CORRESPONDENCE

Advances in the research and development of therapeutic antibodies against the Zika virus

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The Zika virus (ZIKV) is an arbovirus in the same family, *Flaviviridae*, as the dengue virus (DENV), the West Nile virus, the yellow fever virus, and the Japanese encephalitis virus. ZIKV has been a recent research focus because of its close association with severe human diseases and syndromes, particularly congenital Zika syndrome, which includes fetal microcephaly and other brain abnormalities.[1] The ZIKV envelope (E) protein is a major structural protein involved in receptor binding, viral entry, and fusion. It is arranged as a dimer, with each monomer consisting of three domains (DI, DII, and DIII), a stem region, and a transmembrane region.[2] The ZIKV E protein induces the production of potent neutralizing antibodies against ZIKV and is thus a key target for the development of therapeutic ZIKV antibodies.

Several ZIKV E-targeting monoclonal antibodies (mAbs) with therapeutic potential have been developed (Table 1). Except for one mAb, Tyzivumab, which is currently undergoing testing for safety and tolerability in Phase 1 clinical trials (https://clinicaltrials.gov/ct2/show/NCT03443830), these mAbs are in preclinical development. While E-targeting mAbs are typically tested for neutralizing activity against ZIKV in vitro, most have also been evaluated for in vivo protective efficacy in ZIKV-susceptible animal models, including type I interferon (IFN) receptor (Ifnar1)-deficient A129 mice, type I/II IFN receptor-deficient AG129 or AG6 mice, and non-human primates (NHPs).[3–6] The mAbs mainly target the DI, DII, or DIII region of the ZIKV E protein. Depending on the mAb concentrations, virus titers, virus strains, and injection routes, as well as the animal models used for the neutralization evaluation and/or challenge studies, the protective efficacy of these mAbs against ZIKV may vary.

Therapeutic mAbs targeting DI/II of the ZIKV E protein. mAbs targeting the ZIKV EDI/II are categorized as those with or without cross-reactivity and/or cross-neutralizing activity against other flaviviruses, such as DENV. For instance, the human mAbs Z3L1 and Z20 can bind to conformational tertiary epitopes on ZIKV EDI and EDII, respectively, and neutralize the ZIKV (SMGC-1 strain) grown in both Vero and C6/36 cells. However, Z3L1 does not cross-neutralize DENV-1-4 virions, and Z20 does not neutralize them at high mAb concentrations ($IC_{50} > 10$ μg/ml). Intraperitoneal (i.p.) injection of Z3L1 and Z20 can protect Ifnar1-deficient mice challenged with ZIKV (10^6 plaque-forming units: PFU, PRVABC59 strain, i.p. route) with 100% and 80% survival rates, respectively.[6] In addition, the human mAb ZIKV-117, which binds to the ZIKV E wild-type and a mutant E lacking the DII fusion loop epitope, recognizes a quaternary epitope (critical residues D67, Q89, and K118) on EDII at the dimer–dimer interface and neutralizes five ZIKV strains (H/PF/2013, Paraiba 2015, Malaysia P6740, Dakar 41519, and MR 766). It also protects wild-type mice pretreated with the anti-Ifnar1 mAb against challenge with ZIKV (10^3 focus-forming units: FFU, Paraiba 2015 or mouse-adapted Dakar strain, subcutaneous (s.c.) route), contributing to reduced mortality, and pretreatment and post-exposure treatment prevent pregnant mice from placental and fetal infection and fetal demise.[7] In contrast to the aforementioned mAbs, other ZIKV EDI/II-targeting mAbs, such as ZKA3 and ZKA78, cross-react with DENV and only partially neutralize ZIKV infection, leading to severe symptoms and death in mAb-pretreated (i.p.) AG129 mice after DENV infection (intravenous route).[8]

Therapeutic mAbs targeting DIII of the ZIKV E protein. Among the therapeutic mAbs, most are ZIKV-specific and have no cross-reactivity with other flaviviruses, but some EDIII-reactive mAbs against ZIKV present a higher degree of neutralizing activity than the EDI/DII-reactive mAbs.[8] For example, mouse mAbs, including ZV-54 and ZV-67, are highly specific to the ZIKV EDIII due to their recognition of epitopes on the lateral ridge (LR), and they have potent neutralizing activity against divergent ZIKV strains (H/PF/2013, Paraiba 2015, Dakar 41519, and MR 766), completely protecting anti-Ifnar-treated (i.p.) wild-type mice from ZIKV infection (10^5 FFU, Dakar 41519 strain, s.c. route).[5] Human mAbs, including Z23, ZIKV-116, and ZKA190, can also neutralize ZIKV.[6, 7] Z23 binds to a conformational tertiary epitope on the ZIKV EDIII and neutralizes ZIKV (SMGC-1 strain) without exhibiting cross-neutralizing activity against DENV-1-4. Post-treatment (i.p.) of ZIKV (10^6 PFU, PRVABC59 strain, i.p. route)-infected A129 mice with this mAb results in complete protection without weight loss.[6] ZIKV-116, which binds to an epitope (residues T309, E393, and K394) on the EDIII-LR, neutralizes four ZIKV strains (H/PF/2013, Paraiba 2015, Malaysia P6740, and Dakar 41519).[7] ZKA190 binds to an exposed, conserved epitope consisting of the ZIKV EDI-III linker and the LR region of the EDIII, and occupies all 180 copies of the E protein on the viral surface; this mAb neutralizes the MR 766, H/PF/2013, MRS_OPY_Martinique_PaRi_2015, PV10552, and PRVABC59 ZIKV strains with the capacity to prophylactically and therapeutically (i.p.) protect A129 and/or AG129 mice from ZIKV (strain MP1751: 10^2 PFU; Nica 2-16: 10^3 FFU, s.c. route)-caused morbidity and mortality (80–100% survival rates).[4] The human mAbs m301 and m302 specifically bind the adjacent regions of the EDIII C–C′ loop, an intermittently exposed cryptic epitope, with high affinity in each case. The combination of m301 and m302 exerts a synergistic effect on ZIKV (R103451, PRVABC59, PAN2015, FLR, and SZ01 strains) neutralization in vitro and in an AG6 mouse model of ZIKV infection (10^5 PFU, SZ01 strain, i.p. route).[9]

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Table 1. Summary of preclinically developed ZIKV E-targeting neutralizing mAbs with therapeutic potential

Name	Source	Target region	In vitro efficacy	In vivo efficacy	Structure	Ref
Z3L1	Human	EDI tertiary epitopes	Neutralizes ZIKV Asian strain (IC_{50}: 170–240 ng/ml)	Post-exposure treatment protects Ifnar1-deficient mice from ZIKV (American strain)	Yes	6
ZIKV-117	Human	EDII quaternary epitopes	Neutralizes ZIKV African, Asian, and American strains (IC_{50}: 5–25 ng/ml)	Prophylactically and therapeutically protects ZIKV (African and American strain)-infected, anti-Ifnar1-treated wild-type mice from mortality, fetal infection and fetal demise	Yes	7, 10
ZKA190	Human	Part of the EDI-III linker and EDIII-LR region	Neutralizes ZIKV African, Asian, and American strains (IC_{50}: 0.6–8 ng/ml)	Prophylactically and therapeutically protects A129 and/or AG129 mice from ZIKV (African and American strains)-caused morbidity and mortality	Yes	4
SMZAb2 SMZAb1 SMZAb5	Human	EDII EDIII EDIII	Neutralize ZIKV American strain (IC_{50}: 189, 5.4, and 3.8 ng/ml for SMZAb2, 1, and 5, respectively)	Cocktail of LALA mutants of these three mAbs prophylactically protects NHPs from ZIKV (American strain) replication	ND	3
Z23	Human	EDIII tertiary epitopes	Neutralizes ZIKV Asian strain (IC_{50}: 370–560 ng/ml)	Post-exposure treatment protects A129 mice from ZIKV (American strain)	Yes	6
ZV-54 ZV-67	Mouse	EDIII-LR region	Neutralize ZIKV African, Asian, and American strains	Prophylactically protect ZIKV (African strain)-infected, anti-Ifnar-treated wild-type mice from viremia, weight loss, and mortality	ND Yes	5
ZKA64	Human	EDIII	Neutralizes ZIKV African strain (IC_{50}: 93 ng/ml)	ZKA64-LALA mutant prophylactically and therapeutically protects A129 mice against ZIKV (African strain)-caused weight loss and lethality	ND	8
m301 m302	Human	EDIII cryptic C–C′ loop epitope	Neutralize ZIKV Asian and American strains (IC_{50}: ~2 μM)	m301/m302 combination shows moderate prophylactic and therapeutic protection of AG6 mice against ZIKV (Asian strain)-caused lethality	ND	9

IC_{50} median inhibitory concentration, *anti-Ifnar* antibodies against type I interferon receptor, *LALA mutant* incorporation of L234A and L235A mutations into the IgG-Fc, *LR* lateral ridge, and *ND* not determined

It should be noted that mAbs targeting the ZIKV EDI/II, particularly those that cross-react with DENV, such as ZKA3 and ZKA78, may promote antibody-dependent enhancement (ADE) of ZIKV infection in cell culture and/or in vivo.[4, 8] In some cases, EDIII-targeting mAbs, such as ZKA64 and ZKA190, may also induce ADE at low or sub-neutralizing concentrations (e.g., 0.0001–1 nM or < 1 μg/ml).[4, 5, 8] To some extent, such ADE effects can be ameliorated. For instance, LALA mutations (i.e., the substitution of IgG-Fc residues at positions 234 and 235 from leucine (L) to alanine (A)), which abrogate the binding affinity of the Fcγ receptor (FcγR) but retain neonatal Fc receptor (FcRn) interaction, can eliminate Fc effector functions and reduce or block potential ADE without affecting anti-ZIKV neutralization and/or protective abilities.[3, 4, 7, 8] Another issue regarding ZIKV mAbs is escape. Although ZIKV escape mutants have been identified in the presence of the EDIII-targeting mAb ZKA190, a bi-specific antibody (FIT-1) that links ZKA190 and an EDII-targeting mAb, ZKA185, effectively prevents ZIKV escape without reducing the neutralizing activity and protective efficacy of ZKA190.[4] Additionally, pretreatment of NHPs using a cocktail of the human mAbs SMZAb2, SMZAb1, and SMZAb5, which target the ZIKV EDII and EDIII and contain LALA mutations, has shown efficacy in a ZIKV challenge (10^3 PFU, Rio U-1 2016 strain, s.c. route) by inhibiting viral replication.[3]

In summary, ZIKV EDIII-targeting mAbs (and some EDI/II-specific mAbs) that show potent anti-ZIKV neutralizing activity without cross-reactivity or cross-neutralizing activity against other flaviviruses can be developed as safe and effective therapeutics to prevent and treat ZIKV infection. Some ZIKV E-specific mAbs may cross-react with or cross-neutralize DENV and cause ADE, but at certain concentrations, these mAbs are safe and do not cross-react and/or show cross-neutralizing activity.[8] Thus, to eliminate potential harmful responses, it is advisable to test for potential ADE effects of ZIKV E-specific therapeutic mAbs and, if necessary, to remove or diminish the mAb-induced ADE using engineering or other available approaches. In addition, treatment of ZIKV infection using bi-specific neutralizing mAbs or mAb cocktails targeting epitopes from different regions of the ZIKV E protein is expected to reduce mAb escape and to improve the broad-spectrum efficacy of therapeutic mAbs.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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