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Adjuvanting of Fluzone® with JVRS-100 in Mice, Rabbits and Non-Human Primates Demonstrates Increased Immunogenicity and Dose-Sparing

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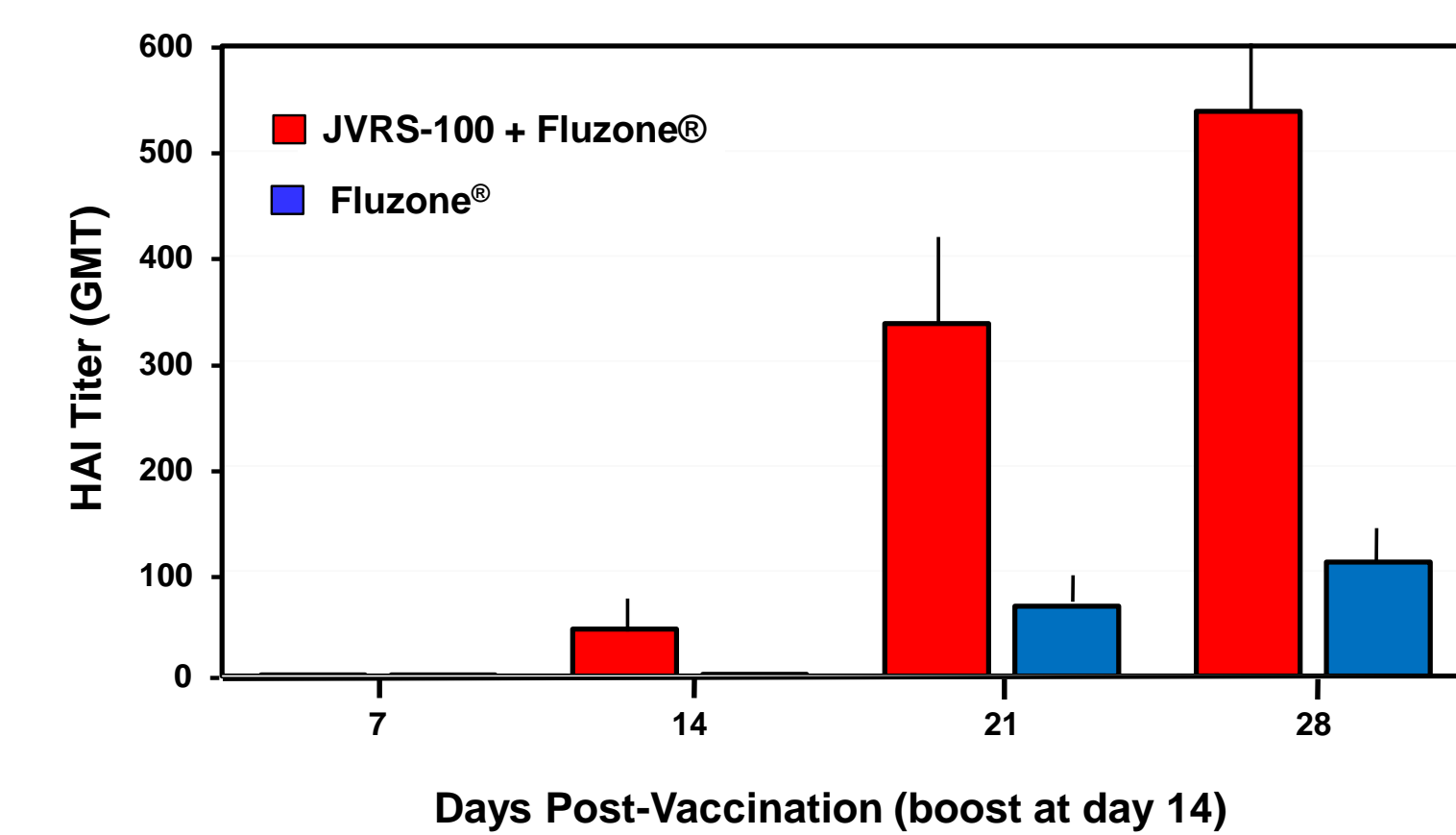
INTRODUCTION

JVRS-100 (lipid-DNA complexes) is a unique and promising adjuvant for vaccine applications that require high levels of antibody and T-cell immunity. The JVRS-100 adjuvant was mixed with a split influenza vaccine (Fluzone®, Sanofi Pasteur) and administered either subcutaneous to mice, or intramuscular to rabbits and non-human primates. Vaccination with JVRS-100-Fluzone® resulted in a significant increase in total IgG, IgG1 and IgG2a influenza antibodies. Furthermore, hemagglutination inhibiting (HAI) antibodies were higher compared with Fluzone® alone. Administration of decreasing amounts of Fluzone® mixed with JVRS-100 resulted in a ~50-fold dose-sparing effect based on HAI titer. In vitro stimulation of splenocytes from JVRS-100-Fluzone® vaccinated mice with Fluzone® demonstrated increased antigen-specific T cell responses (IFN-γ production) compared with Fluzone® alone. Splenocytes from JVRS-100-Fluzone® vaccinated mice responded to unmatched H1N1, H3N2, and influenza B viruses, suggesting induction of cross-reactive T cell responses to conserved viral antigens. Analysis of T-cell responses from vaccinated non-human primates demonstrated significant enhancement of both interferon-gamma positive and IL-2 positive cells (intracellular cytokine staining) indicating the enhancement of both primary and memory responses to influenza antigens.

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RESULTS

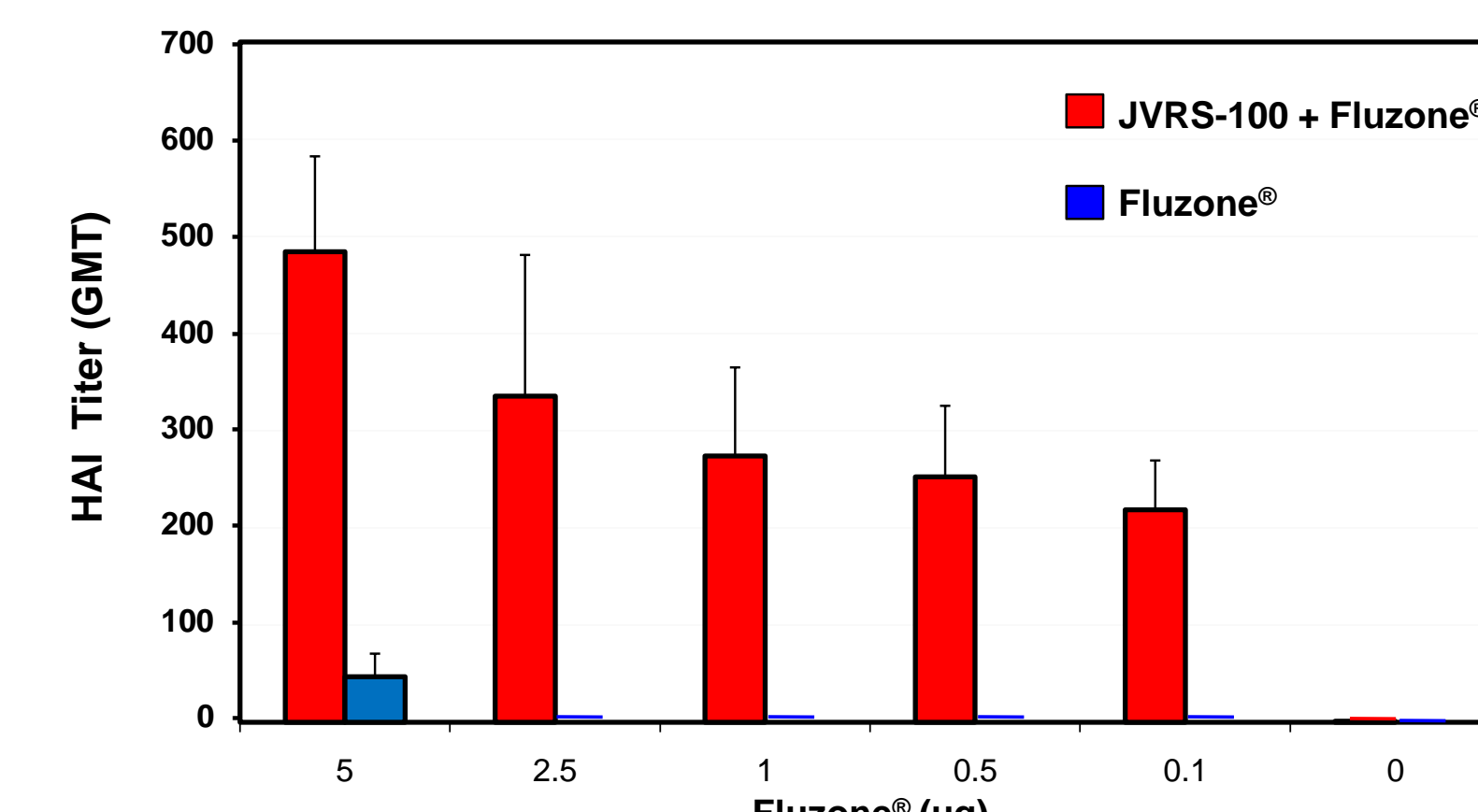
JVRS-100/Fluzone® Vaccination Mouse HAI Antibody Kinetics



Vaccination = Days 0 & 14
Assay = Day 28

CD-1 mice were administered 5ug Fluzone® with or without 20ug JVRS-100 adjuvant on day 0 and 14. Sera were collected at day 7, 14, 21, and 28 for hemagglutination inhibiting (HAI) antibody determination (using Fluzone® as the antigen)

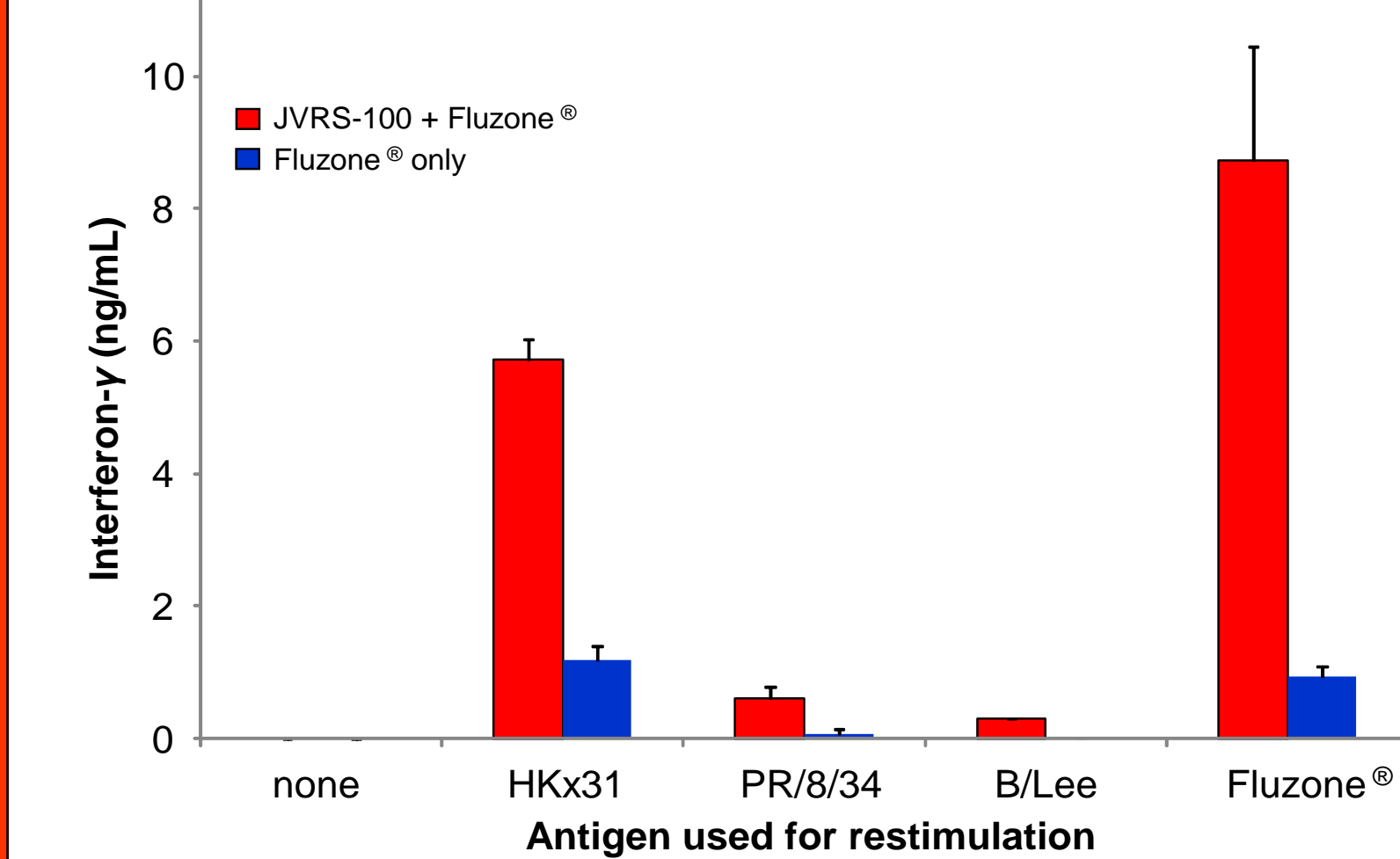
JVRS-100/Fluzone® Vaccination Fluzone® Dose Titration



Vaccination = Days 0 & 14
Assay = Day 28

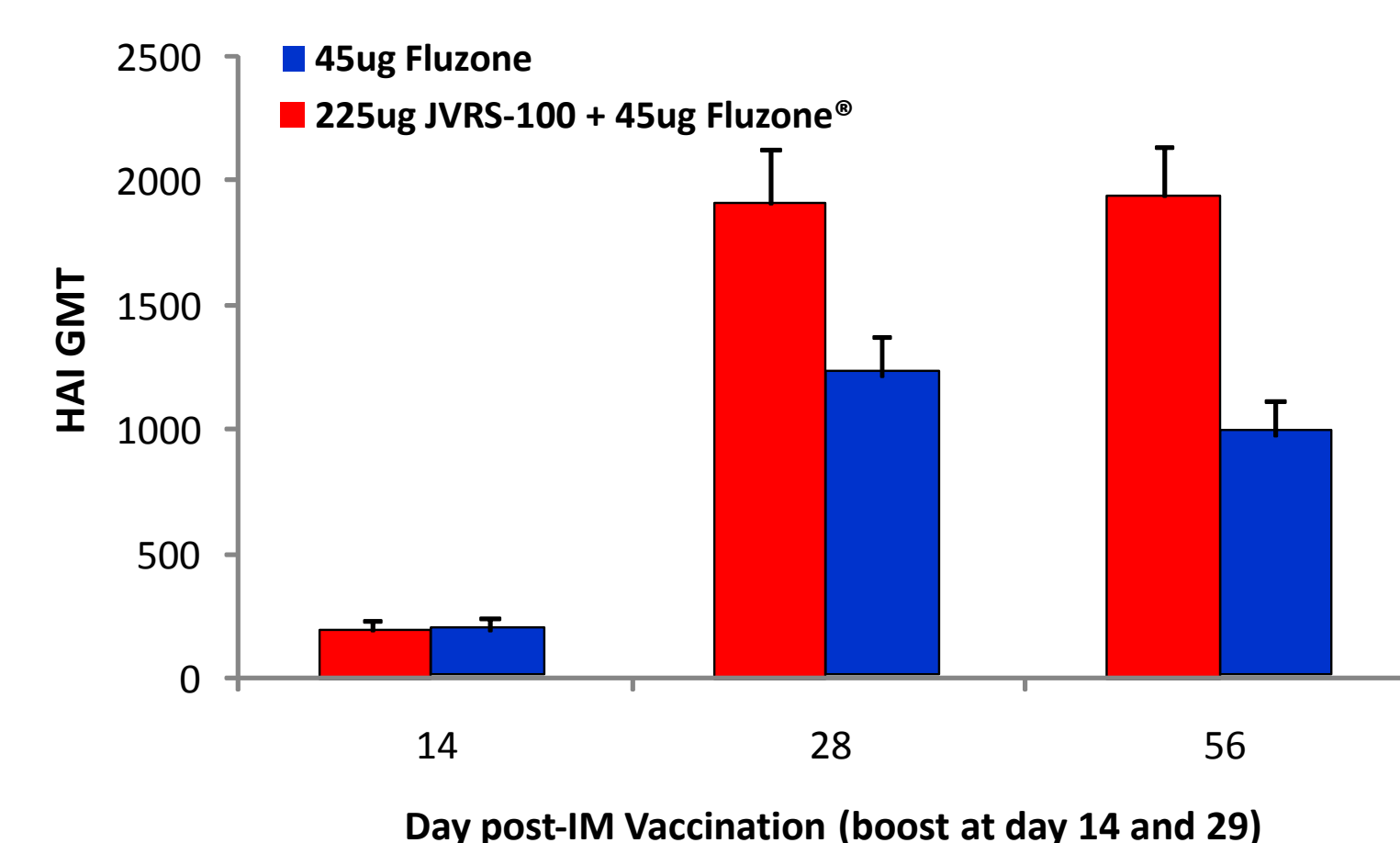
CD-1 mice were administered 20µg of JVRS-100 with 5.0, 2.5, 1.0, 0.5, and 0.1µg of Fluzone® on day 0 and 14. Sera were collected on day 28 for hemagglutination inhibiting (HAI) antibody determination (using Fluzone® as the antigen).

JVRS-100/Fluzone® Results in More Robust Heterotypic and Homotypic T-Cell Responses



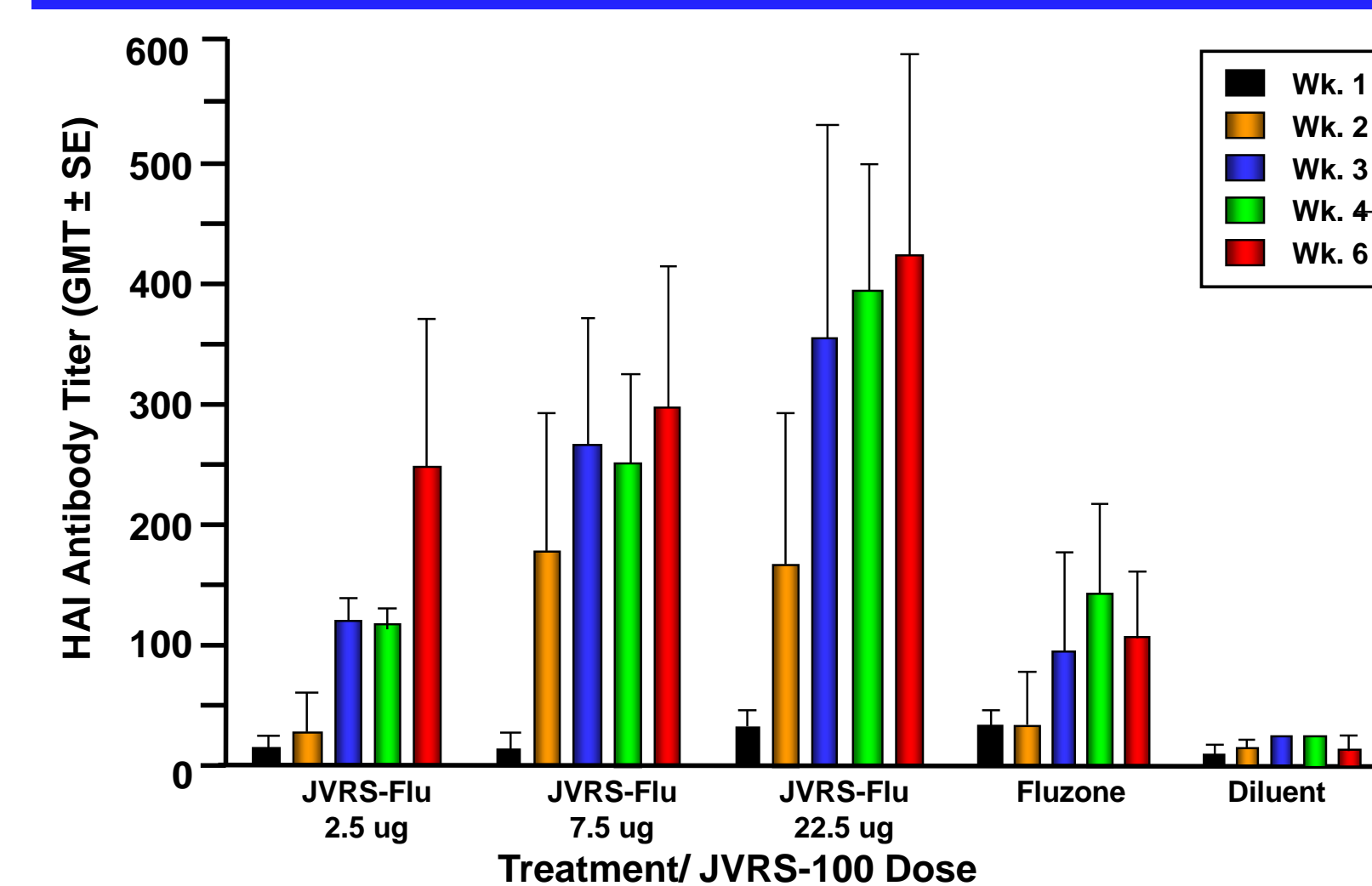
CD-1 mice were administered 5ug of Fluzone® with 20ug JVRS-100 on day 0 and 14. Splenocytes were isolated on day 28 and restimulated with either HKx31, PR/8/34, B/Lee or pediatric Fluzone®.

JVRS-100 Enhances Anti-Fluzone® Responses in Rabbits



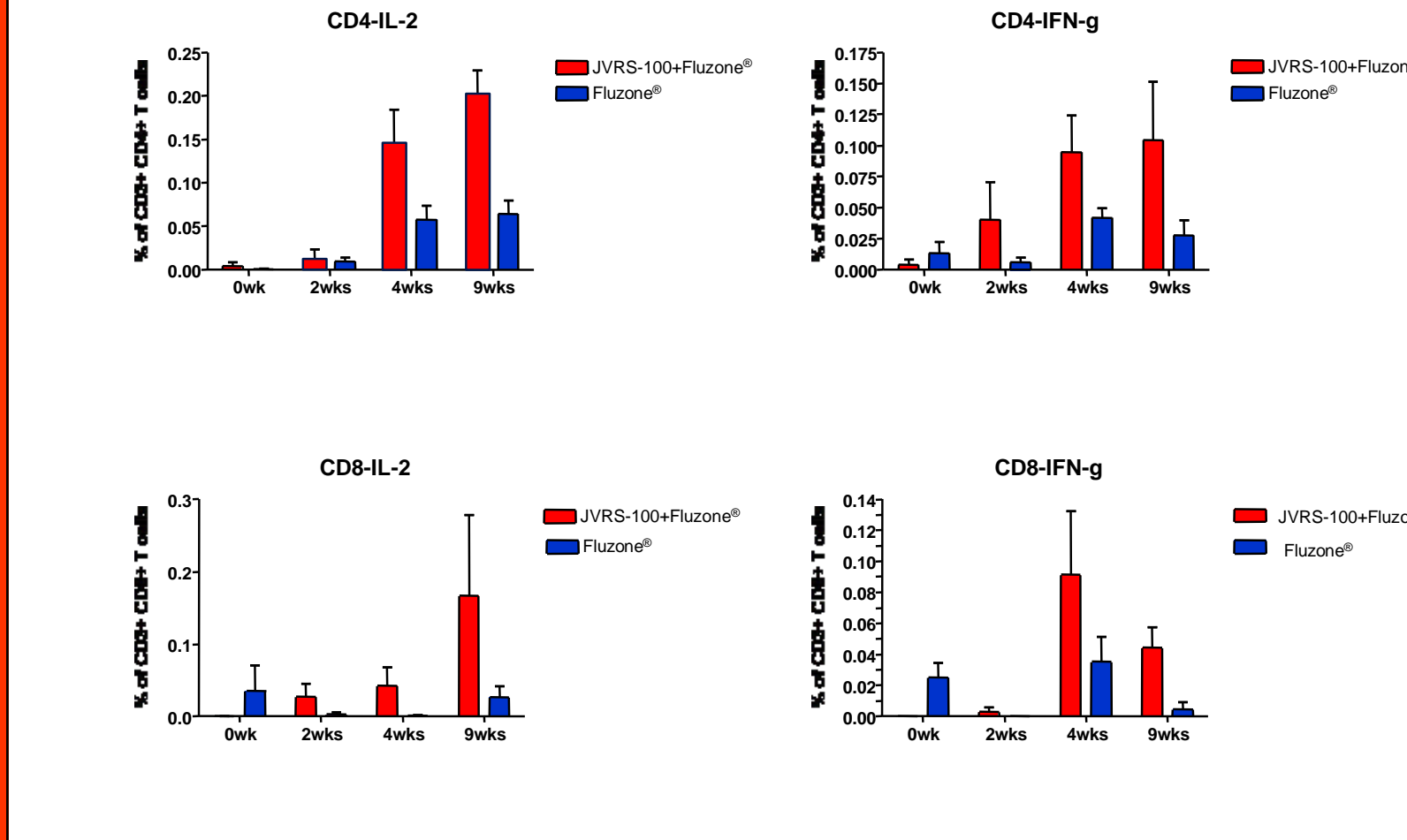
Vaccine was administered at day 0, 14, and 29 and HAI response quantitated at day 14, 28, and 56. The administration of JVRS-100 with Fluzone® increased the antibody response in rabbits both in magnitude and duration.

JVRS-100 (graded doses)/Fluzone® (22.5µg) Vaccination Results in Increased HAI Titers in Non-Human Primates



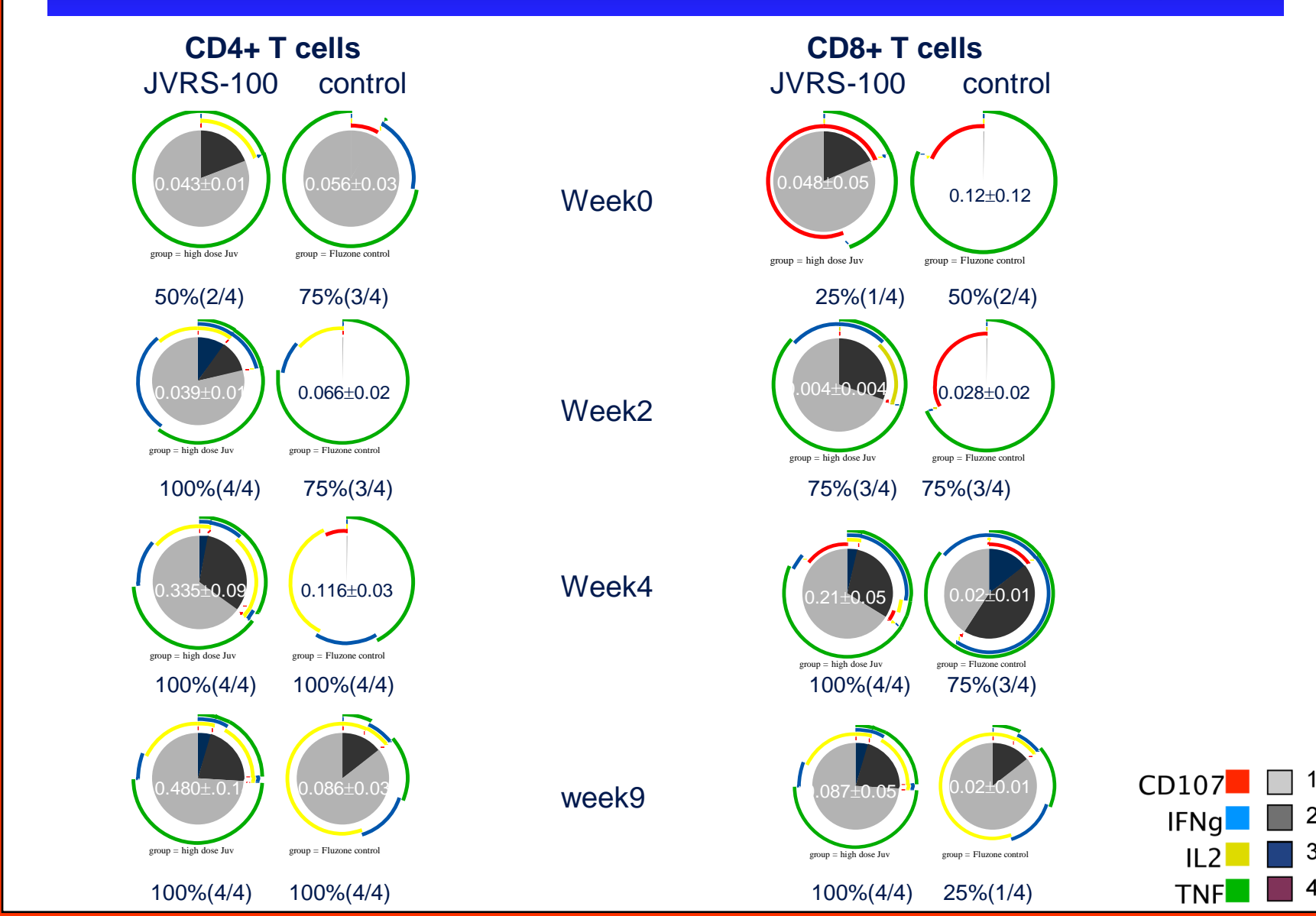
A dose-ranging study was conducted in non-human primates (*Macaca mulatta*). Groups of macaques (n=4) were vaccinated on day 0 and 14 (IM) with graded doses of JVRS-100 adjuvant mixed with a fixed dose (22.5µg) of Fluzone® vaccine.

Fluzone® Specific T-cell Responses in High Dose JVRS-100 vs. No Adjuvant in Non-Human Primates



PBMC samples from non-human primates were restimulated with pediatric Fluzone®, blocked with brifeldin A and analyzed for intracellular accumulation of interferon-gamma or IL-2 as an indicator of primary or memory immune response.

Fluzone specific T cell responses in high dose JVRS-100 and no adjuvant animal groups



Mean influenza specific T cell responses in non-human primate PBMC before and after Fluzone® immunization with or without JVRS-100 adjuvant. The pie charts summarize the mean T cell responses and the extent to which the CD4+ and CD8+ responses were polyfunctional. For each pie chart the percentage (and proportion) responding is indicated below the chart. Mean frequency (± SEM) of each response is shown as a white number in the middle of the pie chart; only animals with a positive response are included. Each portion of a pie chart indicates the percentage of Fluzone-specific T cells that responded with one, two, three, or four functions; and the arcs around the pie show the function or combination of functions to which the specific response corresponds (see color legend).

CONCLUSIONS

These results suggest that use of the JVRS-100 adjuvant enhances immune responsiveness and reduced antigen doses needed for strong immune responses to a licensed flu vaccine. The JVRS-100 adjuvant has also been shown to potentiate immune responses to multiple viral and bacterial antigens, and could be a broadly applicable adjuvant for human and veterinary vaccines.