerular lesions in idiopathic nephrotic syndrome: minimal-change nephropathy and FSGS (2). Response to drugs varies from rapid and permanent disease remission after a short course of oral steroids to forms that are refractory to multidrug combination schemes. The terms steroid-sensitive, steroid-dependent, and steroid-resistant are used to differentiate clinical phenotypes, although other drugs are often used in combination with steroids. In Western countries, idiopathic nephrotic syndrome affects 2–2.7 new children per 100,000 children per year, with a prevalence of 16 cases per 100,000 (3). A substantial proportion of these children have a genetic disorder; almost 20 new genes have been discovered, documenting the involvement of the podocyte structure and function in the mechanisms of the disease (4). Despite genetic evolutions, the pathogenesis of nephrotic syndrome is poorly defined; it has been considered a T cell disorder for years (5), but evolution in basic immunology now suggests a more articulated immune cell interaction (6).

Prednisone is the cornerstone of therapy for idiopathic nephrotic syndrome, inducing remission within 4–6 weeks in approximately 90% of cases (7). However, the risk of relapse can be as high as 85% at 5 years, requiring reiteration of prednisone courses, often with the additional use of calcineurin inhibitors. Given the toxicity of protracted use of these and other immunosuppressive drugs, in recent years the anti-CD20 monoclonal antibody rituximab has gained popularity in the treatment of several immunemediated disorders, including idiopathic nephrotic syndrome (8).

Anti-CD20 Antibodies: Specificity and Mechanism of Action
Anti-CD20 monoclonal antibodies are a class of drugs recognizing a 35-kD integral protein (CD20) expressed on the surface of B lymphocytes at various stages of differentiation, starting from pre–B cells to the mature lineage, at which phase it reaches the highest concentration (9). Rituximab was the first chimeric molecule developed to treat B cell non-Hodgkin lymphomas (10). Its use was then extended to autoimmune anemia, rheumatic diseases (11,12) and, more recently, antibody-mediated conditions affecting the kidney, including ANCA-associated
vasculitis (13) and membranous GN (14). Upon binding, the complex rituximab-CD20 is translocated into the lipid raft of the cell membrane, where it crossreacts with sphingomyelin phosphodiesterase acid-like 3b protein (SMPDL-3b) (9). At this stage, B cells undergo a process that ends with their disappearance and that is postulated to be based on three different pathways: one is apoptosis, and the other two involve external mechanisms based on antibody- and complement-dependent cell-mediated cytotoxicity. Activation of caspase-dependent and independent pathways and of the bcl-2 gene are the two main mechanisms for apoptosis. Complement-dependent cytotoxicity has been demonstrated in vitro and is supported by the observation that rituximab infusion in humans results in rapid and profound depletion of complement. Antibody-dependent cellular toxicity is an important mediator of rituximab activity; it is effected by cells bearing the Fcγ receptor (e.g., natural killer [NK] cells, monocytes, macrophages) that recognize the CD20-rituximab complex and lyse cells mounting the complex. In addition to general interest, these mechanisms are important in considering the possibility of predicting the effect of rituximab (see later discussion of this topic). In addition to the classic view, convincing evidence suggests that other mechanisms linked to the binding of rituximab to SMPDL-3b are active in several settings (Figure 1) that seem more kidney specific. They are important for explaining the unexpected effect of rituximab in idiopathic nephrotic syndrome, which is classically a nonimmune disease, at least in the usual understanding of the term.

Use of Anti-CD20 Antibodies in Idiopathic Nephrotic Syndrome

Observational Data

The interest in rituximab as a potential therapy for nephrotic syndrome followed the observation of a dramatic reduction in proteinuria in children who had nephrotic syndrome and received rituximab to treat idiopathic thrombocytopenic purpura (15) or a post-transplant lymphoproliferative disorder (8,16). Successive retrospective studies reported between 2008 and 2011 (17–26) confirmed these potential benefits in uncontrolled small series of mixed populations with nephrotic syndrome. Although these studies could not assist in decision making because of their observational design, they were key to informing the design of subsequent clinical trials that have been completed in the last 5 years.

One major problem with these retrospective studies is that they included both resistant and dependent forms of the disease on the basis of unclear selection criteria. Some of the resistant forms were resistant ab initio, and others became resistant after a period of drug sensitivity. Another

Figure 1. Proposed mechanisms of action of rituximab in patients with nephrotic syndrome effects on all cells expressing CD20 or sphingomonel phosphodiesterase acid-like 3b (SMPDL-3b) protein. Cells presenting CD20 or SMPDL-3b/acid sphingomyelinase (ASM) as a target and mediator of rituximab effect are included. In addition to B cells (CD20), the schematic includes podocytes that express the SMPDL-3b/ASM complex and Th17-expressing ASM. The classic view of rituximab activity implies that B cells presenting the CD20-rituximab complex undergo apoptosis, become a target of antibodies, or activate complement; their lysis is the final result of all mechanisms. Deficit in B cells produces immunologic rebounds linked to the lack of their activity. In particular, B cells may act at several levels of the immune response: They modulate adaptive immunity and regulate the T-cell compartment via CD80 and CTLA4; CD80 is a costimulatory molecule expressed by antigen-presenting cells and by B cells (6). Via SMPDL-3b/ASM, rituximab also modulates IL-17 production by Th17; CD39 and CD161 may serve as surface markers of IL-17 and modulate, in turn, ASM SMPDL-3b-mediated signal transduction (STAT3) (44). Finally, by interacting with SMPDL-3b/ASM in podocytes, rituximab may stabilize actin remodeling, which is a mechanism for proteinuria (42).
problem is the variable number of treatment infusions given to patients. Overall, these studies recruited 211 patients with steroid-dependent nephrotic syndrome and 90 patients with steroid-resistant nephrotic syndrome, treated them with variable doses of rituximab (from one to ten single or multiple courses of rituximab, 375 mg/m²) and followed them for up to 54 months (Table 1). Response rates were >50% in steroid-dependent nephrotic syndrome (results from ten studies) and <25% in steroid-resistant nephrotic syndrome (five studies). Although these reports included mixed populations with unclear eligibility criteria and were at high risk of bias because of their observational design, they provided key data. Some of these studies anticipated the lack of effects of rituximab in forms that were resistant to a combination of steroids and calcineurin inhibitors (20). Others informed the design of subsequent trials by providing data on how patient history may modify the effect of rituximab, including previous number of relapses or therapies and optimal number of rituximab infusions and infusion methods.

Randomized Controlled Trials

Between 2011 and 2015, five clinical trials on the use of rituximab in idiopathic nephrotic syndrome were reported: four as journal articles (27–30) and one in abstract form. Results of four prospective studies were also published in the same period (see below) (27,31,32). All these trials tested the effects of rituximab as steroid-sparing agent. Tables 2 and 3 summarize the key characteristics and findings from these randomized controlled trials. One trial enrolled children with idiopathic nephrotic syndrome maintained in remission with high-dose steroids alone (27), three trials enrolled children maintained in remission with steroids and calcineurin inhibitors (28,29), and one trial assessed the effect of rituximab in children with nephrotic syndrome resistant to both steroids and calcineurin inhibitors (30). Figure 2 presents a flow chart of treatment of idiopathic nephrotic syndrome based on randomized controlled trials.

Data from these clinical trials suggest that the effects of rituximab in idiopathic nephrotic syndrome vary depending on the clinical characteristics of the disease defined by its sensitivity to standard therapy. In one trial, children who were dependent on high-dose steroids (at least 0.7 mg/kg per day) were randomly assigned to continue steroids alone or receive a single intravenous pulse of rituximab (375 mg/m²) in addition to steroids (27). The steroids were tapered and withdrawn in the following month in both groups. Patients treated with rituximab (375 mg/m²) completed steroid withdrawal as indicated earlier; 80% of these patients maintained drug-free remission at 1 year, whereas patients who did not receive rituximab had recurrence of proteinuria shortly after steroid elimination (27). The median relapse-free survival time after repeated rituximab infusions in the follow-up study of this trial cohort was 18 months (95% confidence interval, 9 to 32 months). Although these data require confirmation in additional randomized controlled trials and more information is needed about optimal intervals and dose of repeated infusions, they suggest that single injection of rituximab can maintain long-term drug-free remission in steroid-dependent nephrotic syndrome.

Results are less convincing in forms that are dependent on both steroid and calcineurin inhibitors. Two trials have studied the effects of rituximab in these more complex forms of idiopathic nephrotic syndrome. One study enrolled 54 children with at least a 1-year history of nephrotic syndrome who had been maintained in remission for at least 6 months (28). In this study, participants were stratified on the basis of the presence or absence of signs of drug toxicity versus steroids (i.e., growth retardation, cataract, and osteoporosis) and/or calcineurin inhibitors (i.e., hypertension and neurologic and renal signs) and were randomly assigned to continue the combination therapy alone or to receive one (if toxicity was absent) or two (if toxicity was present) doses of rituximab (375 mg/m² each). After 30 days from randomization, the combination therapy was tapered off in both groups. All patients in the control group had disease recurrence within a few weeks from the drug withdrawal. At 3 months, relapse rates were 18.5% and 48.1% in the intervention and control groups, respectively (P=0.03), and the probabilities of being drug free were 62.9% and 3.7%, respectively (P<0.001). About half of the children treated with rituximab were in drug-free remission at 6 months.

These results were confirmed in a second trial that included 47 children maintained in remission with combination therapy consisting of steroids, cyclosporine, mycophenolate, and/or mizoribine and one child receiving high-dose steroids alone (29). Children were randomly assigned to receive one rituximab infusion (375 mg/m²) or placebo and were followed for 1 year, during which oral drugs were tapered. The median relapse-free period in the intervention group (about 6 months after complete standard drug withdrawal) was twice as long as in the placebo group. A final study on drug-dependent nephrotic patients is now in progress in Korea. Interim results published in abstract form confirm the findings of the previous two studies.

Only one clinical trial has been conducted to determine whether rituximab can induce disease remission in patients with multidrug-resistant forms of nephrotic syndrome (30). In this trial, 31 children who were resistant to steroids and calcineurin inhibitors for 6–12 months were randomly assigned to continue the combination therapy alone or add to the combination therapy two doses of rituximab (375 mg/m²) administered 2 weeks apart. Levels of proteinuria at 3 months remained the same in both groups. The persistent decrease in the CD20 count to <1% during the whole study period suggested an adequate dose of rituximab in the intervention group.

Preliminary data from a recent multicenter open-label trial performed in 19 children who were resistant to steroids and calcineurin inhibitors and reported in abstract form indicate complete remission in seven children after a single dose of rituximab (375 mg/m²).

Although both these studies have limited power and relatively short follow-up duration, differences in design may explain conflicting findings. For example, some patients may respond to the association of steroids and calcineurin inhibitors after the 12-week period proposed in the Kidney Disease Improving Global Outcomes guidelines (33), and therefore enrollment of patients with delayed sensitivity
Table 1. Retrospective studies on the efficacy of rituximab in nephrotic syndrome with dependence and resistance to drugs

<table>
<thead>
<tr>
<th>Author, Year (Reference)</th>
<th>Category</th>
<th>Patients, n</th>
<th>Age, yr[^a]</th>
<th>Male/Female Patients, n/n</th>
<th>Disease Duration[^b]</th>
<th>No. of Relapses before Rituximab[^a]</th>
<th>No. of Doses of Rituximab, 375 mg/m[^2]</th>
<th>Patients in Remission, n[^b]</th>
<th>Time of Relapse, mo[^a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guigonis et al., 2008 (17)</td>
<td>DDNS</td>
<td>22</td>
<td>14 (6–22)</td>
<td>13/9</td>
<td>11 (4–16)</td>
<td>2.5 (1–8) last year NA</td>
<td>3–8</td>
<td>16</td>
<td>12 (7–17)</td>
</tr>
<tr>
<td>Peters et al., 2008 (18)</td>
<td>DRNS</td>
<td>3</td>
<td>20 (15–20)</td>
<td>2/1</td>
<td>8 (8–18)</td>
<td>NA</td>
<td>2</td>
<td>2</td>
<td>0–12</td>
</tr>
<tr>
<td>Kamei et al., 2009 (19)</td>
<td>DDNS</td>
<td>12</td>
<td>13 (5–19)</td>
<td>8/4</td>
<td>7 (2–11)</td>
<td>2.5 (1–5) last year NA</td>
<td>1–2[^c]</td>
<td>11</td>
<td>5 (3–8)</td>
</tr>
<tr>
<td>Fernandez-Fresnedo et al., 2009 (20)</td>
<td>DRNS</td>
<td>8</td>
<td>26 (19–55)</td>
<td>7/1</td>
<td>3 (2–9)</td>
<td>NA</td>
<td>4–10</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Fujinaga et al., 2010 (21)</td>
<td>DDNS</td>
<td>10</td>
<td>12 (4–19)</td>
<td>5/5</td>
<td>5 (3–11)</td>
<td>10 (4–17)</td>
<td>1</td>
<td>4</td>
<td>9 (0–17)</td>
</tr>
<tr>
<td>Gulati et al., 2010 (22)</td>
<td>DDNS</td>
<td>24</td>
<td>12 (5–17)</td>
<td>19</td>
<td>9 (4–16)</td>
<td>NA</td>
<td>2–4</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Prytula et al., 2010 (23)</td>
<td>DRNS</td>
<td>28</td>
<td>NA</td>
<td>25/3</td>
<td>6 (2–41)</td>
<td>NA</td>
<td>NA</td>
<td>1–5</td>
<td>17 (6 (1–16))</td>
</tr>
<tr>
<td>Sellier-Leclerc et al., 2010 (25)</td>
<td>DDNS</td>
<td>22</td>
<td>13 (6–20)</td>
<td>17/5</td>
<td>11 (3–17)</td>
<td>NA</td>
<td>1–4</td>
<td>13</td>
<td>3–12</td>
</tr>
<tr>
<td>Kemper et al., 2010 (24)</td>
<td>DDNS</td>
<td>37</td>
<td>13 (6–18)</td>
<td>25/12</td>
<td>NA</td>
<td>NA</td>
<td>1–4</td>
<td>26</td>
<td>10 (5–64)</td>
</tr>
<tr>
<td>Sellier-Leclerc et al., 2012 (25)</td>
<td>DDNS</td>
<td>30</td>
<td>13 (4–20)</td>
<td>21/9</td>
<td>10 (0.3–17)</td>
<td>NA</td>
<td>1–4</td>
<td>19[^d]</td>
<td>Only reported from CD20 recovery</td>
</tr>
<tr>
<td>Ito et al., 2013 (26)</td>
<td>DDNS</td>
<td>55</td>
<td>4 (1–16)</td>
<td>13/3</td>
<td>5 (0.2–15)</td>
<td>NA</td>
<td>1–2</td>
<td>27</td>
<td>16 (7–31)</td>
</tr>
<tr>
<td>Sinha et al., 2012 (50)</td>
<td>DRNS</td>
<td>19</td>
<td>NA</td>
<td>NA</td>
<td>2.3±1.4[^d]</td>
<td>2.6 ± 6[^d]</td>
<td>6</td>
<td>6 (1–12)</td>
<td></td>
</tr>
</tbody>
</table>

Ten studies reported results in at least ten children and/or young adults with drug dependence. Five studies described results in small series of patients with drug resistance. DDNS, drug-dependent nephrotic syndrome; DRNS, drug-resistant nephrotic syndrome; NA, not available.

[^a]Years, median and range.

[^b]Remission was defined as absence of proteinuria based on different criteria (i.e., protein excretion < 10 mg/m[^2] per h; protein-to-creatinine ratio < 0.2; protein excretion < 150 mg/24 h).

[^c]Rituximab dose, 1000 mg.

[^d]Mean ± SD.
Table 2. Randomized controlled trials in children with nephrotic syndrome with different sensitivity to drugs: Dependence on steroids alone, multidrug dependence on steroids and calcineurin inhibitors, and multidrug resistance to both therapies

<table>
<thead>
<tr>
<th>Author, Year (Reference)</th>
<th>Allocation</th>
<th>Patients, n</th>
<th>Age, yr</th>
<th>Male/Female Patients, n/n</th>
<th>Duration, yr</th>
<th>Histologic Findings Showing FSGS/MCD</th>
<th>No. of Doses of Rituximab, 375 mg/m²</th>
<th>Follow-Up, mo</th>
<th>Patients in Remission (Duration of Remission), % (mo)</th>
<th>Relapse-Free Period, days</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steroid-dependent nephrotic syndrome</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ravani et al., 2015 (27)</td>
<td>Rituximab</td>
<td>15</td>
<td>6.9±3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10/5</td>
<td>2.7±2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>1</td>
<td>12</td>
<td>80 (12)</td>
<td>480 (210–900)</td>
<td>1 mild&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>6.9±3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9/6</td>
<td>2.0±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>0</td>
<td>12</td>
<td>0 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Multidrug-dependent nephrotic syndrome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ravani et al., 2011(28)</td>
<td>Rituximab</td>
<td>27</td>
<td>10.2±4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24/3</td>
<td>7.9±4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/21</td>
<td>1 in 15</td>
<td>12</td>
<td>50 (6)</td>
<td>NA</td>
<td>3 mild&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>27</td>
<td>11.3±4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19/8</td>
<td>8.0±5.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/23</td>
<td>0 in 12</td>
<td>12</td>
<td>0 (6)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Iijima et al., 2014 (29)</td>
<td>Rituximab</td>
<td>24</td>
<td>11.5±1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18/6</td>
<td>5.7±3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/21</td>
<td>1</td>
<td>12</td>
<td>71 (12)</td>
<td>267 (233–374)</td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>24</td>
<td>13.6±6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16/8</td>
<td>7.8±4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/23</td>
<td>0</td>
<td>12</td>
<td>8 (12)</td>
<td>101 (70–155)</td>
<td>7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ahn et al., 2014 (Supplemental Material)</td>
<td>Rituximab</td>
<td>24</td>
<td>13±4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1</td>
<td>6</td>
<td>77 (6)</td>
<td>NA</td>
<td>24 mild&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>18</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>6</td>
<td>39 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Multidrug-resistant nephrotic syndrome</strong></td>
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<td></td>
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<tr>
<td>Magnasco et al., 2012 (30)</td>
<td>Rituximab</td>
<td>15</td>
<td>8.5±4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10/5</td>
<td>2.5 (0.5, 12)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9/4</td>
<td>2</td>
<td>3</td>
<td>3 (3)</td>
<td>NA</td>
<td>2 mild&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.3±3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9/6</td>
<td>1.4 (0.5,8.1)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10/3</td>
<td>0</td>
<td>3</td>
<td>3 (3)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

MCD, minimal-change disease; NA, not available.

<sup>a</sup>Mean±SD.
<sup>b</sup>Mild infusion reactions.
<sup>c</sup>Serious events.
<sup>d</sup>Median (interquartile range).
<table>
<thead>
<tr>
<th>Author, Year (Reference)</th>
<th>Category</th>
<th>Patients, n</th>
<th>Age, yr</th>
<th>Male/Female Patients, n/n</th>
<th>Duration, yr</th>
<th>Histologic Findings Showing FSGS/MCD</th>
<th>No. of Doses of Rituximab, 375 mg/m²</th>
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<th>Patients in Remission (Duration of Remission), % (mo)</th>
<th>Relapse-Free Period, days</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ravani et al., 2013 (34)</td>
<td>DDNS</td>
<td>46</td>
<td>9.9±4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29/17</td>
<td>6.3±4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/10</td>
<td>1-5</td>
<td>36</td>
<td>48 (6) 20 (12) 20 (18)</td>
<td>168 (129–243)&lt;sup&gt;b&lt;/sup&gt; first course 258 (195–351)&lt;sup&gt;b&lt;/sup&gt; Subsequent course</td>
<td>5 bronchospasm, 3 arthritis, 2 neutropenia</td>
</tr>
<tr>
<td>Ruggenenti et al., 2014 (31)</td>
<td>DDNS</td>
<td>30</td>
<td>22.7 (14–43)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15/15</td>
<td>8.5 (4.6–15.4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8/22</td>
<td>1</td>
<td>12</td>
<td>10 (24) 57 (12)</td>
<td>225 (15–321)&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 gastroenteritis, 3 viral infection, 3 noninfectious, 1 melanoma</td>
</tr>
<tr>
<td>Iwabuchi et al., 2014 (32)</td>
<td>DDNS</td>
<td>25</td>
<td>30.1±20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/21</td>
<td>13±8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>7</td>
<td>54</td>
<td>100 (24) 100 (36) 100 (54) 37</td>
<td>NA</td>
<td>13 cough, 1 exanthema, 1 leukopenia</td>
</tr>
<tr>
<td>Ahn et al., 2014 (Supplemental Material)</td>
<td>DRNS</td>
<td>19</td>
<td>9.2±4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>22 mild infusion reactions</td>
</tr>
</tbody>
</table>

Three studies were performed in patients with multidrug-dependent nephrotic syndrome; one study was performed in patients with multidrug-resistant nephrotic syndrome. MCD, minimal-change disease; DDNS, drug-dependent nephrotic syndrome; DRNS, drug-resistant nephrotic syndrome; NA, not available.

<sup>a</sup>Mean±SD.

<sup>b</sup>Median (interquartile range).

<sup>c</sup>Time of tapering was not subtracted.
(i.e., children who respond to standard therapy in 3–6 months) could explain positive results in previous observational studies and this most recent trial.

**Cohort Studies**

Overall, the findings from randomized controlled trials are consistent with results deriving from three observational prospective studies (Table 3) limited to children (34) or including adult patients (31,32). These data suggest that the duration of oral drug–free remission after rituximab infusion increases with age and that oral drug–free remission after the second and subsequent infusion of rituximab lasts longer than after the first infusion (34). Iwabuchi et al. (32) have followed a different strategy based on sequential infusions (up to seven) of rituximab every 6 months and showed persistence of stable remission during 54 months follow-up.

In general, rituximab seems to be less effective in forms of idiopathic nephrotic syndrome that are dependent on both steroids and calcineurin inhibitors (50% probability of oral drug–free remission at 6 months) than in forms that are dependent on steroids alone (80% probability of oral drug–free remission at 12 months). The two categories probably overlap because most children who require multiple drugs to maintain remission were originally dependent on steroids alone and have developed double dependence over time. Disease duration is, in fact, shorter in children who are dependent on steroids alone than in the other group (2 versus 7 years). Some steroid-sensitive forms of idiopathic nephrotic syndrome may become progressively drug resistant over time as disease progresses and would potentially benefit from earlier rituximab therapy. Response to multiple drugs, as opposed to steroids alone, may lead to a classification of the disease with more meaningful prognostic implications.

**Safety Data**

Rituximab has a limited number of adverse effects, the most common of which occur during the infusion. Early symptoms (i.e., nausea, skin rash, and bronchospasm) are related to the speed of rituximab infusion and can be minimized with pretreatment medications and by slowing the infusion rate. Cytopenia and acute arthritis may occur in the weeks after the administration of rituximab. Among all studies (27,29,31,32,34), four patients had neutropenia, four had lymphocytopenia, and one had leukopenia (Table 3). Four patients had acute arthritis associated with fever after 1–3 months that recovered within 72 hours; other symptoms were gastroenteritis, infectious and noninfectious symptoms (seizures, biliary colic, and in situ melanoma) (31), and other symptoms not specifically linked with rituximab (29).

Pneumonia has been reported in 121 patients receiving rituximab as a part of combination chemotherapy for large B cell lymphoma or non-Hodgkin lymphoma (35). Only two cases of rituximab-related lung injury occurring in patients with new-onset nephrotic syndrome have been described in the literature (36,37). The first was a 9-year-old child who presented with segmental atelectasia a few days before rituximab therapy and developed acute respiratory distress after infusion; he died of respiratory failure after 5 weeks. The second patient was a teenager who developed progressive dyspnea, fever, hypoxemia, and fatigue 18 days after rituximab and recovered spontaneously after empirical antibiotic therapy.
Long-term complications are less common but include potentially serious events. Progressive multifocal leukoencephalopathy has been reported in patients with B cell lymphoproliferative disorders and autoimmune diseases (38) who were treated with a prolonged course of immunosuppressive medications; progressive multifocal leukoencephalopathy has never been reported in people with renal disorders.

Monitoring and Molecular Testing

Monitoring clinical variables is clearly needed in patients receiving rituximab because of their potential susceptibility to infection. The CD20-positive cell count can be used to monitor the duration of rituximab’s effects on B cells. The CD20 count rapidly decreases after rituximab infusion, and CD20 reconstitution seems slower in patients with longer remission. In one study (34), CD20 remained undetectable (<2.5%) for about 200 days after rituximab infusion and CD20 reconstitution was delayed in children with better response (190 versus 110 days). Another study reported similar results (29). New studies are now in progress to better define the panel of T and B cells that respond to rituximab, the kinetics of response, and any relationship of recurrence with specific cell lineages. This topic has had limited clinical impact so far and should therefore be studied further.

Predictors of rituximab sensitivity have been studied in detail in hematologic disorders and only partially analyzed in nephrotic patients. They have been considered on the basis of the mechanisms of action of rituximab. One is the FcγRIII protein (39) encoded by the FCGR3A gene, which is expressed by monocytes/macrophages and NK cells and is involved in the basic mechanism for antibody-mediated action of rituximab. A phenylalanine in place of a valine decreases the affinity of rituximab for NK cells and has been associated with resistance to rituximab in patients with follicular lymphoma (FCG3A-rs396991 and FCGRA2-rs1801274). A second potential biomarker directly involves the affinity of rituximab for CD20 and is related to polymorphisms in the M54A1 gene coding for the CD20-binding site on B cells (40); in fact, variations in the binding sites of rituximab with CD20 (loops Rp15-C9 and Rp5-L) may modify drug activity. A third is SMPD-3b (41,42), which is the target of rituximab on the membrane of human podocytes specifically at the 156ELWKPW161 epitope (the binding site). Molecular analysis for mutations or variants at this site showed no change (34). As already reported, molecular tests for predicting rituximab effects in patients with nephrotic syndrome have been performed in our laboratory in a small number of cases (34). Therefore, expanded studies are needed (34).

Implications for Disease Management

The evidence supporting the use of rituximab in nephrotic syndrome has limitations. Few clinical trials are available, all are small, and most come from one center. Consistent data from two trials exist to inform the management of children with complex forms of steroid-dependent nephrotic syndrome (i.e., requiring both steroids and calcineurin inhibitors), but benefits of rituximab in this patient population are smaller than expected. Relatively short periods of drug-free remission may be achieved in these patients (i.e., about 6 months), requiring repeated courses of rituximab or the reuse of previous drugs. Current data do not support the use of rituximab in resistant forms of the disease (34). Considering the larger effects of rituximab in forms of nephrotic syndrome that are dependent on steroids alone and the progressively smaller effects in more complex forms of the disease, earlier use of rituximab once steroid dependence is demonstrated (for at least 6 months) may maximize its steroid-sparing benefits, reduce episodes of recurrence, and modify the clinical impact of the disease. More data are needed to support this hypothesis and provide information about benefits and harms of repeated infusions, including long-term safety data. A clinical trial in progress seems to confirm the utility of rituximab in children who are dependent on steroids alone (G.M. Ghiggeri, unpublished observations).

Implications for Research

Rituximab represents a unique situation in which the clinical evidence of a therapeutic effect has modified the view of the disease. This is of particular interest because idiopathic nephrotic syndrome in children has historically been considered a T cell disorder, whereas outcome improvement after rituximab treatment points to a multifactorial pathogenesis (6). B cells may be just one of the
possible targets of rituximab (Figure 1). Recent data suggest that rituximab may interfere with different mechanisms leading to the disease, including SMPLD3B. Fornoni et al. (42) showed direct binding of rituximab with SMPLD-3b, which regulates acid sphingomyelinase in the raft of podocytes and partially colocalizes with synaptopodin, a regulator of the cell cytoskeleton. It has been suggested that rituximab interaction with SMPLD-3b prevents podocyte actin remodeling induced by serum of patients with FSGS preserving the structure and function of podocytes. It is also of interest that both SMPLD-3b and ASM are expressed on Th17 (44), a cell lineage whose activation is considered a key element in the pathogenesis of nephrotic syndrome (6). Other studies also demonstrated that rituximab reduces Th17 cell response in rheumatoid arthritis (45), thus making a logical connection among rituximab, Th17, and nephrotic syndrome.

Future Directions
A goal of potential rapid evolution is the development of molecular tests for predicting rituximab effects. This could offer an option to treat patients who are more sensitive to the drug and limit potential risks in other cohorts. Defining cell lineages that are more sensitive to rituximab would add to the research on mechanisms and could help define sensitivity. Studies in large and homogeneous series of patients with nephrotic syndrome that focus on cellular and molecular aspects should be done in a reasonable time.

Humanized anti-CD20 antibodies have been developed on the basis of the idea that the chimeric structure of rituximab may limit its efficacy. Ofatumumab (2F2, HuMax-CD20; Genmab and GlaxoSmithKline, Brentford, UK) has recently been licensed for human use. It is a completely humanized antibody that recognizes a membrane epitope in the human CD20 molecule distinct from the target of rituximab and whose structure allows the binding between antigen and antibody to occur more closely to the B cell membrane. This property may explain the significantly higher efficacy of ofatumumab in causing B cell death (46). Two randomized controlled phase 2 trials in patients with rheumatoid arthritis nonresponsive to methotrexate showed safety of ofatumumab and demonstrated clinical benefits (47). Basu recently reported (48) a potent effect of ofatumumab given in six infusions of 2 g/1.73 m² each in six patients with idiopathic nephrotic syndrome who were resistant to other drugs, including previous infusions of rituximab. In all cases persistent normalization of proteinuria occurred. We used low-dose ofatumumab (two pulses of 300 and 700 mg/1.73 m² given 2 weeks apart) in six children with idiopathic nephrotic syndrome unresponsive to other treatments (including rituximab) and monitored urinary and serum measures for 6 months. Remission of proteinuria was observed in the two patients with normal renal function (49). The treatment failed in four children with reduced renal function. Although ofatumumab may work only in children with normal kidney function, it may have advantages over rituximab in the treatment of idiopathic nephrotic syndrome and may be used at lower doses than originally proposed (48). Ofatumumab is currently being tested in clinical trials (trial identifiers NCT02394119 and NCT02394106).

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None.

References


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