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The anti-CCR5 mAb004 Inhibits HIV-1 Replication Synergistically in Combination with Other Antiretroviral Agents but does not Select for Resistance during In Vitro Passage

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ABSTRACT

Background: The monoclonal antibody (mAb) CCR5mAb004 is an anti-CCR5 mAb with potent anti-HIV-1 activity in vitro. We evaluated the anti-HIV-1 interactions between CCR5mAb004 and various reverse transcriptase, protease and fusion inhibitors in vitro. To determine the pattern/rate of emergence of resistance to this mAb, R5 clinical isolates were passaged in human peripheral blood mononuclear cells (PBMC) in the presence of drug for up to 6 months.
Methods: Susceptibility of R5 clinical isolates to CCR5mAb004 was tested in PBMC. Activity of CCR5mAb004 in combination with zidovudine, lamivudine, efavirenz, indinavir and enfuvirtide (T-20) was tested against isolates RM and JC. Results were analyzed using the median-effect principle and expressed as combination indices (CI). R5 clinical isolates RM and JC were passaged in PMBC in the presence of increasing concentrations of CCR5mAb004; cultures maintained in the absence of drug served as controls.
Results: No cellular toxicity was seen in PBMC at mAb concentrations up to 10 μM. Geometric mean IC_{50} values for CCR5mAb004 against 8 primary isolates of HIV-1 ranged from 0.014 to 0.202 μM. The geometric mean IC_{50} for RM was 0.055 μM ± .032 μM; for JC it was 0.202 μM ± .152 μM. CI values showed synergistic interactions for all combinations tested. In the resistance selection experiments, the IC_{50}'s of isolates passaged in the presence of antibody did not differ significantly from the IC_{50}'s of viruses from control (no drug) cultures at similar passage number. Passage of JC in PBMC was halted after 19-20 weeks because of the emergence of syncytium-inducing (SI) viruses in the control (no drug) cultures. Emergence of X4 virus was confirmed by inoculation onto U87-CXCR4 cells. Isolate RM continued to Week 24 (passage 33) before a tropism switch, observed both in the absence and presence of drug.
Conclusions: Synergistic activity of CCR5mAb004 in combination with drugs from each of the four approved classes of antiretroviral agents was observed in the R5 patient isolates RM and JC. The inability to select HIV-1 variants with increased IC_{50} values after in vitro passage in the presence of CCR5mAb004 for up to 24 weeks suggests that resistance requires multiple mutations and may be slow to emerge in vivo. These results support clinical evaluation of CCR5mAb004 in combination with current antiretroviral drugs.

METHODS

Viruses: R5 clinical HIV-1 isolates were derived from subjects with acute HIV-1 primary infection syndrome. They were shown to be R5 by replication in U87 MG-CD4 cell lines expressing CCR5 and absence of replication in U87 MG-CD4 cell lines expressing CXCR4. They were also non-syncytium-inducing (NSI) in MT-2 assays. HIV-1 (RAS) harbors multiple mutations conferring resistance to RTI and PI.

Mathematical Analysis: The multiple-drug effect analysis of Chou and Talalay, based on the median effect principle and the isobologram technique, was used to analyze combined-drug effects. Synergy is defined as inhibition greater than the expected additive effect and antagonism as inhibition less than the expected additive effect. CI values < 0.9 = synergy, between 0.9 and 1.1 = nearly additive effects and > 1.1 = antagonism.

Susceptibility and synergy studies: Drug susceptiiblity and in vitro synergy assays were performed in PHA-stimulated peripheral blood mononuclear cells (PBMC) from HIV-1 seronegative donors. Drugs were added simultaneously with the HIV-1 inoculum. Serial dilutions of drugs were tested singly or in fixed-ratio combinations. Each condition was tested in duplicate, and each experiment was conducted at least two times. Uninfected drug-treated toxicity controls were maintained at the highest concentration of each agent tested. Cell-free culture supernatant fluids were harvested and analyzed by ELISA (PerkinElmer) for HIV-1 p24 antigen production on day 7 of culture. Cell proliferation and viability were assessed by the trypan-blue dye exclusion method.

In vitro selection experiments. Viruses were cultured on PHA-stimulated PBMC in the presence of increasing concentrations of CCR5mAb004 as indicated in the figures. Cultures were passaged every 4-7 days. HIV-1 cultures passaged in parallel in the absence of drug served as controls. At intervals, culture supernatants were harvested, titered to determine HIV-1 infectivity and assayed to determine the IC_{50} for CCR5mAb004. Cultures showing cytopathic effect were tested on MT-2 cells to determine syncytium formation and on U87.CD4.CXCR4&CCR5 cell lines (provided by the NIH AIDS Reference and Research Reagent Program) to determine chemokine co-receptor usage.

RESULTS

Table 1: Geometric Mean of the 50%, 75% and 90% inhibitory concentrations of CCR5mAb004 (μM) against a panel of R5 HIV-1 clinical isolates.

Isolate	IC_{50}	IC_{75}	IC_{90}
RM	0.055+/-0.03	0.267+/-0.11	>1μM
JC	0.241+/-0.25	>1μM	>1μM
JFM	0.013+/-0.01	0.077+/-0.05	0.472+/-0.23
KS	0.020+/-0.03	0.124+/-0.24	0.774+/-2.05
MB	0.018+/-0.03	0.116+/-0.25	0.759+/-2.05
ND	0.014+/-0.004	0.110+/-0.04	0.884+/-0.33
RAS	0.067+/-0.03	0.429+/-0.14	>1μM
RC*	0.04	0.23	>1μM

(*) Result of only one determination.

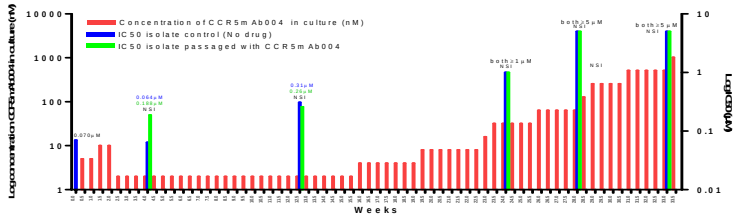
Table 2: Mean combination indices (CI) of CCR5mAb004 tested at various inhibitory concentrations with zidovudine (AZT), lamividine (3TC), indinavir (IND), efavirenz (EFV) and enfuvirtide (T20) against CCR5 HIV-1 clinical isolate JC.

		Combination Indices			
DRUGS	**N**	**ED50**	**ED75**	**ED90**	**ED95**
CCR5mAb004+AZT+3TC	2	0.73+/-0.32	0.51+/-0.21	0.42+/-0.16	0.38+/-0.15
CCR5mAb004+AZT	2	0.60+/-0.35	0.38+/-0.32	0.32+/-0.29	0.31+/-0.29
CCR5mAb004+3TC	2	0.51+/-0.49	0.36+/-0.44	0.34+/-0.43	0.36+/-0.47
AZT+3TC	2	0.88+/-0.15	0.77+/-0.08	0.72+/-0.25	0.69+/-0.36
CCR5mAb004+IND+EFV	3	0.86+/-0.22	0.76+/-0.11	0.75+/-0.10	0.78+/-0.17
CCR5mAb004+IND	3	0.59+/-0.22	0.55+/-0.08	0.63+/-0.07	0.73+/-0.21
CCR5mAb004+EFV	3	0.78+/-0.05	0.64+/-0.19	0.63+/-0.26	0.66+/-0.32
IND+EFV	3	1.24+/-0.06	1.15+/-0.14	1.08+/-0.25	1.03+/-0.32
CCR5mAb004+T20	2	0.41+/-0.21	0.32+/-0.25	0.33+/-0.31	0.35+/-0.37

Table 3: Mean combination indices of CCR5mAb004 tested at various inhibitory concentrations with zidovudine (AZT), lamividine (3TC), indinavir (IND), efavirenz (EFV) and enfuvirtide (T20) against CCR5 HIV-1 clinical isolate RM.

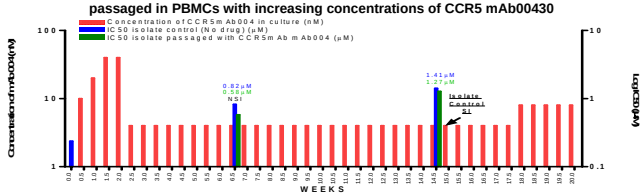
		Combination Indices			
DRUGS	**N**	**ED50**	**ED75**	**ED90**	**ED95**
CCR5mAb004+AZT+3TC	2	0.72+/-0.06	0.52+/-0.02	0.42+/-0.01	0.37+/-0.04
CCR5mAb004+AZT	2	0.74+/-0.02	0.54+/-0.05	0.44+/-0.06	0.40+/-0.04
CCR5mAb004+3TC	2	0.73+/-0.32	0.52+/-0.11	0.42+/-0.01	0.39+/-0.03
AZT+3TC	2	0.92+/-0.03	0.76+/-0.00	0.63+/-0.01	0.56+/-0.03
CCR5mAb004+IND+EFV	2	0.93+/-0.14	0.69+/-0.03	0.62+/-0.04	0.60+/-0.04
CCR5mAb004+IND	2	0.85+/-0.17	0.61+/-0.13	0.58+/-0.27	0.60+/-0.39
CCR5mAb004+EFV	2	0.90+/-0.02	0.66+/-0.07	0.61+/-0.02	0.62+/-0.02
IND+EFV	2	1.20+/-0.00	1.07+/-0.10	0.96+/-0.15	0.91+/-0.17

Figure 1: CCR5 HIV-1 Clinical Isolate (RM) passaged in PBMCs with increasing concentrations of CCR5mAb004



Experimental Condition	Passage Number	WEEK# Number	CCR5 mAb 004 IC50 (uM)	MT2 assay
RM INITIAL	P3D7	0	0.07	NSI
No drug	P7D4	4	0.064	n/a
CCR5mAb004 **2nM**	P7D4	4	0.188	n/a
No Drug	P17D7	12	0.31	NSI
CCR5mAb004 **2nM**	P17D7	12	0.26	NSI
No Drug	P33D7	24	>1	NSI
CCR5mAb004 **16nM**	P33D7	24	>1	NSI
No Drug	P40D7	29	>5	NSI
CCR5mAb004 **64nM**	P40D7	29	>5	NSI
No Drug	P43D7	33	>5	NSI
CCR5mAb004 **512nM**	P43D7	33	>5	NSI

Figure 2: CCR5 HIV-1 Clinical Isolate (JC) passaged in PBMCs with increasing concentrations of CCR5 mAb00430



Experimental Condition	Passage Number	Week Number	CCR5mAb004 IC50 (uM)	MT2 Assay	U87-X4 P24 (ng/ml)	U87-R5 P24 (ng/ml)
JC INITIAL	P3D4	0	0.237	NSI	0.3	45.4
No Drug	P12D4	6.5	0.82	NSI	1.9	88.2
CCR5mAb004 4nM	P12D4	6.5	0.58	NSI	0.5	82.1
No Drug	P22D7	14.5	1.41	n/a	0.7	160.5
CCR5mAb004 4nM	P22D7	14.5	1.27	n/a	1.5	39.2
No Drug	P32D3	15	n/a	SI	0	51.9
CCR5mAb004 4nM	P23D3	15	n/a	NSI	0.2	25.8
No Drug	P25D8	17	n/a	NSI	n/a	n/a
CCR5mAb004 4nM	P25D8	17	n/a	SI	n/a	n/a
No Drug	P28D4	19	n/a	SI	8.3	154
CCR5mAb004 8nM	P28D4	19	n/a	SI	0	51.1
No Drug	P30D4	20	n/a	SI	145.4	163.4
CCR5mAb004 8nM	P30D4	20	n/a	SI	0.7	235
No Drug	P33D4	22	n/a	SI	13.7	151.7
CCR5mAb004 8nM	P33D4	22	n/a	SI	15.7	150.7

CONCLUSIONS

- CCR5mAb004 inhibits R5 HIV-1 clinical isolates at sub-micromolar concentrations.
- Synergy demonstrated between CCR5mAb004 and drugs from each class of currently approved antiretroviral drugs.
- In vitro passage in PBMC resulted in emergence of SI virus or adaptation to PBMC culture and increases in IC50 for CCR5mAb004, both in the absence and presence of drug
- No evidence from these studies of resistance selection by CCR5mAb004.
- Additional experiments including passage on CCR5+ cell lines are needed to define in vitro emergence of resistance to CCR5mAb004.

ACKNOWLEDGMENTS

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