Effects of Gamma Irradiation of Human Serum Samples from rVSVΔG-ZEBOV-GP (V920) Ebola Virus Vaccine Recipients on Plaque-Reduction Neutralization Assays

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Abstract. Gamma irradiation (GI) is included in the CDC guidance on inactivation procedures to render a group of select agents and toxins nonviable. The Ebola virus falls within this group because it potentially poses a severe threat to public health and safety. To evaluate the impact of GI at a target dose of 50 kGy on neutralizing antibody titers induced by the rVSVΔG-ZEBOV-GP vaccine (V920), we constructed a panel of 48 paired human serum samples (GI-treated versus non-GI-treated) from healthy participants selected from a phase 3 study of V920 (study V920-012; NCT02503202). Neutralizing antibody titers were determined using a validated plaque-reduction neutralization test. GI of sera from V920 recipients was associated with approximately 20% reduction in postvaccination neutralizing antibody titers. GI was not associated with any change in pre-vaccination neutralizing antibody titers.

INTRODUCTION

Recombinant vesicular stomatitis virus–Zaire Ebola virus envelope glycoprotein (rVSVΔG-ZEBOV-GP) vaccine (V920) demonstrated robust immunogenicity in early-phase studies in Europe, North America, and Africa in countries unaffected by the 2013–2016 Ebola outbreak.^{1–4} Robust vaccine immunogenicity was also observed in phase 2/3 studies in African countries directly affected by the outbreak,^{5,6} where one phase 3 study demonstrated vaccine efficacy.⁷

In 2019, the European Medicines Agency granted conditional marketing authorization for V920 (ERVEBO[®], Merck & Co., Inc., Kenilworth, NJ; November 11),⁸ prompting WHO prequalification (November 12)⁹ and Food and Drug Administration approval (December 20).¹⁰ In Africa, V920 has been registered by health authorities within the Democratic Republic of the Congo, Burundi, Ghana, and Zambia; efforts to obtain licensure for V920 use in additional African countries affected by Ebola virus are ongoing.¹¹

The CDC guidance on inactivation procedures to render select agents nonviable includes gamma irradiation (GI) and outlines the need for validated processes suitable for clinical samples potentially infected with select agents before shipping, processing, and clinical testing.¹² Given its potential as a severe threat to public health and safety, Ebola virus falls within this group of biological select agents and toxins.

Two independent, prospective studies evaluated the GI effect (target dose: 50 kGy) on antibodies against recombinant ZEBOV GP in a validated ELISA, using pre- and post-V920 vaccination serum samples from participants in a North American phase 1 clinical study.¹³ GI resulted in approximately 20% higher and 20% lower antibody concentrations in pre- and postvaccination samples, respectively. We concluded the decreased probability of transmitting viable Ebola virus to testing personnel far outweighs the effect on antibody titer.

In this study, we evaluated the GI impact (same target dose of 50 kGy) on neutralizing antibody titers in human serum samples from a phase 3 study conducted in North America and Europe, as assessed by a validated plaque-reduction neutralization test (PRNT).

METHODS

This prospective study investigated any measurable change in neutralizing antibody in serum following GI treatment versus non-GI-treated aliquots using validated PRNT. A panel of paired samples from 48 participants (GI-treated versus non-GI-treated; 96 total samples) from a phase 3 study of the V920 vaccine in healthy adults from the United States, Canada, and Spain (NCT02503202)^{14,15} was evaluated. Samples were selected to provide a broad range of neutralizing antibody titers, based on their historic PRNT result, and included 12 historic pre-vaccination and 36 historic postvaccination samples (prior PRNT performed by Q² Solutions [San Juan Capistrano, CA]; Supplemental Table 1).

Full details on sample preparation, handling, and shipping were described previously.¹³ In brief, serum samples were prepared at Merck & Co., Inc. testing lab (West Point, PA) before shipping and/or treatment. Duplicate tubes from each sample were placed in separate boxes (Supplemental Figure 1); Box 1 was shipped on dry ice to Sterigenics (Corona, CA) for GI treatment at a target dose of 50 kGy, as described previously,¹³ and subsequently shipped on dry ice to Q² Solutions. A replicate untreated box (Box 2) was shipped directly to Q² Solutions on dry ice.

Neutralizing antibody titers were measured at Q² Solutions using the same PRNT reported previously.¹⁶ The assay uses rVSVΔG-ZEBOV-GP as the target virus, and was developed and validated to quantify neutralizing antibodies post-V920 vaccination.

Serum was serially diluted and mixed with an equal volume of live rVSVΔG-ZEBOV-GP for final dilutions (range: 1:10–1:20, 480). Internal quality controls were included. Neutralization proceeded over 18 hours at 2–8°C, after which the serum/virus mixture was used to inoculate Vero cells. Viral adsorption was performed at 37 ± 2°C for 60 minutes, after which a methylcellulose overlay was added. Infected cells were incubated at 37 ± 2°C 5% CO₂ for 2 days. Plaques were visualized by crystal violet stain and counted automatically using an AID Reader (Autoimmun Diagnostika GmbH, Strassberg, Germany); neutralizing titers were calculated based on the percent reduction in viral plaques in the presence of serum versus virus control without serum. Results are reported as plaque-reduction neutralization of 60% (PRNT₆₀), with the reciprocal serum dilution resulting in a 60% reduction in plaque number. The PRNT has completed validation, and the report has

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undergone Center for Biologics Evaluation and Research review. PRNT₆₀ was used because of improved total assay precision over a less conservative endpoint of plaque-reduction neutralization of 50% (PRNT₅₀).

Geometric mean fold differences in titer between GI and non-GI and 95% CIs were estimated and statistically assessed using a paired *t*-test. The correlation between assay measures was estimated by the Pearson correlation coefficient and Lin's coefficients for accuracy and concordance, respectively.^{17,18} To assess if GI effect is dependent on the titer level, the natural log-transformed GI titers were regressed against the natural log-transformed non-GI titers using errors-in-variables regression.¹⁹

Qualitative comparisons between assay methods were based on 2 × 2 cross-classification tables about the PRNT limit of detection (LOD) of 20. From the 2 × 2 cross-classification tables, the agreement rate (proportion of double-positive and double-negative samples relative to the total number of samples) was reported. The 95% CI for the estimated agreement rate was computed based on the binomial distribution, and imbalance in the distribution of discordant samples was assessed using an exact McNemar's test.

RESULTS

Figure 1 displays the V920 PRNT titers, and Table 1 shows the statistical comparisons between antibody titers of GI-treated samples and corresponding non-GI samples. On average, GI was associated with a small, but statistically significant, reduction (approximately 20%) in postvaccination PRNT titers (1.19-fold reduction; 95% CI: 1.06–1.34). Fold reduction in titer and its 95% CI are the reciprocals of the geometric mean fold ratio in titer (GI/non-GI) and its 95% CI (Table 1). The proximity of the estimated concordance slope to

1.0 (95% CI: 0.85–1.09) suggests the effect is fairly constant throughout the positive titer range evaluated. The closeness of Pearson's correlation coefficient (0.94), Lin's accuracy coefficient (0.98), and Lin's concordance correlation coefficient (0.93) to 1.0 provides additional support that GI effect is small and consistent.

Qualitative comparisons between the two procedures used for GI and non-GI sample sets showed little discordance. Based on the 2 × 2 cross-classification table about the PRNT LOD of 20, all 36 of the historic postvaccination samples had detectable titers (i.e., were positive) in both GI and non-GI conditions (Supplemental Table 2). Similarly, none of the 12 historic pre-vaccination samples had detectable titers (i.e., no negative samples became positive with GI).

DISCUSSION

We have described a controlled inactivation procedure enabling the high-throughput treatment of potentially infectious clinical specimens before performing immunogenicity assays essential to support clinical trials in a vaccine program.

Our results suggest GI of pre-vaccination samples is not associated with detectable changes in neutralizing antibody titers, as measured in the validated PRNT; none of the 12 non-GI samples with neutralizing antibody titers less than the PRNT LOD had detectable titers following GI. Furthermore, GI of postvaccination sera across a wide range of titers was associated with consistently minor decreases in neutralizing antibody titers. Results are consistent with the previously reported effect on antibody concentrations using ELISA with postvaccination antibody-positive sera.¹³

An explanation for why antibodies in pre-vaccination samples are increased with GI for ELISA but not PRNT while measurements in postvaccination samples are decreased

FIGURE 1. Concordance plot displaying the effect of GI on neutralizing antibody titer of human clinical samples from vaccinated subjects as measured in the V920 PRNT. ^aFor ease of data visualization, pre-vaccination samples (titers < 20), which were excluded from the analyses, were randomly assigned a titer close to 10. GI = gamma irradiation; LOD = limit of detection; PRNT = plaque-reduction neutralization test; rSVΔG-ZEBOV-GP = recombinant vesicular stomatitis virus–Zaire Ebola virus envelope glycoprotein; V920 = rSVΔG-ZEBOV-GP vaccine.

TABLE 1
Summary measures of agreement between GI and non-GI procedures

Concordance parameter	N	Estimate or P-value	95% CI
Serostatus agreement (%)		100	92.6–100
Discordance imbalance P-value	48	1.0000	
Geometric mean fold ratio in titer (GI/non-GI)		0.84	0.75–0.95
Pearson's correlation coefficient		0.94	0.89–0.97
Lin's accuracy coefficient		0.98	0.97–0.99
Lin's concordance correlation coefficient	36	0.93	0.87–0.96
Concordance slope		0.96	0.85–1.09
Concordance intercept		1.07	0.51–2.26

GI = gamma irradiation.

with GI for both ELISA and PRNT is that for ELISA, GI may break down cellular debris or cause nonspecific antibodies, present in all normal human sera, to fragment. Without competition from specific rVSVΔG-ZEBOV-GP antibodies, debris or fragments could nonspecifically bind ZEBOV-GP coating antigen, producing a false-positive signal above lower limit of quantitation. In postvaccination sera, nonspecific binding would be overwhelmed by specific antibodies with higher affinity, some of which are also broken down by GI, as evidenced by slightly impaired specific binding. Plaque-reduction neutralization test, however, measures functional neutralizing antibody not expected to have nonspecific activity, and only demonstrates loss of specific activity after GI.

As described previously,¹³ study limitations include use of specimens from North American and European subjects with minimal pre-vaccination antibody levels; potential GI impact on sera from subjects living in regions where Ebola circulates, who may have higher baseline antibody titers, was not evaluated. Future studies using sera from subjects with possible current or prior wild-type Ebola virus infections would require appropriate biosafety level (BSL) laboratory conditions.

This GI study may support future vaccine trials in regions where BSL 3/4 organisms require sample testing. GI may provide additional safety for laboratory personnel against lethal pathogens present in study samples.¹² Current and prior results indicate GI effect is small and consistent across a range of antibody titers.¹³ Assuming all study samples receive the same GI treatment, our results suggest GI effect is unlikely to bias treatment comparisons within a study.

CONCLUSION

GI of V920-vaccinated subject sera was associated with approximately 20% reduced neutralizing antibody titers as measured by PRNT postvaccination and was not associated with any change in neutralizing antibody titers pre-vaccination.

GI is recommended to reduce the transmission of viable Ebola virus to testing personnel when specimens may contain wild-type virus. Any GI impact on immunogenicity should be considered when interpreting PRNT data.

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