The substitutions detected (such as K226R in several raccoon and African dog RABV isolates; L231P/S in several skunk, raccoon, and various bat RABV lineages; etc.) did not preclude virus neutralization from previously published studies. No substitutions that abolished binding of MAb CR57 in escape mutant studies were detected in naturally occurring field RABV isolates. In contrast, numerous substitutions were detected in the binding site of MAb CR4098 (AA 330-338 of the G ectodomain). Examples include a K330N substitution in a bat isolate from Brazil; V332I/F substitutions in several RABV lineages, associated with big brown bats; N336D in several viruses associated with big brown bats in North America, in South-African mongoose RABV, in one African and one Korean dog RABV isolate; N336G/S in several raccoon isolates; E337D in several canine RABV from Serbia and in the southcentral skunk RABV isolates; I338T in the canyon bat and Arctic RABV isolates. Substitutions in position 336, particularly the N336D, were detected earlier in escape virus studies and precluded neutralization of such viruses by MAb CR4098. Nevertheless, no isolates had substitutions in binding sites for MAbs CR57 and CR4098 simultaneously. There is no reason to expect that any of the viruses from our study would escape neutralization by a combination of these MAbs in vivo. The situation is different for HuMAb RAB1 (also referred to as 17C7). We confirmed numerous substitutions, particularly in position 336, which may abolish binding of MAb RAB1 as was shown previously by escape virus generation. The RAB1 was proposed as a single MAb for use in human rabies PEP, claiming that there are no natural RABV isolates which harbor critical substitutions in its binding site (the combination 336D-346K in the G ectodomain). We encountered this combination in the majority of viruses from one of the lineages associated with big brown bats distributed broadly in North America. Our findings clearly demonstrate that the proposed use of a single MAb for rabies PEP is inappropriate, in line with international recommendations.

CO.21
NEUTRALIZATION ANTIBODIES IN COMBINATION OF MCP-1 ARE AS EFFECTIVE AS LIVE-ATTENUATED RABIES VIRUS IN PREVENTING MICE FROM DEVELOPING RABIES

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Rabies virus (RABV) is a neurotropic virus that causes fatal disease in humans and animals. Currently there is no cure for rabies once clinical signs appear. It has been hypothesized that once the virus enters the central nervous system (CNS), neutralizing antibodies in the periphery cannot cross the blood–brain barrier (BBB) into the CNS. Previous studies have demonstrated that treatment with live-attenuated RABV via the intracerebral route 5 days after infection with wild-type viruses can lead to the clearance not only the attenuated, but also the wild-type virus. Direct administration of liveattenuated RABV stimulated high levels of neutralization antibodies and enhanced the BBB permeability. However, direct intracerebral administration of live-attenuated RABV possesses safety concerns. In the present study, neutralization antibodies were administered in conjunction with a chemokine, MCP-1 (known to enhance the BBB permeability), into mice after infection with wild-type virus. Significantly more protection was found in mice treated with this combination when compared to treatment with neutralization antibodies alone without MCP-1. Furthermore, the combined treatment with neutralization antibodies and MCP-1 is as effective as the live-attenuated RABV in preventing mice from developing rabies. These studies further demonstrate that enhancement of the BBB is critical for immune effectors in the periphery to enter into the CNS to clear RABV.

CO.22
DEVELOPMENT OF CL184 HUMAN MONOCLONAL ANTIBODY COMBINATION FOR RABIES POST-EXPOSURE PROPHYLAXIS, FROM PRECLINICAL DESIGN TO CLINICAL EVALUATION

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The currently recommended prophylaxis for individuals exposed to rabies virus is the combined administration of rabies vaccine and rabies immune globulin (RIG). However, limited supply hampers the availability of RIG, particularly in enzootic areas. To circumvent the global RIG limitation we aimed to develop a human monoclonal antibody combination, CL184, for rabies post-exposure prophylaxis (PEP) that would replace the plasma origin RIG. CL184 consists of two human IgG1 mAbs, CR57 and CR4098, which are directed against non-overlapping rabies virus (RV) glycoprotein epitopes. Previously, we have shown that the *in vitro* breadth of neutralization of CL184 against a large panel of street RV of various animal origins as well as *in vivo* protection by CL184 in a Syrian hamster rabies challenge model was comparable to results obtained with human RIG. A detailed preclinical selection procedure was applied to establish the CL184 antibody combination. Efforts on RV surveillance to ensure adequate coverage by CL184 continue. In addition, encouraging data from the Phase I (US and India) and Phase II (US and Philippines) clinical evaluation of CL184 have been obtained. In preparation of the pivotal Phase III evaluations for CL184, a final Phase IIb evaluation has been executed for which data analysis is ongoing. The future availability of CL184 may help to ensure consistent supply of pivotal life-saving biologics to rabies endemic areas and could substantially contribute to the reduction of human rabies deaths, when combined with educational measures and efforts to eliminate canine rabies.

CO.23
GM-CSF OR FLAGELLIN IMPROVES THE EFFICACY OF RECOMBINANT RABIES VIRUSES FOR BOTH PARENTAL AND ORAL IMMUNIZATIONS

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Our previous studies indicated that recombinant rabies viruses expressing chemokines and cytokines (including GM-CSF) could enhance the immunogenicity by inducing innate immunity and recruiting/activating dendritic cells and B cells. In this study, bacterial flagellin was cloned into the rabies virus genome and recombinant virus rLBNSE-Flic was rescued. To compare the immunogenicity of rLBNSE-Flic with recombinant virus expressing GM-CSF (rLBNSE-GMCSF), mice were immunized with each of these recombinant rabies viruses by i.m. or the oral route. The parental virus (rLBNSE) without expression of any foreign molecules was included for comparison. The i.m.-immunized mice were bled at three weeks after the immunization for the measurement of virus neutralizing antibodies (VNA) and then challenged with 50 LD50 CVS-24. The orally immunized mice were boostered after three weeks and then bled and challenged one week after the booster immunization. It was found that both the recombinant viruses LBNSE-GMCSF and LBNSE-Flic induced higher levels of VNA and protected more mice against rabies challenge than the parental rLBNSE in both the i.m.- and the orally immunized groups. Together, these studies suggest that recombinant rabies viruses expressing GM-CSF or flagellin are better vaccines than the parent virus for both parental and oral immunizations, most likely by recruiting/activating dendritic cells.