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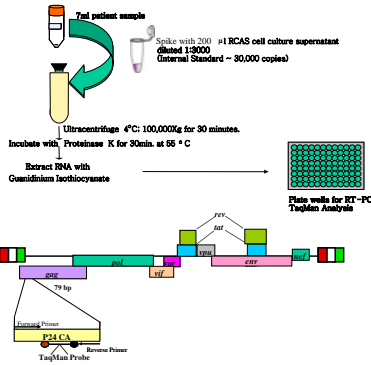
Introduction

In a small proportion (up to 5-10%) of HIV-1 infected individuals immunodeficiency progresses slowly. These patients have CD4+ T cell counts of >500 cells/ μ l and some, known as "elite controllers," have very low plasma HIV-1 RNA levels, often undetectable by standard assays. In the present study, we used a real time RT-PCR assay with single copy sensitivity to quantify and compare plasma HIV-1 RNA levels in elite controllers to those in patients with <50copies/ml on suppressive antiretroviral therapy.

Single Copy Detection of HIV-1 virion RNA

Predicted value (Ct)	Amplicor Ultra-Sensitive Assay					Modified Ultra-Sensitive Assay					Single Copy Assay							
	Run 1	Run 2	Run 3	Run 4	Mean	SD	Run 1	Run 2	Run 3	Run 4	Mean	SD	Run 1	Run 2	Run 3	Run 4	Mean	SD
200	173	176	170	127	162	23	190	149	140	196	179	29	187	200	188	232	202	39
100	80	137	150	90	117	31	87	45	107	88	82	26	85	84	75	139	96	29
50	24	70	60	59	53	20	68	27	61	44	50	19	32	33	39	62	42	14
25	31	28	Neg	Neg	30	2.1	27	18	28	17	23	5.8	15	25	11	30	20	8.8
12.5	Neg	Neg	Neg	Neg	Neg	Neg	9	11	15	11	12	2.5	11	8	10	12	10	1.1
6.25	Neg	Neg	Neg	Neg	Neg	Neg	17	12	5	7	10	5.4	2	9	8	6	8	3.2
3.125	Neg	Neg	Neg	Neg	Neg	Neg	10	Neg	Neg	Neg	10	0	2	5	4	3	3.5	1.3
1.56	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2	1	1	6	2.5	2.4
0.781	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	0.3	1	0.8	ND	0.7	0.4
0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

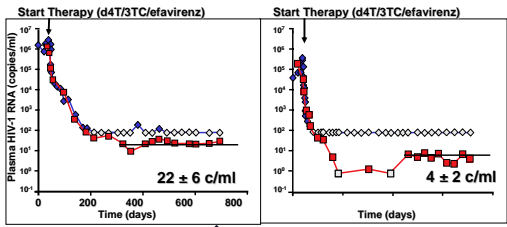
Real Time PCR for HIV-1 RNA



Methods

Plasma samples from 49 elite controllers who had three viral RNA levels of <50 copies/ml for at least a year without treatment were analyzed. Their HIV-1 RNA levels were compared to samples from 163 patients suppressed on therapy to <50 copies/ml for 4 to 333 weeks. Plasma samples were spiked with an internal virion control (RSV), ultra-centrifuged, extracted and quantified in triplicate using real-time RT-PCR (Palmer et al. JCM 2003). Results from samples with less than 50% recovery of the internal control were censored.

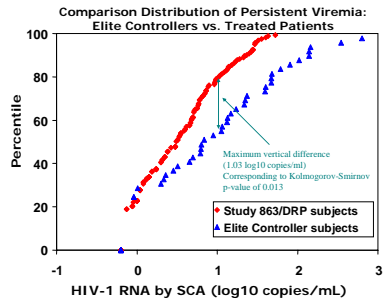
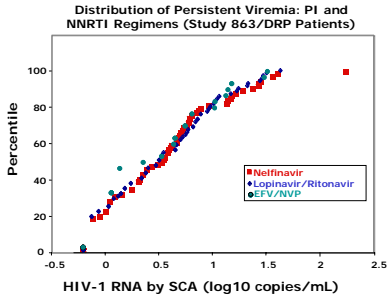
Viremia Persists after Suppression by Antiretroviral Therapy



◆ bDNA
◇ bDNA <75 copies/ml
■ Single-Copy Assay
□ Single-Copy Assay < 1 copy/ml

Results

Viremia was detected in 75% of the samples from both elite controllers (ranging from 1-614 copies/ml) and patients on suppressive therapy (ranging from 1-43 copies/ml). The median HIV-1 RNA value for the elite controllers and treated patients (irrespective of their treatment regimens) was 6.2 and 3.0 copies/ml, respectively ($p = 0.001$, one-way ANOVA), and the overall distribution of HIV RNA levels was also significantly different ($p = 0.013$, Kolmogorov-Smirnov test). Testing of longitudinal samples (2 – 3 samples/patient) from five elite controllers showed spontaneous changes in viremia of 2-5 fold.



Patient	Timepoint	SCA Result (copies/ml)	SCA Result (copies/ml)	SCA Result (copies/ml)	MIT	Total ml. Assayable	SCA Average (copies/ml)	SCA Average (copies/ml)
1	1/1/2004	111	272	200	0	8.3	261	229
1	2/1/2004	19	758	103	0	8.3	261	12.9
1	3/1/2004	0	0	0	0	8.5	20.0	0.0
1	4/1/2004	19	8	0	0	8.5	0	0.0
1	5/1/2004	27	52	27	0	8.5	37	24
1	6/1/2004	20	24	20	0	8.5	0	0.0
1	7/1/2004	20	24	20	0	8.5	25	16
1	8/1/2004	20	24	20	0	8.5	25	16
1	9/1/2004	20	24	20	0	8.5	25	16
1	10/1/2004	20	24	20	0	8.5	25	16
1	11/1/2004	20	24	20	0	8.5	25	16
1	12/1/2004	20	24	20	0	8.5	25	16
1	1/1/2005	20	24	20	0	8.5	25	16
1	2/1/2005	20	24	20	0	8.5	25	16
1	3/1/2005	20	24	20	0	8.5	25	16
1	4/1/2005	20	24	20	0	8.5	25	16
1	5/1/2005	20	24	20	0	8.5	25	16
1	6/1/2005	20	24	20	0	8.5	25	16
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