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ABSTRACT Ebola viruses (EBOV) cause severe disease in humans and nonhuman primates with high mortality rates and continue to emerge in new geographic locations, including several countries in West Africa, the site of a large ongoing outbreak. Phosphorodiamidate morpholino oligomers (PMOs) are synthetic antisense molecules that are able to target mRNAs in a sequence-specific fashion and suppress translation through steric hindrance. We previously showed that the use of PMOs targeting a combination of VP35 and VP24 protected rhesus monkeys from lethal EBOV infection. Surprisingly, the present study revealed that a PMOplus compound targeting VP24 alone was sufficient to confer protection from lethal EBOV infection but that a PMOplus targeting VP35 alone resulted in no protection. This study further substantiates recent data demonstrating that VP24 may be a key virulence factor encoded by EBOV and suggests that VP24 is a promising target for the development of effective anti-EBOV countermeasures.

IMPORTANCE Several West African countries are currently being ravaged by an outbreak of Ebola virus (EBOV) that has become a major epidemic affecting not only these African countries but also Europe and the United States. A better understanding of the mechanism of virulence of EBOV is important for the development of effective treatments, as no licensed treatments or vaccines for EBOV disease are currently available. This study of phosphorodiamidate morpholino oligomers (PMOs) targeting the mRNAs of two different EBOV proteins, alone and in combination, demonstrated that targeting a single protein was effective at conferring a significant survival benefit in an EBOV lethal primate model. Future development of PMOs with efficacy against EBOV will be simplified if only one PMO is required instead of a combination, particularly in terms of regulatory approval.
sapiens-tc/COD/1995/Kikwit) and then administered 40 mg/kg of AVI-7537, 40 mg/kg of AVI-7539, or 40 mg/kg of AVI-6002 intravenously (0.5 ml/kg animal weight in 0.9% sodium chloride) for 1 h ± 30 min after viral challenge once daily for 14 consecutive days (Table 1). Animals in the saline control group were administered sterile saline. Monkeys were randomized at the time of group assignment, and study personnel were blinded to the treated group. Quantitative reverse transcriptase PCR (qRT-PCR) analysis of individual animals' viral genome levels on day 8 showed that animals treated with AVI-7537 alone had significantly lower viral RNA than those treated with AVI-7539 alone (P = 0.043), similar to levels observed with the combination treatment (Fig. 1C).

As liver and kidney damage are hallmarks of filovirus infection in humans and nonhuman primates, we examined the serum liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and blood urea nitrogen (BUN) in these animals (Fig. 2). On day 8, serum BUN levels were significantly reduced (P < 0.05) in animals treated with AVI-7537 or the AVI-

### TABLE 1: Study design

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>PMOplus dose (mg/kg)</th>
<th>Gene target(s)</th>
<th>No. of monkeys (male/female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AVI-7537</td>
<td>40</td>
<td>VP24</td>
<td>8 (4/4)</td>
</tr>
<tr>
<td>2</td>
<td>AVI-7539</td>
<td>40</td>
<td>VP35</td>
<td>8 (4/4)</td>
</tr>
<tr>
<td>3</td>
<td>AVI-6002</td>
<td>40</td>
<td>VP24, VP35</td>
<td>8 (4/4)</td>
</tr>
<tr>
<td>4</td>
<td>Saline</td>
<td>0</td>
<td>NA</td>
<td>6 (3/3)</td>
</tr>
</tbody>
</table>

*All animals were challenged with EBOV (Homo sapiens-tc/COD/1995/Kikwit) by the intravenous route and treated daily from day 0 to day 13. NA, not applicable.*
6002 combination (i.e., targeting both VP24 and VP35) compared to treatment with AVI-7539 alone or the saline controls (Fig. 2A). Similar to the BUN levels, both liver enzymes were significantly reduced (P < 0.05) in animals treated with AVI-7537 or the combination of AVI-6002 compared to treatment with AVI-7539 alone (Fig. 2B and C).

These data show that no antiviral synergy exists between AVI-7537 and AVI-7539 and, more importantly, reveal that targeting VP24 alone is sufficient to confer protection against lethal EBOV infection. This result is quite surprising, as VP35 is often viewed as an attractive therapeutic target due to its many critical roles in viral infection, and targeting VP35 in previous work conferred efficacy in vitro and in intraperitoneally infected rodent survival studies. Specifically, treatments with 20 mg/kg of AVI-7539, AVI-7537, and AVI-6002 led to 38%, 49%, and 83% survival, respectively (12). In contrast, in the present study, no protection against lethal EBOV infection was seen in the NHPs treated with AVI-7539. We postulate that these differences may be explained by increased sensitivity of mouse-adapted EBOV to IFN inhibition (13). In addition to its critical roles in immune evasion and host adaptation, recent studies have indicated that VP24 plays a larger role in the viral cycle than previously thought (7, 14, 15), indicating that VP24 is a viable therapeutic target. Indeed, the results of the present study suggest that impairment of VP24 alone is enough to protect against EBOV infection and that targeting VP24 may lead to the development of more effective countermeasures against this important viral pathogen. Furthermore, AVI-7537 has recently been shown to be safe and well tolerated in humans (16) and should be further developed as an effective EBOV therapeutic.

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Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

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