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Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies

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ABSTRACT

Lassa fever is a significant health threat to West African human populations with hundreds of thousands of annual cases. There are no approved medical countermeasures currently available. Compassionate use of the antiviral drug ribavirin or transfusion of convalescent serum has resulted in mixed success depending on when administered or the donor source, respectively. We previously identified several recombinant human monoclonal antibodies targeting the glycoprotein of Lassa virus with strong neutralization profiles *in vitro*. Here, we demonstrate remarkable therapeutic efficacy using first-in-class human antibodies in a guinea pig model of Lassa infection thereby presenting a promising treatment alternative.

Lassa Fever (LF) is a major public health problem in Western Africa where hundreds of thousands of infections are estimated to occur annually. For those who present to a hospital for treatment and are referred to a ward specific for the treatment of viral hemorrhagic fevers, the mortality rate approaches 70% (Shaffer et al., 2014). Elevated mortality, environmental stability that favors artificial aerosol infection, and a dearth of medical countermeasures for LF also make Lassa virus a clear biodefense concern (Stephenson et al., 1984). The nucleoside analog ribavirin, convalescent sera, and supportive therapy are the only treatments options currently in use for LF. Safety profile concerns, lack of controlled and randomized clinical studies, and short window of efficacy for therapeutic ribavirin or convalescent sera use in LF patients support the need for development of alternative and robust therapeutic options (Bausch et al., 2010). Single monoclonal antibody therapies against viral agents have recently proven highly effective; however, only one has been approved for use in humans (Mejias and Ramilo, 2008). The 2013–2016 Ebola hemorrhagic fever epidemic in West Africa has, however, potentiated the accelerated development and clinical evaluation of ZMapp, a cocktail of three chimeric monoclonal antibodies against distinct epitopes on the Ebola virus glycoprotein (McCarthy, 2014).

Since 2008, the Viral Hemorrhagic Fever Consortium (http://www.VHFC.org) has concentrated efforts at characterizing protective or pathogenic roles of B cells in LF directly from human patients (Robinson et al., 2016). This work has resulted in the derivation of the largest known collection of fully human monoclonal antibodies (huMAbs) from convalescent West African LF patients. Here, we describe the protective efficacy of select LASV glycoprotein specific huMAbs *in vivo* where a panel of 11 huMAbs targeting the

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glycoprotein of Lassa virus (LASV) was evaluated in an outbred guinea pig (GP) model of LF based on their *in vitro* neutralization profiles.

Optimized bicistronic IgG1 backbone vectors for high level, transient expression in mammalian cells were used to generate huMAbs for therapeutic evaluation *in vitro* and in a GP model of LF. Scaled production of antibodies for *in vivo* studies is detailed in the supplementary materials. Antibodies for therapeutic studies were formulated in a buffer for injection at >20 mg/mL, and assayed for purity, concentration, identity, and endotoxin levels prior to lot release.

A panel of 125 independently derived LASV glycoprotein-specific huMAbs were extensively characterized for binding, epitope grouping, and neutralization potential *in vitro* (Robinson et al., 2016). Eleven huMAbs consistently registered greater than 50% neutralization in a lentiviral entry assay system pseudotyped with the LASV (Josiah strain, Clade IV) GPC, a lymphocytic choriomeningitis virus (LCMV) backbone pseudotyped with LASV GPC, or in a live LASV plaque reduction neutralization test (PRNT) (Robinson et al., 2016).

Antibodies segregated into two distinct performance clusters of IC50 and IC80 values (Supplemental Table 1, Fig. 1A, B). Neutralizing antibodies (NAbs) 37.2D, 8.9F, 12.1F, 37.7H, 25.6A, 36.1F, and 25.10C clustered in the lowest combined Log10 IC50 (Fig. 1A.) and Log10 IC80 (Fig. 1B.) values range. A second cluster of NAbs comprised of 19.7E, 10.4B, 37.2G, and 2.9D recorded significantly combined higher Log10 IC50 and Log10 IC80 values range (Fig. 1A, B). Ten of the 11 huMAbs bound mammalian cell-generated LASV glycoprotein immobilized on ELISA plates. The single exception, huMAb 8.9F was identified via a pseudovirus neutralization assay performed as previously reported (Robinson et al., 2016), and did not bind purified glycoprotein in ELISA.

Binding efficacy to different clades of LASV was assessed using flow cytometry to detect the glycoprotein complex (GPC) genes of LASV Josiah (Clade IV), and Nigerian strains NigA19-08 (Clade III) and NigA18-237 (Clade II) which were transiently expressed on the surface of transfected HEK-293T/17 cells for 24 h prior to flow cytometric analysis (FACS). Histograms were generated that display the log fluorescence intensity for each antibody bound to GPC on the surface of live transfected cells. All 11 huMAbs bound to HEK-293T/17 cell surface-expressed LASV GPC by flow cytometry (Fig. 1C). Three LASV huMAbs (8.9F, 12.1F, and 37.2D) also bound cell surface expressed GPCs from Clade II (strain Nig A18-237) and III (strain Nig A19-08) strains (Fig. 1C, D). These binding profiles suggest that these 3 LASV huMAbs may have therapeutic utility across LASV Clades. These data guided the identification and prioritization of leading candidate therapeutic huMAbs for *in vivo* studies.

Animal studies were completed under biosafety level (BSL)-4 biocontainment at the Galveston National Laboratory and were approved by the University of Texas Medical Branch Institutional Laboratory Animal Care and Use Committee. Female outbred Hartley strain GP (~350–400 g, ~5–6 weeks old, Harlan Sprague Dawley, Houston, TX) were acclimatized for ~1 week prior to challenge intraperitoneally (IP) with 1000 plaque forming units of guinea pig adapted (GPA) LASV-Josiah (N = 5/treatment group). The GPA LASV infection model of outbred Hartley GP has been described recently for testing therapeutics against LASV (Safronetz et al., 2015). The advantage of using outbred animals to model human infection is inferred from the higher variability of immune responsiveness inherent in outbred populations allowing for increased countermeasure testing stringency versus those inbred for genetically tractable immune deficiencies which may, in turn, result in reduced predictive efficacy of the treatment in question by only testing a single immune phenotype rather than multiple at one time, as would be present in an outbred test population.

Animals were administered huMAbs (30 mg/kg of body weight) IP immediately after challenge (day 0) and on days 3 and 6 post infection (PI). Control animals were not treated. Guinea pigs were monitored for clinical observations, appearance, behavior, activity, and signs of paresis. Clinical scores were determined as Bright, Alert, Responsive = 0, Rough = 4, Sick = 8, Moribund = 12, or Expired = 16 over the course of 28 days after which survivors were euthanized at the study endpoint per IACUC protocol. Animals which were scored rough had roughed fur, delayed responsiveness, and some evidence of weight loss. Sick animals had marked lack of grooming, unstable gait, and clear evidence of weight loss. Blood was collected into EDTA containing tubes on days 7 and 14 and viremia was assessed from separated plasma using classical titration methods as outlined in supplemental materials. Kaplan-Meier survival curves were generated in Prism 5 and survival curves were compared using Log-rank (Mantel-Cox) test (GraphPad Software, La Jolla, CA) for treated groups versus untreated controls for each study (Fig. 2A).

Eleven LASV huMAbs tested in a Hartley GP model of LF segregated the antibodies into three distinct protection groups. One group of huMAbs, 25.6A, 2.9D, 8.9F, 12.1F, and 37.7H conferred 100% protection and no change in clinical score in GPs. In a second group, 37.2D, 19.7E, and 37.2G protected 80–90% of animals. A third group comprised of 10.4B, 25.10C, and 36.1F, conferred 40, 30, and 20% protection, respectively. An irrelevant recombinant human isotype control (IgG1) antibody did not confer protection (0% survival). From the tested antibodies, no significant differences were observed between protection level and epitope groups; however, more antibodies from each respective treatment group will need to be tested in order to verify the potential importance of any one particular epitope group in regard to protection (Supplemental Table 1).

The combined *in vitro* IC50 and IC80 data generally correlated well with protection *in vivo* for the top tier antibodies (100% protection). All but 2.9D neutralized pseudovirus in the nanomolar range, and registered a PRNT IC50 mean of 13.6 nM. Despite conferring 100% protection in GP huMAb 2.9D registered high IC50 values *in vitro* (Supplemental Table 1). 37.2D and 19.7E generated distinct IC50 values in pseudovirus assays, with the former neutralizing in the low nanomolar range, and the latter at >20 nM. Both antibodies neutralized LASV in PRNT at IC50 ~ 18 nM, and conferred 90% overall protection in GPs. It should be noted that 19.7E and 37.2D antibodies conferred 100% protection yet showed some clinical signs in an initial study (5/5) so a repeat study was performed where 80% (4/5) survived and similar clinical values were observed.

37.2G protected 80% of animals tested, despite high IC50 values in pseudovirus and PRNT assays (16.0 and >100 nM, respectively). 10.4B neutralized poorly in all assays and conferred only 40% protection in GPs. Surprisingly, the most potent antibody identified *in vitro*, 25.10C, with subnanomolar activity in all pseudovirus platforms, and 6.24 nM in PRNT, conferred an average 30% (3/10) protection in GPs. In our initial assessment, 25.10C protected 40% (2/5) of GPs, yet only 20% (1/5) in a repeat study. The difference in results could be attributed to inherent immune variability of outbred animals where differences in individual's susceptibility to the virus contributes to disease course. Importantly, these data indicate that *in vitro* neutralization assays have value in selecting antibodies with protective efficacy *in vivo*, but are not necessarily predictive of levels of protection.

Viremia was compared by Kruskal-Wallis test supported by Dunn's Multiple comparison posttest (Prism 5, GraphPad Software, La Jolla, CA) to detect differences from the control group for time points relevant to onset (day 7) or peak viremia (day 14) as determined from historical data (Geisbert, T.W. unpublished data).