Author's response to reviews

Title: High titer West Nile IVIG from selected donors for treatment of West Nile infection

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High titer West Nile IVIG from selected donors for treatment of
West Nile virus infection.

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Abstract

**Background:** West Nile Virus (WNV) is endemic in Israel and a significant level of antibodies is present in the population due to natural exposure. Anecdotal cases suggested that the presence of anti-WNV antibodies, in IVIG from Israeli donors assisted the recovery of patients with severe WNV infection.

**Methods:** In order to enhance the therapeutic efficacy of IVIG against WNV infection, OMRIX strategized for selection of plasma units from a 10% fraction of blood donors with anti-WNV antibodies. Positive units were processed into a pharmaceutical grade WNV IVIG (WNIG). Following inoculation with WNV, mice received i.p. injections of different doses (0.01-8mg/mouse) of IVIG or WNIG according to the specific experimental protocol.

**Results:** WNIG was about 10 times more potent than regular IVIG when tested by ELISA and neutralization assay. In a mouse lethal model WNIG was at least 5-10-fold more potent compared to regular IVIG, in a prophylactic model. Treatment with WNIG during active encephalitis, three or four days after challenge had a significant protective effect. WNIG was also very effective for protection of immunosuppressed mice. Treatment of dexamethasone-immunosuppressed mice with 0.2 or 1.0 mg WNIG 4h after virus infection led to 100% survival.

**Conclusion:** IVIG produced from selected plasma donated in WNV endemic regions can be used to produce WNV IVIG with superior activity for therapy and prophylactic measures.
Background

Passive transfer of antibodies has been shown to be effective for prevention and treatment of many infectious diseases including those caused by viruses [1]. Intravenous human immunoglobulin produced from pooled plasma (IVIG) is the major source for antibody therapy by virtue of the diverse repertoire of immunoglobulins which include a wide spectrum of antibacterial and antiviral specificities [2].

Pooled plasma of subjects that were naturally exposed to pathogens has been used for production of IVIG preparations containing specific antibodies for treatment of viral diseases including Cytomegalovirus, Hepatitis A, B and C, HIV, Respiratory Syncytial Virus, Measles and Varicella Zoster virus [1].

West Nile virus (WNV), a mosquito transmitted flavivirus, was first isolated from a febrile adult woman in the West Nile District of Uganda in 1937 [3]. WNV is a single stranded plus RNA virus, a member of the Japanese encephalitis antigenic complex of the genus Flavivirus, family Flaviviridae [4, 5]. Until 1999, West Nile Virus was found in Africa, the Middle East, parts of Asia, Southern Europe and Australia. It then suddenly emerged in New York, and rapidly spread throughout the United States and since than has caused considerably acute mortality and morbidity [6]. The clinical manifestations in humans range from asymptomatic seroconversion to fatal meningoencephalitis with symptoms including cognitive dysfunction, muscle weakness and flaccid paralysis [7-10]. Depressed immunity, age and genetic factors [11, 12], correlated with greater risk for neurological disease. Currently there is no effective therapy or vaccine for WNV infection.

Evidence suggests that WNV might be more susceptible to antibody–mediated immunity than cell mediated immunity. Indeed, passive transfers of specific
antibodies (Ab) or immunoglobulin have been shown to abort or modify West Nile virus infections in animal models in a dose dependent manner [13-15]. WNV is endemic in Israel and significant levels of anti-WNV Ab are found in commercial preparations of IVIG prepared from plasma of Israeli donors. Anecdotal cases suggested that the presence of anti-WNV Ab, in IVIG from Israeli donors assisted the recovery of patients with severe infection [16, 17]. We have previously shown that Israeli IVIG protected mice against lethal doses of WNV while the low exposure to the virus among US donors resulted in no effect of US IVIG [13, 18]. Recently however, it has been shown that some IVIG preparations produced from plasma collected from US donors during the epidemic years had antibodies against WNV and thus was protective in an animal model for WNV infection [19].

In order to enhance the therapeutic efficacy of IVIG against WNV infection, OMRIX strategize for selection of plasma units from the 10% fraction of blood donors with WNV antibodies. Positive units were processed into a pharmaceutical grade WNV IVIG (WNIG). The potency of WNIG for neutralization of WNV NY99 strain was tested in vitro by cell neutralizing assay and in vivo by using a mouse lethal model. WNIG was at least 5-10-fold more potent compared to regular IVIG. Treatment with WNIG three or four days after challenge was also efficacious. We conclude that blood from selected donors in WNV endemic regions improve the potency of IVIG and should be developed for use for therapy and prophylactic measures.
Methods

Mice
Female BALB/cOlaHsd mice (Harlan Laboratories, Israel) were used at the age of 4-5 week (15-17 gr) unless otherwise stated. Mice were maintained in isolation cages throughout the study and fed and watered ad libitum. The mouse experiments were approved and performed according to the Kimron Veterinary Institute guidelines for animal experimentation with arboviruses.

Cell Cultures
Vero cell line (ATCC #CRL-1587) were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1% nonessential amino acids, 1.2% NaHCO₃ and antibiotics. The cells were maintained in a humidified atmosphere at 37°C, 5% CO₂ and were used for growing virus stocks and virus titration.

Virus stocks and virus titrations
WNV, 3rd passage in Vero cells of strain NY 385-99 (WNV-NY99) [20], was kindly provided to Omrix, by M.S. Diamond, Washington Univ., School of Medicine, St. Louis, USA. Virus plaque assays were performed on Vero cells as previously described [21] and virus titers were expressed as plaque-forming units (PFU) /ml. A single virus stock containing 5x10⁸ PFU/ml was stored in aliquots at -70°C and was used in all studies.

Infection and challenge of mice
In all experiments (unless otherwise stated), 4-5 week old mice were inoculated intraperitoneally (i.p.) with 0.2 ml/mouse containing different quantities of stock virus. Lethal dose 50 (LD₅₀), was calculated according to the method of Reed and Muench [22].
Immunoglobulin (IVIG) preparations

Omrix IVIG preparations (Omr-IgG-am™, OMRIX Biopharmaceuticals, Israel) contain 5% protein consisting more than 99% IgG and a very small quantities of IgA and IgM. Three IVIG preparations were used (Table 1): (a) High titer WNV-IVIG (WNIG), Batch #K44G511. This product was prepared during 2006 from Israeli plasma that was pre-screened by ELISA for specific anti WNV antibodies. About 10% of the plasma units from the general blood donor population had > 100 arbitrary units/ml (AU/ml) of anti-WNV antibodies. These units were pooled for the production of WNIG. (b) Commercial IVIG batch #H23131, prepared in 2003, from pooled plasma of Israeli blood donors. (c) Commercial IVIG (IVIG-US), Batch #K30G370, prepared in 2006 from pooled plasma of US blood donors.

Antigen preparation

Inactivated WNV antigen was prepared by using β-Propiolactone inactivation of the stock virus (0.001%final concentration) [18, 23].

Detection of anti-WNV antibodies by ELISA

a. ELISA for human antibodies

A quantitative ELISA was developed at Omrix based on the method described earlier [18]. To allow quantitative measurements, specific positive plasma sample was assigned a value of 2000 arbitrary units per ml (AU/ml) of WNV antibodies. This sample was used as a calibration standard. The range of 1–10 AU/ml was found to have linear characteristics when the log-transformation of concentration was related to the observed optical density at 405 nm. Briefly, microtiter plates were coated with inactivated WNV antigen in carbonate buffer, pH 9.6. The plates were than blocked with 0.5% I-block (Tropix, Bedford, MA) and 10% goat serum. Samples and controls were diluted in blocking buffer to fit the standard curve range. Alkaline phosphatase –
conjugated goat anti-human IgG (Sigma, Israel) was added as a detector followed by PnPp substrate (Sigma, Israel). The titer of each serum and control sample was calculated by using the absorbance at 405 nm plotted against the log transformation of calibrator concentration.

b. ELISA for mouse sera

ELISA test was performed according to the method described by Martin et al. [24] with slight modifications [18]. The specific titers were expressed as the reciprocal of the highest dilution giving a reading above the cut off [25].

**Plaque Reduction Neutralization Titer 50% (PRNT<sub>50</sub>)**

The titer of neutralizing antibodies was determined using a plaque reduction test based on the method described earlier [18]. Briefly, samples were diluted by serial two-fold dilutions (1:10 – 1:10240) in DMEM, 2% FCS and mixed with equal volume of similar medium containing approx. 450 pfu/ml of WNV NY99. The mixtures were incubated overnight at 4°C on a roller and the virus-antibody mixtures (400 µl) were then added onto Vero cells that were pre-seeded in 6-well plates at a concentration of 5x10<sup>5</sup> cells/well and pre-grown over night at 37°C, 5% CO<sub>2</sub>. After 60 minutes at 37°C, 5% CO<sub>2</sub>, the monolayers were overlaid with 3 ml of MEME x2 and tragacanth (Sigma, Israel) containing 4% FCS and 2.4% Sodium bi-carbonate. The cultures were incubated for additional 72 hours at 37°C, 5% CO<sub>2</sub>. The cells were then fixed with ethanol, stained with Fucsin, and plaques were counted. PRNT<sub>50</sub> were expressed as the reciprocal of the highest dilution giving 50% reduction in plaque numbers.

**Treatment with Immunoglobulins**

Following inoculation with WNV, mice received i.p. injections of different doses (0.01-8mg/mouse) of IVIG or WNIG according to the specific experimental protocol.
WNIG Therapy of immunosuppressed mice

Seven to 8 weeks old mice received two subcutaneous (s.c.) doses of dexamethasone (Sigma, Israel, 60µg/mouse each). First dose was given 2 hours before inoculation with 5-10 PFU of WNV-NY99 and a second dose was given 1 day thereafter. Four hours after inoculation with WNV the animals were treated i.p. with 1 mg or 0.2 mg/mouse of WNIG.

Statistics

Survival rates in different groups were compared by Fisher’s exact test. Average days for death were compared by Student’s T-test.
Results

The protective effects of WNIG against lethal WNV infection

To study the relative protective efficacy of WNIG compared to IVIG-IL or IVIG-US, mice were inoculated i.p. with 10-20 LD$_{50}$ (50-100 PFU) of WNV-NY99, followed 4 hours later by treatment with a single injection of 2, 0.5 or 0.1 mg of WNIG, IVIG-IL or IVIG-US. Treatment with 2 mg of WNIG/mouse or IVIG-IL was sufficient to confer 88-100% protection against 10-20 LD$_{50}$ of the virus (Table 2). As expected, IVIG-US showed lower protective efficacy, with only 63% survival after treatment with 2.0 mg/mouse. The superiority of WNIG was clearly demonstrated at a dose of 0.1 mg/mouse where IVIG-IL conferred a low 44% protection compared to 94% of WNIG.

These results suggest that the protective efficacy of 0.1 mg WNIG was similar to that obtained with 0.5 mg of IVIG-IL, i.e. at least 5 fold more efficacious. WNIG at 0.1 mg had at least similar efficacy as IVIG-US at 2.0 mg, i.e. about 20 fold more efficacious. Interestingly, when considering the total specific antibody units that were given to each animal, there was good correlation between WNIG and IVIG-IL such that 7-15 AU (IVIG-IL and WNIG respectively) were sufficient to give 87-94% protection. However, in the case of IVIG-US, 7 AU gave 63% protection, possibly pointing to qualitative differences between the specific antibodies in US and Israeli donors. The superiority of WNIG was also demonstrated by comparing the levels of viremia in mice treated with 0.1 mg IVIG or WNIG before inoculation with 50 PFU of WNV. IVIG treatment moderately decreased the virus levels in blood (compared to infected control mice) from 3.4 to 2.3 log$_{10}$ PFU/ml on day 2 and from 2.9 to 1.8 log$_{10}$ PFU/ml on day 3. Nevertheless, no virus was detected in blood of mice treated with WNIG.
The previous experiments showed that there is direct correlation between the quantity of antibody and the resulting protection. Therefore, we further investigated the therapeutic efficacy using lower doses of WNIG and IVIG-IL. Groups of mice were infected with 10 LD$_{50}$ (50 PFU) of WNV and treated 4 hours later with a single injection consisting of 0.05, 0.025 or 0.01 mg of WNIG or 0.25 mg of IVIG-IL. As shown in Figure 1, the ratio of 5-10 fold increase in protective efficacy between the WNIG and IVIG-IL was maintained by comparing treatment with 0.25 mg IVIG-IL to 0.05-0.025 mg WNIG. Nonetheless, mortality in these groups was delayed compared to that of the control infected mice. In the untreated WNV-NY99 infected mice, all of the deaths occurred until day 8 while in the IVIG treated mice mortality was delayed and occurred between days 8-15. Similar delayed mortality pattern was shown after treatment with 0.05 or 0.025 mg of WNIG or 0.25 mg of IVIG-IL.

**Detection of anti-WNV antibodies in surviving mice:** The induction de novo of anti-WNV antibodies in WNIG treated, surviving mice was tested 21, 40 and 60 days after challenge and treatment. Animals were challenged with 10 LD$_{50}$/mouse and 4h later were treated with WNIG or IVIG-IL (0.1-0.5mg/mouse). Pooled serum samples were collected from surviving mice on days 21, 40 and 60 after treatment and antibody titers were assayed by ELISA. The surviving animals developed high levels of anti WNV antibody titers ranging from 1:8,000 -1:16,000 which were maintained for at least 60 days.

**Therapeutic efficacy of WNIG:** In the mouse model, active encephalitis can be detected 3 days after challenge with WNV [18]. To study the therapeutic efficacy of WNIG, mice were injected with 10 LD$_{50}$ of WNV, and treated with 2 mg WNIG or IVIG-IL on days 2 and 4 or 3 and 5 after infection. As shown in Figure 2 injection of 2 mg of WNIG on days 2 and 4 after infection protected 100% of the animals
compared with 75% protection in mice receiving IVIG-IL. Treatment with WNIG on days 3 and 5 delayed significantly the mortality of the infected mice. In this group 62% of the animals survived for 17 days. The average period of death in the WNIG group treated on days 3 and 5 was 12.5 days (SD=5.7), while the average period of death in the IVIG-IL group treated 3 and 5 days after infection and in controls untreated mice, was 7.1 days (SD=1.1) and 6.6 days (SD=0.5) respectively (p=0.032 for WNIG compared to IVIG-IL; p=0.048 for WNIG compared to control group).

After demonstrating a delayed therapeutic effect of treatment with a dose of 2 mg/mouse, we assessed the therapeutic effect of a higher dose of WNIG. A single dose of 8 mg/mouse was given on days 3, 4, or 5 after infection. This dose protected all the animals when administered 3 days after infection (Table 3). When given 4 days after infection the survival rate was 63% compared to 25% in the non-treated control group. Treatment on day 5 after infection was not effective, suggesting that at this point the damage cause by the virus was irreversible.

**WNIG therapy of immunosuppressed mice:** Adult mice are relatively resistant to WNV but this resistance can be abolished by treatment with glucocorticoids such as dexamethasone [21, 26]. In this experiment we tested whether WNIG therapy can protect mice from WNV infection after treatment with dexamethasone. Seven to 8 weeks old mice were injected s.c with two doses of 60µg/mouse of dexamethasone. First dose was given 2h before, and second dose was given 1 day after infection with 5-10 PFU of WNV NY-99 strain.

Dexamethasone treatment increased the susceptibility of adult's mice to WNV thus lowering the survival rate from 58% to less than 19%. Treatment of dexamethasone - immunosuppressed mice with 0.2 or 1.0 mg WNIG 4h after virus infection led to 100% survival (Table 4).
**Discussion**

Antibody based therapy against viral and bacterial infections has been practiced successfully since the end of the 19th century [27]. Since the emergence of West Nile virus (WNV) as an important pathogen in a number of regions including the continental USA, the potential use of specific antibodies for protection and treatment of infections associated with WNV was studied using several animal models [13, 18, 28-30]. Previously, we showed by using a mouse model that commercial preparations of intravenous immunoglobulin (IVIG) from normal Israeli blood donors allows protection of mice against viral infection. The emergence of WNV in many regions of the continental USA after 1999 has allowed others to show the efficacy in animal models of IVIG produced from specific USA regions [19]. Using another approach, humanized monoclonal antibodies against specific epitopes in the viral envelope were developed and showed high potency in-vivo [31]. Nevertheless, it is easier to develop a new formulation which is derived from a known pharmaceutical product with an excellent safety profile such as IVIG [1]. Therefore, the use of IVIG with high WNV antibody levels for treatment of WNV infection is considered a leading approach. Intravenous immunoglobulin has become a first or second line treatment choice for many chronic inflammatory disorders, including disorders that involve the CNS. Although IVIG is commonly used for autoimmune diseases, it can be speculated that IVIG will have beneficial effect in infectious diseases such as WNV where encephalitis represents a major cause for severe symptoms including death [32]. Although the potential use of IVIG from regions endemic for WNV was reported, a serious limiting factors in applying this concept is the relatively low levels of specific antibodies, and very high batch-to-batch variance, especially in IVIGs that are
produced in countries such as the USA where the exposure of donors to WNV is limited and not homogenous [19]. Even in hyper endemic region such as Israel, only a fraction of the population has history of exposure to WNV [17]. Our finding that only 8-10% of the plasma units from the normal donor population contributed almost all of the WNV antibodies into the plasma pools allowed the development of highly effective treatment option for WNV. The IVIG produced from positive WNV antibody plasma, WNIG, showed promising potential in-vitro with about 10-fold higher WNV antibody levels compared to regular Israeli IVIG preparations which were produced during the same year. Tests in mice confirmed the ELISA and cell neutralizing assay by showing 5-10 fold enhancement of efficacy when compared to regular IVIG produced from Israeli donors and at least one log increase compared to IVIG produced from US donors. Our results suggest that there are qualitative difference between WNV specific antibodies from US and Israeli donors. We showed that 7-15 AU of antibodies from Israeli donors were more potent than 7 AU of antibodies originated in US donors.

However, the transport of antibodies across the blood brain barrier (BBB) is very inefficient and the possibility to treat active encephalitis is of major concern. It is speculated that during active disease the BBB becomes partially permeable thus allowing the penetration of significant quantities of antibodies to the infected site.

Nevertheless, the level of antibodies in the serum is directly correlated to the level in the brain thus the goal is to supply as much antibodies as possible, especially when trying to treat active disease. The protective effect of IVIG and monoclonal antibodies against infection that is already established in the brains was shown previously in animal models [15, 18, 28]. In human, intravenous immunoglobulin treatment was reported to be associated with improvement and elimination of signs and symptoms of
WNV infection [16, 17]. Our results support the superiority of WNIG over regular IVIG when given to mice even 3 or 4 days after infection, at a point where WNV has already established infection in the brain [18, 33]. Augmenting the level of antibodies against the WNV may prove to be very important treatment strategy for infection, particularly for elderly and those with immune systems deficiencies [34]. Immunocompromised subjects consists a major target population for passive antibody treatment or prophylaxis against WNV infection. It was shown that a broad range, non-specific immunosuppressant with cyclophosphamide increases the sensitivity of golden hamsters to WNV infection [35].

In an earlier paper it was shown that B-cell deficiency, alone or combined with T-cell deficiency increases the sensitivity of mice to WNV infection [28]. The natural resistance of adult mice to WNV infection is probably based on non-specific innate immunity mechanism since the development of specific immunity is longer than the critical time needed for development of lethal disease. Engle and Diamond [28] suggested that antibodies can only partially restore the protection in immunocompromised animals. However, we show here that WNIG was able to establish very effective protection in the presence of aggressive immuno-suppression agent such as dexamethasone which affects the innate immunity [26]. Glucocorticoids administration during viral infection increases the mortality and virus titers [21] and lead to widespread suppression of the innate immune response to a variety of pathogens [26]. In the present study, WNIG treatment offered complete protection to mice injected with dexamethasone while the mortality among control infected mice that were injected with dexamethasone exceeded 80%.

When considering the practical application of passive immunotherapy for WNV infection, the total quantity of specific antibodies administered is limited by the
maximum quantity of IVIG allowed for IV infusion (up to 2 gr/kg). The use of WNIG will allow to administer 5-10 times more specific antibodies compared to regular IVIG thus provide significantly better therapeutic potential.

**Conclusion**

The use of IVIG containing high titer WNV antibodies offer great potential for controlling active infection even in the CNS. The possibility to take advantage of the level of specific antibodies in small fraction of the population by selection of plasma units before processed into IVIG allow to use acceptable doses of IgG in patients and increase their chances for survival or lower the risk of immediate and long term adverse effects.

**Competing interests**

DBN, OGY, IS, YK, AG, MS, AP and BRZ declare that they have no competing interests. NO, IN and OL are employees of OMRIX Biopharmaceuticals.

**Authors' contributions**

DBN and NO were responsible for the animal studies design, results interpretation and drafting the manuscript; IN, OL and NO were involved in concept development, IVIG production and characterization and study design. OGY carried out the mouse ELISA assays under the supervision of AP. IS, YK and MS were involved in the animal study designs and had a critical technical contribution to the animal model. BRZ contributed in all aspects of the animal study design and data interpretation. All of the authors were involved in critical reading of the manuscript and read and approved the final manuscript.
Acknowledgments

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**TABLES and FIGURES**

**Table 1.** Anti-WNV antibodies in the IVIG preparations used for treatment

<table>
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<th>ELISA</th>
<th>PRNT&lt;sub&gt;50&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td>AU/ml</td>
<td>AU/mg</td>
</tr>
<tr>
<td>K44G511 (WNIG)</td>
<td>7608</td>
<td>152</td>
</tr>
<tr>
<td>H23131 (IVIG-IL)</td>
<td>668</td>
<td>13</td>
</tr>
<tr>
<td>K30G370 (IVIG-US)</td>
<td>179</td>
<td>4</td>
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Table 2. Protective efficacy of WNIG and IVIG-IL in mice infected with WNV NY99

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific Ab dose</th>
<th>Survival % (live/treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/mouse)</td>
<td></td>
<td>10-20 LD$_{50}$</td>
</tr>
<tr>
<td>No treatment</td>
<td>0</td>
<td>7 (1/14)$^b$</td>
</tr>
<tr>
<td>WNIG (0.1)</td>
<td>15</td>
<td>94 (15/16)$^c$</td>
</tr>
<tr>
<td>IVIG-IL (0.1)</td>
<td>1.3</td>
<td>44 (7/16)</td>
</tr>
<tr>
<td>WNIG (0.5)</td>
<td>76</td>
<td>100 (16/16)</td>
</tr>
<tr>
<td>IVIG-IL (0.5)</td>
<td>7</td>
<td>87 (14/16)</td>
</tr>
<tr>
<td>WNIG (2.0)</td>
<td>304</td>
<td>100 (8/8)</td>
</tr>
<tr>
<td>IVIG-IL (2.0)</td>
<td>27</td>
<td>88 (7/8)</td>
</tr>
<tr>
<td>IVIG-US (2.0)</td>
<td>7</td>
<td>63 (5/8)</td>
</tr>
</tbody>
</table>

$^a$Arbitrary units of WNV antibodies per mouse (by ELISA).

$^b$p<0.039 compared to all treatment groups.

$^c$p=0.006 compared to IVIG-IL 0.1 mg
Table 3. Therapeutic efficacy of high dose WNIG treatment

<table>
<thead>
<tr>
<th>Treatment day (after challenge)</th>
<th>Treatment dose (mg/mouse)</th>
<th>Survival % (live/treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>-</td>
<td>25 (2/8)</td>
</tr>
<tr>
<td>Day 0</td>
<td>2</td>
<td>100 (8/8)</td>
</tr>
<tr>
<td>Day 0</td>
<td>8</td>
<td>100 (8/8)</td>
</tr>
<tr>
<td>Day 3</td>
<td>8</td>
<td>100 (8/8)</td>
</tr>
<tr>
<td>Day 4</td>
<td>8</td>
<td>63 (5/8)</td>
</tr>
<tr>
<td>Day 5</td>
<td>8</td>
<td>38 (3/8)</td>
</tr>
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</table>
Table 4. Therapy of immunosuppressed mice by WNIG

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Survival % (live/treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNV control</td>
<td>58 (7/12)</td>
</tr>
<tr>
<td>Dex.</td>
<td>19 (3/16)$^a$</td>
</tr>
<tr>
<td>Dex. + WNIG 1.0mg/mouse</td>
<td>100 (17/17)</td>
</tr>
<tr>
<td>Dex. + WNIG 0.2 mg/mouse</td>
<td>100 (16/16)</td>
</tr>
</tbody>
</table>

$^a p<0.001$ compared to WNIG 0.2-1.0 mg.
FIGURES

Figure 1. Dose dependent protection by WNIG

Figure 2. Therapeutic efficacy of WNIG
Figure legends

Figure 1.

Groups of 5-week old BALB/c mice were treated i.p. with IVIG (0.5 or 0.25 mg/mouse) or with WNIG (0.01, 0.05, or 0.025 mg/mouse) 4hrs after infection with 10 LD₅₀ of WNV NY99. Mice were observed for mortality for 21 days. One group of infected mice received no treatment (control).

Figure 2

Groups of 5-week old BALB/c mice were treated i.p. with 2mg/mouse IVIG-IL or WNIG on days 2 and 4, or days 3 and 5 after infection with 10 LD₅₀ of West Nile virus. Mice were observed for mortality for 21 days. One group of infected mice received no treatment (control).