Four days a week or less on appropriate anti-HIV drug combinations provided long-term optimal maintenance in 94 patients: the ICCARRE project

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ABSTRACT **Short, intraweekly cycles of anti-HIV combinations have provided intermittent, effective therapy (on 48 patients) (1). The concept is now extended to 94 patients on treatment, 4 days per week or less, over a median of 2.7 discontinuous treatment years per patient. On suppressive combinations, 94 patients volunteered to treatment, 5 and 4 days per week, or reduced stepwise to 4, 3, 2, and 1 days per week in 94, 84, 66, and 12 patients, respectively, on various triple, standard, antiviral combinations, or nonregistered, quadruple, antiviral combinations. Ninety-four patients on treatment 4 days per week aggregated 165 intermittent treatment years; no viral breakthrough was observed over 87 average treatment weeks per patient, 63 of 94 having passed 2.5 intermittent treatment years on any of the antiviral combinations prescribed. On the hyperintermittent treatment of 3, 2, and 1 days per week, HIV RNA surged >50 copies, 4 weeks apart, in 18 instances (6.8 viral escapes/100 hyperdiscontinuous maintenance years). Viral escapes could have been a result of erratic adherence (EA) to regimen or follow-up (3 patients)—drug taken at half of the daily recommended dosage (8 patients) and/or overlooked archival-resistant HIVs from antecedent treatment failures (6 patients). Aside from the above circumstances, HIV unexpectedly rebounded in 3 patients on 2 days per week treatment and 1 patient on 1 day per weektreatment, posting 2.2 intrinsic viral escapes/100 highly discontinuous treatment years. All 18 escapes were eventually reversed by 7 days per week salvage combinations, and 11 of 18 patients have been back for a second course of intermittent therapy, 4 days per week or less. Both cell-activation markers on the surface of T lymphocytes and cell-bound HIV DNA levels remained stable or declined. CD4/CD8 ratios rose to ≥1 in 35% of patients, whereas CD4 counts went ≥500/μl in 75%. These values were previously 7 and 40%, respectively, on 7 days per week therapy. In our aging, long, HIV-enduring, multitreated patient cohort, treatment 4 days per week and less over 421 intermittent treatment years reduced prescription medicines by 60%—equivalent to 3 drug-free/3 virus-free remission year per patient—actually sparing €3 million on just 94 patients at the cost of 2.2 intrinsic viral failure/100 hyperintermittent treatment years. At no risk of viral escape, maintenance therapy, 4 days per week, would quasiuniversally offer 40% cuts off of current overprescriptions.—Leibowitch, J., Mathez, D., de Truchis, P., Ledu, D., Melchior, J. C., Carcelain, G., Izopet, J., Perronne, C., David, J. R. Four days a week or less on appropriate anti-HIV drug combinations provided long-term optimal maintenance in 94 patients: the ICCARRE project. *FASEB J.* 29, 000–000 (2015). www.fasebj.org**

Key Words: medical ethics · deontology · adjusted posology

COMBINED ANTIRETROVIRAL (ARV) THERAPY (ART), resulting in undetectable plasma HIV, has drastically amended HIV-related morbidity and mortality in patients, while essentially abrogating the mucosal transmission of the AIDS virus to the uninfected partner (2, 3). Yet, under effective ART or highly active ARV treatment (HAART), HIV persists as proviral DNA, forming a latent reservoir within resting memory $CD4^+$ T cells ever since the time of primary HIV infections (4–6), recalcitrant to current antiviral therapy. As long as a fraction of such latently infected cells can carry inducible, replication-competent proviruses, maintained throughout a patient's lifetime by the proliferation of infected memory T cells or by their intermittent, antigen-driven clonal expansion (7–9), reactivation may rekindle systemic HIV activity at any time, precluding permanent cure or its functional equivalent: a lasting viral remission upon antiviral cessation. Accordingly, antiviral recommendations have required no less than 95% optimal adherence to 7 days per week antiviral therapy, as more relaxed adherence has, in the past, predicted poorer

Abbreviations: ARS, archival-resistant strain; ART, antiretroviral therapy; ARV, antiretroviral; EA, erratic adherence; EFV, efavirenz; ETV, etravirine; HAART, highly active antiretroviral treatment; HLA-DR, human leukocyte antigen-DR; ICCARRE, intermittent, in careful short cycles, antiretrovirals may retain efficacy (derived from French, intermittents,

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outcomes (10–12). Yet, the unremitting treatment doctrine was somewhat refuted by a landmark study, in which 8 patients maintained optimal control on their HIV for 1 year on a 7 day off/7 day on weekly antiviral regimen (13). Spurred by such results, which also highlighted physicians' obligation to adjust prescriptions so as not to overtreat a patient (14), in 2003, we asked our patient volunteers if they were willing to take their antiviral treatment 6, 5, and 4 days per week while having their HIV viral load carefully monitored. Having considered the effectiveness of these intermittent regimens, we went to a 3 day treatment each week and eventually reduced to 2 and 1 days per week. The present report consolidates and adds to our report on 48 patients (1), extended here to 94 patients, on intermittent ARV treatment, 4 days per week or less for 6 months to 9 years, aggregating 420 intermittent treatment years for an average 173 treatment week (median, 142 treatment weeks) per patient.

MATERIALS AND METHODS

Patients and weekly treatment regimens

Prescriptions for intermittent maintenance therapy were written by one of us (J.L.) at Hospital Raymond-Poincaré (Garches, France). This was done under the supervision of our institution's Ethics Committee in conformity with Article VIII bis of the French Code of Ethics, which makes prescribers responsible for off-label prescriptions, granted on the condition of scientific precedents. Patient volunteers received written information on the exploratory nature of the proposal to which they consented in writing.

Of 115 ambulatory patients attending our clinic, intermittent maintenance therapy was sustained for no less than 6 months in 94 volunteers. Before entering the discontinuous maintenance period, all patients had been on continuous suppressive therapy with triple or quadruple ARV combinations for the classic 7 days per week treatment, yielding <50 HIV-1 RNA copies in plasma for 5 months or more. On 126 treatment episodes, 94 patients omitted antiviral drugs each Friday, Saturday, and Sunday. Forty-seven of the 94 had an intermediary 5 days on/2 days off treatment period over 2626 cumulated weeks, as reported previously (1); 47 patients reduced their weekly medicine intakes directly from 7 to 4 days. On 111 treatment episodes, 84 patients experienced treatment 3 days per week. On 87 treatment episodes, 66 patients experienced treatment, 2 consecutive days per week. On 14 treatment episodes, 12 patients experienced treatment 1 day per week. At each intermittent treatment station, 2 or more plasma viremia (typically 2–4 months apart) had shown plasma HIV-1 RNA below the 50 copy-detection threshold before proceeding further down.

Monitoring HIV viremia, T lymphocyte blood counts

HIV replication status in plasma was monitored by an average of 4 samplings per year, drawn out from the patient just before intermittent therapy was resumed. For those on the 2 days per week treatment schedule, plasma viremias were checked every 8 to 10 wk. For those on a 1 day per week regimen, plasma HIV levels were obtained every other week. Plasma viral RNA was measured by the Amplicor HIV-1 Monitor test (Roche Diagnostics GmbH, Mannheim, Germany), followed by the Cobas AmpliPrep/Cobas TaqMan HIV-1 test (Roche Diagnostics GmbH). Plasma HIV spikes, over 50 copies/ml at 2 consecutive dosages within 1 month, defined viral escape, prompting treatment readjustments. Blood lymphocyte counts and subsets were assayed on fresh EDTA blood before treatment and at distant times under therapy by use of an automated cytofluorometric system (Beckman Coulter, Fullerton, CA, USA). Absolute white cell counts and relative percentages to whole lymphocyte counts were determined with an automated Beckman Coulter counter.

Antiviral drug combinations

Standard, triple antiviral compositions, prescribed for 4, 3, and 2 days per week treatment, added 1 pair (of 6) of nucleoside analog RT inhibitors (NRTIs) to 1 HIV-1 protease inhibitor (PI) or to 1 non-nucleoside RT inhibitor (NNRTI) backbone. NRTI preferred pair order, exclusive of thymidine analogs, follows emtricitabine + tenofovir; emtricitabine + didanosine; emtricitabine + abacavir; abacavir + tenofovir; didanosine + abacavir; or didanosine + tenofovir. PI ritonavir-boosted follows 800 mg lopinavir (LPV); 300 mg atazanavir; 1200 mg amprenavir; 600–800 mg darunavir; or 400 mg unboosted atazanavir. NNRTI follows efavirenz (EFV) preferably to etravirine (ETV), with nevirapine (NVP) excluded as a base drug as standard triple combination.

Unregistered, quadruple, anti-RT compositions, prescribed for 4, 3, 2, and 1 days on treatment per week, combined 1 (of 3) NNRTI with 1 of 3 NRTI triads. The preferred NRTI triad order follows emtricitabine + tenofovir + didanosine; emtricitabine + tenofovir + abacavir; or didanosine + abacavir + tenofovir. The preferred NNRTI order follows NVP (400 mg), EFV (600 mg or less), or ETV (400 mg).

HIV-1 integrase inhibitor [raltegravir (RAL)]-based compositions for 4 and 3 days per week treatment added ETV (NNRTI) + 1 ritonavir-boosted PI (15) in 5 patients; ETV + 2 or 3 NRTIs in 4 patients; emtricitabine + tenofovir (NRTIs) in 3 patients; emtricitabine + tenofovir + didanosine (NRTIs) in 4 patients; or ritonavir-boosted LPV (PI) + didanosine (NRTI) in 1 patient.

Prescriptions recommended taking the medications at 1 time per day, preferably at bedtime. Antiviral compositions in the individual patient might have changed on 1 or more occasions, because of unwanted side effects, a promising new drug combination, or patients' preferences (16).

HIV-1 genotypes

Genotyping used the Trugene HIV-1 Genotyping Kit (Siemens, Munich, Germany), according to the manufacturer's specifications. Amplified viruses were derived from a frozen archival plasma specimen obtained <3 months before the 7 day treatment period; upon viral escape, ≥500 copies/ml; or weeks after momentary interruptions of antiviral therapy.

Archival HIVs acquired through antecedent treatment failures

Genotypes from preintermittent treatments were available for 70 patients. In 25 patients, the dominant HIV species selected under past treatments bore mutations in the RT gene, suggestive of clinical resistance to lamivudine/emtricitatine ($n = 25$); abacavir ($n = 12$); NVP/EFV ($n = 12$); tenofovir ($n = 9$); didanosine ($n = 8$); and zidovudine/stavudine ($n = 7$).

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en cycles courts, les antirétroviraux restent efficaces); LPV, lopinavir; NNRTI, non-nucleoside RT inhibitor; NRTI, nucleoside analog RT inhibitor; NVP, nevirapine; PD-1, programmed cell death 1; PE, phycoerythrin; PI, HIV-1 protease inhibitor; RAL, raltegravir; SOD, suboptimal daily drug dosage; TRIO, Translational Research in Oncology; WT, wild-type

Lymphocyte surface phenotypes

Freshly EDTA-collected blood was placed on a Ficoll-Hypaque gradient, and the mononucleated fraction was isolated, washed, and stored frozen in liquid nitrogen as part of our regular clinical repository. Conjugated mAb were obtained from Beckman Coulter [CD3-extracellular domain tagged, CD4-phycoerythrin (PE)-Cy5, CD45RA-FITC, CD38-PE, human leukocyte antigen (HLA)-DR-PE], Becton Dickinson (San Diego, CA, USA; CCR7-PE-Cy7), and BioLegend [San Diego, CA, USA; programmed cell death 1 (PD-1)-PE]. Frozen cell samples were thawed, washed, and stained with the relevant pre-mixed, 5-color antibody combinations for 15 min. Antibodies to CD3, CD4, CD45RA, and CCR7 characterized the relevant lymphocyte subpopulations; the 5th antibody PD-1-PE, CD38-PE, or HLA-DR-PE marked cell activation. CD4-negative cells represented the CD8 population. A Cytomics FC500 instrument, under CellQuest software (Beckman Coulter), was used for analysis. Cell activation markers (CD38, HLA-DR, PD-1 molecules on the surface of live T cells) were numbered after calibration with QuantiBRITE beads (17). Memory T cell counts were deduced from cells not expressing a naive phenotype. Accordingly, lymphocyte subpopulations were characterized as follows: total $CD4^+$ T cells, $CD3^+CD4^+$; terminally differentiated effector memory CD4 T cells, $CD3^+CD4^+CD45RA^+CCR7^-$; CD4 naïve T cells, $CD3^+CD4^+CD45RA^+CCR7^+$; effector memory CD4, $CD3^+CD4^+CD45RA^-CCR7^-$; central memory CD4, $CD3^+CD4^+CD45RA^-CCR7^+$; total $CD8^+$ T cells, $CD3^+CD4^-$; terminally differentiated effector memory CD8 T cells, $CD3^+CD4^-CD45RA^+CCR7^-$; CD8 naïve, $CD3^+CD4^-CD45RA^+CCR7^+$; effector memory CD8, $CD3^+CD4^-CD45RA^-CCR7^-$; central memory CD8, $CD3^+CD4^-CD45RA^-CCR7^+$.

Quantifying cell-associated HIV-1 DNA

In brief (18), quantitation was performed by real-time PCR by use of TaqMan probes on a LightCycler instrument (Roche Diagnostics, Meylan, France). Total HIV-1 DNA copy number was determined on viral DNA molecules that achieved the second strand transfer of RT by use of primers that annealed in the U5 region of the long-terminal repeat and in the 5′ end of the gag gene. Copy numbers of total HIV-1 DNA were determined in reference to a standard curve prepared by amplification of quantities ranging from 10 to 105 copies of cloned HIV DNA.

RESULTS

Patient characteristics at baseline

The 94 volunteers were part of an ambulatory cohort followed for an HIV-1 infection over an average of 17 years (**Table 1**). One or more clinical or immunologic AIDS-defining events were in the history of 59 patients. Nadir T-CD4 blood count below 200 or 100 cells/μl were found in 55 (58%) and 26 patients (27%), respectively. The zenith of the plasma retroviral copies per milliliter before therapy was $\geq$100,000 in 75 patients (80%). Previous conventional antiviral treatments (1–9 episodes) were seen in 71 patients (75%), which were interspersed in many patients with momentary treatment-free intervals. Only 17 patients had their first 7 day per week attack treatment once before discontinuous maintenance therapy. All 94 patients went through a continuous 7 days per week treatment for 5 to 75 months on diverse triple or quadruple drug combinations before commencing discontinuous maintenance therapy. Examples of patients under intermittent, in careful short cycles, ARV may retain efficacy (ICCARRE) can be seen in **Fig. 1*A–D***.

TABLE 1. *Patient characteristics at baseline*

Women	26
Men	68
Age, mean yr 1 ± SD (range)	52.4 ± 11.6 (21–84)
Nadir T CD4/μl	181 ± 98 (5–713)
% CD4 (mean ± SD)	12 ± 8 (1–45)
Below 100/μl, patients (%)	23 (24%)
Below 200/μl	55 (59%)
Maximum plasma viremia, mean log 1 ± SD (range)	5.4 ± 0.6 (3.5–7)
<5 logs	19 (20%)
5–5.5	40 (43%)
>5.5 logs	35 (37%)
Clinical HIV follow-up, mean yr 1 ± SD	17 ± 6.7 (1.7–30.7)
Prior AIDS events and/or CD4 < 200 μl	59 (63%)
Antiviral treatment before study	71 (76%)
Duration of antecedent treatment, mean yr 1 ± SD (range)	6.3 ± 4 (0.2–16.3)
Prior treatment interruptions	72 (77%), 1–9
Duration of interruptions, mean month 1 ± SD (range)	50 ± 68 (1–410)

On intermittent treatment 4 days per week: antiviral performance, all drug combinations

HIV was optimally controlled without any failures in 160 attempts extended over 8164 intermittent treatment weeks in 94 patients on standard triple combinations (63% of the 4 days per week on treatment), novel anti-RT quadruple compositions (25%), or RAL-based triple or quadruple combinations (11%; **Fig. 2**). Standard triple combinations combined 2 NRTIs to a PI in 50% of prescriptions at that level of intermittence or to a NNRTI, mostly EFV (36% of all standard triple combinations prescribed at that level). Four RT inhibitor compositions associated 3 NRTIs + 1 NRTI base, 72% NVP based. RAL was combined to a boosted PI + 1 NNRTI, mostly ETV [as in TRIO (15)], in 59% of prescriptions with that HIV integrase inhibitor.

On super short cycles of ARVs, 3, 2, or 1 days per week: antiviral performances, all combinations

Three days per week, super intermittent therapy was carried out over 115 treatment episodes, optimally maintaining antiviral effectiveness for 5694 treatment weeks in 88 patients (**Tables 2 and 3**). HIV escaped control in 10 instances at a rate of 9.2 per 100 highly intermittent treatment years. Two days per week on super intermittent therapy, over 74 treatment episodes maintained effectiveness in 66 patients over 7176 treatment weeks, with control failing on 7 instances at a rate of 5 escapes per 100 super intermittent treatment years. One day per week on super intermittent therapy, more than 14 attempts in 12 volunteers maintained HIV under control over 851 treatment weeks, with only 1 failure at a rate of 6 viral escapes per 100

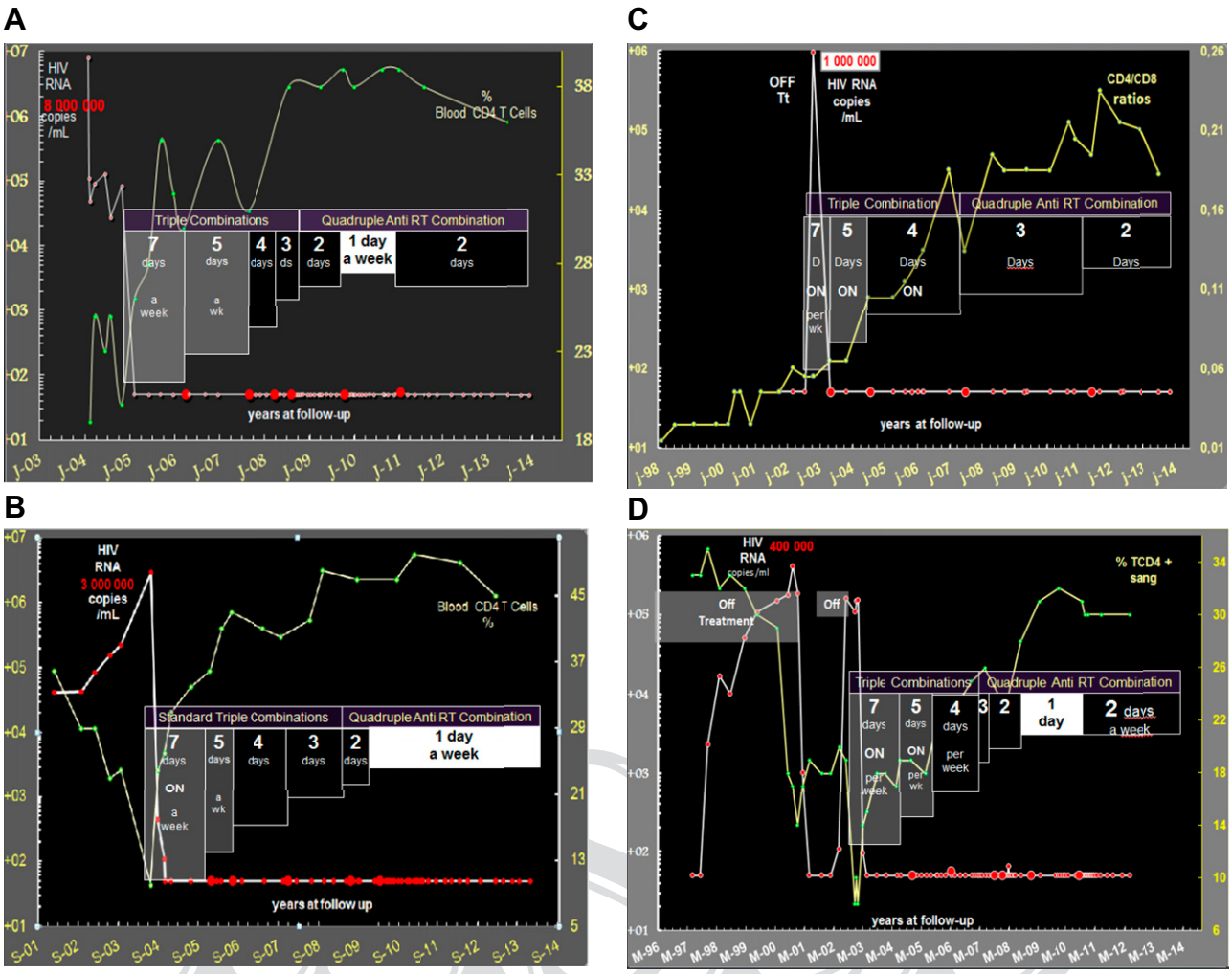

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Figure 1. *A* and *B*) CD4 and HIV RNA follow-up derived from 4 patients on maintenance therapy under ICCARRE. ds, days; Tt, treatment; TCD4^{+}, CD4^{+} T cell; sang, blood in French. *C* and *D*) CD4 and HIV RNA follow-up derived from 2 patients on maintenance therapy under ICCARRE.

super intermittent treatment years, leading to our termination of the 1 day per week regimen (with the exception of 5 self-determined volunteers). Altogether, the super intermittent treatment regimens cumulated 13,728 treatment weeks at the overall cost of 7 viral escapes per 100 super intermittent treatment years.

On super short cycles of ARVs, 3, 2, or 1 days per week: antiviral performances depending on the medicinal combination

On standard triple combinations, given 3 and 2 days per week to, respectively, 46 patients on 48 occasions, HIV was optimally controlled for 3328 treatment weeks, interrupted by 2 failures, leading to 3 viral escapes per 100 super intermittent treatment years (Tables 2 and 3). Furthermore, on 4 anti-RT combinations given 3, 2, and 1 days per week to 60, 60, and 12 patients on 57, 76, and 14 occasions, respectively, HIV was fully controlled over 10,192 treatment weeks, interrupted by 6 + 7 + 1 HIV escapes on 3, 2, and 1 days per week treatments, respectively, for an overall failure rate of 7 per 100 super intermittent treatment years under such combinations. In contrast, on anti-integrase-based combinations given 3 days per week to 9 patients for 185 treatment weeks, HIV eventually rebounded in 3 of 3 patients under RAL + 2 NRTIs, causing high viral escape rates under this anti-integrase-based combination at a deterring 84 per 100 super intermittent treatment years.

Viral escapes under super intermittent maintenance therapy: contextual circumstances and outcomes

HIV escapes in the context of a physician's or patients' blunders and errors

Of the 18 viral escape episodes encountered at 3, 2, and 1 days per week treatment, 14 occurred in the context of 3 plausibly etiological circumstances with a plausible role in viral outcome: 1) overlooked, resistant viruses from past treatment failures (in patients 1, 2, 3, 4a, 4b, and 6); 2) a drug base given below the recommended daily dose (in treatment episodes 4a and 6, patients 4 and 6 cumulated an archival-resistant virus and suboptimal base-drug dosage when HIV escaped on a 3 and 2 days per week regimen):

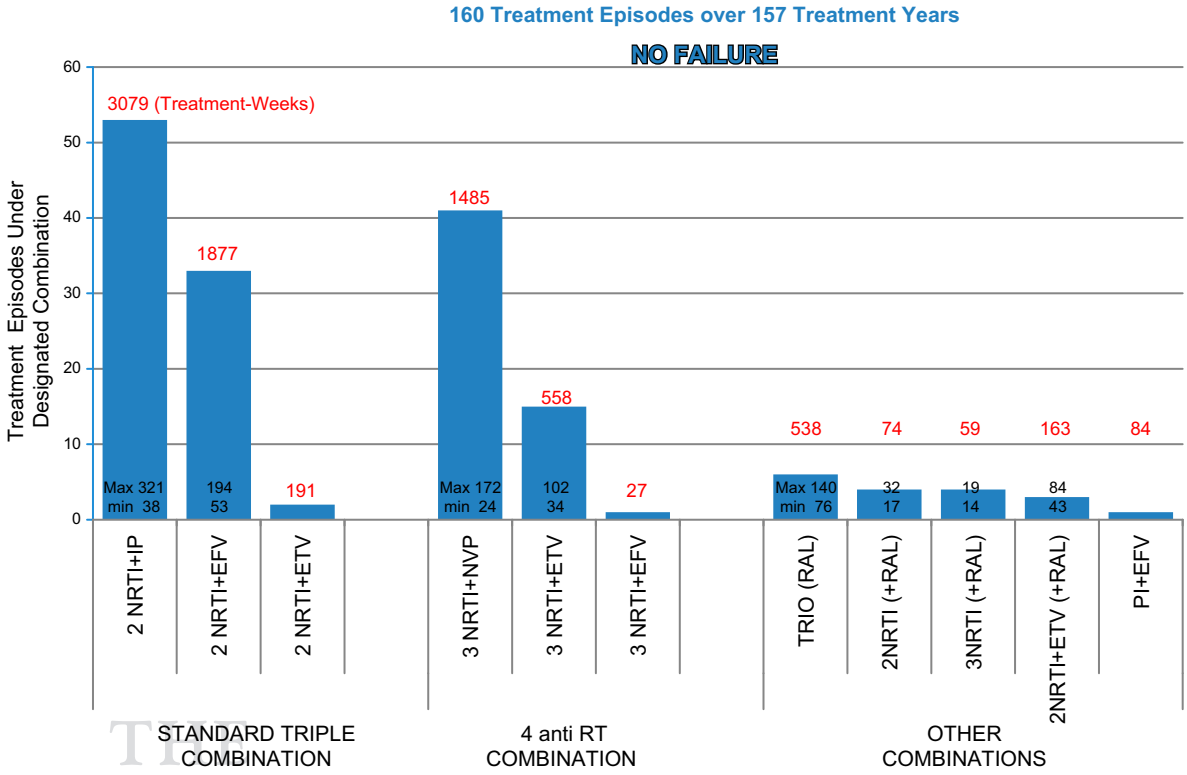

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Figure 2. Intermittent maintenance therapy 4 days a week in 94 patients. PI, protease inhibitor; TRIO, Translational Research in Oncology.

400 mg RAL once a day instead of 400 mg twice per day (patients 4a, 10, and 11a), 200 mg daily instead of 400 mg NVP (patient 6) or ETV (patient 7), and 400 mg LPV in 1 daily intake instead of 800 mg in 2 administrations (patient 8); and 3) patient 9 failed a full dose of her ETV-based combination after she developed clinical steatorrhea from an insoluble carbohydrate fiber diet, altogether entailing the malabsorption of the lipid-soluble NNRTI (19–21). Acute EA to treatment and/or follow-up were noted in patients 11b, 13, and 14 (see Tables 2 and 3).

Viral escapes with no particular etiological context

Of 49 patients on our preferred NVP-based composition taken for 6916 treatment weeks, HIV in 4 patients escaped for reasons other than from the reportable circumstances described above but from the intrinsic weakness of the combination. Patients 15, 16, and 17 were on a 2 day per week treatment regimen, and patient 18 was on a 1 day per week treatment regimen. This was a rate of 2.2 intrinsic failures per 100 super intermittent treatment years.

TABLE 2. *On highly intermittent (3 days)/ultra (2 days)/hyper (1 day) treatment cycles*

	Cumulating 13728 super Intermittent Treatment Weeks					
	3 days per week			2 days per week		1 day per week
Treatment (ARV combinations)	Standard triple combination	3 NRTIs + 1 NNRTI	Others	Standard triple combination	3 NRTIs + 1 NNRTI	3 NRTIs +1 NNRTI
Patients once or more on that combination	46	60	9	14	60	14
Cumulated treatment weeks	2752	2755	185	576	6598	851
Median	38	28	16	27	87	31
Max	208	127	42	152	215	196
Viral escapes	1	6	3[a]	1	6	1
per 100 treatment yr	2	11	84	9	5	6
Intrinsic failures on proper treatment[b]	–	–	–	–	3	1
per 100 treatment yr				3	3	

yr, year. [a]Three patients on emtricitabine + tenofovir + RAL. [b]Aside from EA/archival-resistant strains (ARS)/suboptimal daily drug dosage (SOD); same legend in Table 5.

TABLE 3. *Viral escapes under super intermittent maintenance therapy: contextual circumstances and outcomes*

Patient	On treatment at viral escape (days per week)			Weeks before escape		Past/actual contextual circumstances[b]	Antecedent resistance mutations to NRTI; *NNRTI*	HIV genotype around viral escape	Weeks under second ICCARRE since first viral escape
	3	2	1	On designated regimen	Prior 4 days or less on that regimen[a]			*de novo* Acquired mutations	Current treatment status
1	NVP+[c]			30	130	ARS	M41L D67N K70R M184V K219E; *K103N*	Wild-type (WT) RT[d]	28 4 day per week
2	NVP+[c]			15	38	ARS	L74V M184V; *K101E Y181C G190S*	L74V M184V; *K101E Y181C G190S*	109 4 day per week
3	NVP+[c]			4	.	ARS	M41L D67N M184V L210W K215Y; *K101E*	WT RT[d]	52 3 day per week
4a	RAL+[d]			16		ARS, SOD	K70E L74V Y115F M184V; *L100I K103N*	**K65R** L74V Y115F M184V; *L100I K103N*; WT integrase	114 3 day per week
4b	NVP+[c]			114		ARS	K65R L74V Y115F M184V; *L100I K103N*	K65R L74V Y115F M184V; *L100I K103N*	Momentary interruption
6		NVP+[c]		23	41	ARS, SOD	L74V Y115F M184V; *K103R E138Q V179D*	184V; *K103R E138A V179D*	24 4 day per week
7	ETV+[c]			45	50	SOD		NA	142 3 day per week
8		LPV+[c]		12	75	SOD		**M184I**	97 2 day per week
9	ETV+[c]			9	62	SOD		**M184V; *V106I E138K***	95 3 day per week
10	RAL+[e]			33		SOD		**M184I**; WT integrase	168 2 day per week
11a	RAL+[e]			6		SOD		**M184I**; WT integrase	149 3 day per week
11b	NVP+[c]			26	149	EA	M181I	**M184I; *Y181C***	Momentary interruption
13	EFV+[c]			72	158	EA		***K103N***	84 4 day per week
14		NVP+[c]		34	73	EA		***Y188C***	5 day per week
15		NVP+[c]		32	97	NRC		**K65R M184V; *K101E V179I G190A***	7 day per week
16		NVP+[c]		91	97	NRC		**M184V; *K103N***	Momentary interruption
17		NVP+[c]		22	150	NRC		**M181I; *Y181C***	7 day per week
18			NVP+[c]	25	68	NRC		**K65R M184I; *Y188C***	Momentary interruption

[a]Weeks on combination before to escape. [b]NRC, No relatable context. [c]Didanosine + tenofovir + emtricitabine. [d]3 wk after momentary treatment interruption. [e]Tenofovir + emtricitabine.

Viral escapes under intermittent therapy: outcomes

Of the 18 viral escapes, HIVs bearing newly acquired resistance mutations emerged in 11 instances with 1 mutation to NTRIs (patients 4a, 8, 10, and 11a) or to NNRTIs (patients 18 and 19; Table 3), 2 mutations to NRTI and NNRTI (patients 11b, 16, and 17), 3 mutations (patients 9 and 18), and 5 mutations (patient 15). All escaped mutants were effectively countered by adjusting the antiviral regimens to 7 days per week triple or quadruple salvage combinations, the composition of which was chosen to circumvent the emerged mutations. Six months after a 7 day per week reinduction period, intermittent maintenance schedules were reinstated successfully (see Table 3, HIV genotype around viral escape column), eventually down to discontinuous treatment: 5 days per week (patient 14), 4 days per week (patients 1, 2, 6, and 13), 3 days per week (patients 4a and 7), or 2 days per week (patients 8, 9,

10, and 11a). As taken up further in our discussion, viral escape rates on 3, 2, or 1 days per week therapy matched those reported in most studies with 7 days per week maintenance treatment (1–21).

Lymphocyte activation markers and discontinuous maintenance therapy

Fluorescent mAb to CD3, CD4, CD8, CD38, HLA-DR, and PD-1 surface proteins, evocative of an "ongoing activation" process, were applied to sequential live, frozen blood mononucleated cell specimens. These were obtained before treatment; upon treatment 7 days per week; on intermittent treatment 4 days per week; and on 3, 2, or 1 days per week and had been stored in our clinical specimen repository (**Table 4**). The cytofluorometric evaluation of the 5 most representative T lymphocyte subpopulations are presented in **Table 5**. Before ARV treatment, cell-surface expression levels ranged from 2 to 10 times higher than those of HIV-negative control subjects; under continuous treatment in the patients, the levels plummeted 0.6- to 5-fold in $CD8^+$ or $CD4^+$ T cell subpopulations, respectively. On intermittent treatment, cell activation levels subsided further (CD38) or remained as low and stable (HLA-DR, PD-1) compared with 56 patients (from an independent, local patient cohort) on unremittent, 7 day per week treatment for a matched median treatment time (see legend to Table 5).

Cell-associated HIV DNA and discontinuous maintenance therapy

Proviral DNA was extracted from live-frozen blood mononucleated cell specimens obtained from patients over years of follow-up and kept deep frozen in our live cell clinical specimen repository (Table 5). Before antiviral treatment, cell-associated HIV DNA had ranged at average high levels relative to other published cohorts (22); under continuous, 7 days per week treatment regimen, levels plummeted by 70–75%, subsiding further or remaining low and stable under discontinuous treatment at 20% of pre-ART values.

DISCUSSION

High levels of adherence to ARV treatment have generally been deemed necessary for optimal viral responses, as the probability of long-term viral suppression statistically

TABLE 4. *Cytofluorometric cell-surface markers of lymphocyte activation before/under intermittent maintenance treatment*

	Median percentage of cells expressing <500 surface molecules of:				
ART	$CD8^+$	CD8 EM	CD8 TDEM	$CD4^+$	CD4 EM
CD38					
1. 27 before ART	68	70	72	58	39
2. 27 on ART 7 days per week	19	15	26	38	16
3. 23 on ART 4 days per week	19	13	21	36	13
4. 22 on ART 3, 2, and 1 days per week	18	12	19	35	13
5. 56 on continuous ART 7 days per week	17	10	20	33	12
6. 31 HIV neg	12	7	19	33	11
HLA-DR					
1. 27 before ART	25	34		10	19
2. 27 on ART 7 days per week	9	13		6	13
3. 23 on ART 4 days per week	8	15		6	11
4. 22 on ART 3, 2, and 1 days per week	8	13		6	13
5. 56 on continuous ART 7 days per week	9	12		5	8
6. 31 HIV neg	5	9		3	5
PD-1					
1. 27 before ART	40	60		31	53
2. 27 on ART 7 days per week	26	40		20	39
3. 23 on ART 4 days per week	26	43		20	34
4. 22 on ART 3, 2, and 1 days per week	27	39		20	34
5. 56 on continuous ART 7 days per week	21	36		20	31
6. 31 HIV neg	19	29		12	22

1, 27 patients before ARV. 2, 27 patients, last specimens under treatment 7 days per week, median duration 67 weeks. 3, 23 patients, last specimens under treatment 4 days per week, median duration 223 weeks. 4, 22 patients among the precedents under treatment 3, 2, or 1 days per week, median duration 360 weeks. 5, 56 control patients with continuous treatment 7 days per week, median duration 345 weeks. 6, 31 HIV-negative control. CD8 EM and CD4 EM, effector memory; CD8 TDEM, terminally differentiated effector memory.

TABLE 5. *Total cell-associated HIV DNA before/under intermittent maintenance treatment*

Measurement	Before ART	7 day treatment	4 day treatment	3, 2, and 1 day treatment
Mean (log_{10})	3.33	2.66	2.71	2.73
Median	3.47	2.80	2.73	2.66
Standard	0.49	0.47	0.50	0.35
Range	2.29–3.93	1.6–3.24	1.56–3.51	2.1–3.44
No. of patients	25	21	15	20
Percent residual HIV DNA				
Mean		28	24	19
Median		23	19	17

followed (close to) perfect adherence to 7 days per week ARV regimens (23–25). However, unrelenting lifetime HAART, with its physiologic and psychological constraints, pending toxicities, soaring costs, and the challenges with unremitting, long-term adherence (26–28), has been calling for treatment alleviation.

ICCARRE 4 day per week treatment: not a single HIV escape

Our study of short, intermittent antiviral treatment cycles of 4 days per week [1 step down from the previously administered 5 day per week regimen (29)] struck a bonanza: 94 patients took intermittent therapy for 157 cumulated years, i.e., 87 treatment weeks per patient, 63 patients having passed 2.5 intermittent treatment years. It should be noted that despite archived, resistant viruses from past failures in 25 patients or the administration of half of the daily recommended dose of a drug base in 11 patients, there was not 1 viral escape over 8164 weeks. The average patient benefited from 262 virus-free/drug-free days. Treatment of 4 days per week removed 40% of current overmedications, conforming to the ethical obligation to adjust medicinal treatment only to the amount necessary to obtain the best results.

A majority of patients presently on suppressive ART 7 days per week should benefit from ICCARRE 4 days per week

Our long-term virus carriers, infected with HIV for an average of 17 years, came to intermittent maintenance therapy following long medical histories encompassing AIDS-defining conditions, low CD4, high pretreatment viral loads, past treatment failures, archived resistant HIVs, dense cell-activation markers, and abundant cell-borne, proviral DNA (in 6 patients who successfully endured 2 or 1 day per week treatment regimen over 825 treatment weeks, proviral DNA at the onset of ART had totaled an average high 6500 copies/million cells), the latter purportedly predictive of treatment failure (30–32). The very patchiness and overall vulnerability of this uncontrolled selection of patients would support a general applicability of the 4 days per week regimen to many if not all persons currently under steady ART, still excluding patients in whom multiple resistant viruses would preclude an eventual salvage composition with 2 synergistic ARVs, should ICCARRE fail. Likewise, patients wantonly blundering with ARVs or medical follow-up should not be enrolled (33).

Super intermittent treatment, 3, 2, and 1 days per week: work in progress still requiring caution

In 84 patients, ARVs were reduced to 3 days per week over 70 average treatment weeks met with 10 viral escapes; in 66 patients, treatment was reduced to 2 days per week over an extended 109 average treatment weeks, encountering 7 viral escapes; 12 patients went on a 1 day per week schedule over 71 treatment weeks, ending in 1 viral escape (Tables 2 and 3). Altogether, over 264 extendedly discontinuous treatment years, HIV escaped control in 18 instances, amounting to 7 escapes per 100 highly discontinuous treatment years, within the range of viral escapes noted in a number of 7 days per week combinations (34–44), yet lower than on unremitting PI monotherapy (45–48). Congruent with the tentative exploratory nature of our prescriptions, 14 of the 18 escapes could reasonably be linked to unfitting prescriptions or EA (Table 3). Had appropriate combinations and proper follow-up been the rule, failure rates inherent to the intrinsic antiviral weakness of the super intermittent modality would fall to 1.5 per 100 treatment years, in line with 7 day treatment standards and way upbeat from PI monotherapies.

Treatment outcomes following viral escapes

In the super intermittent treatment situation, 12 patients had their HIVs rebounding with newly acquired mutations, upsetting the antiviral efficacy of NRTIs in patient 4, NNRTIs in patients 7 and 9, or both in patients 6, 8, 12, and 13. Antiviral treatment was momentarily interrupted in patients 5, 6, 8, 11, 12, and 13 for 6 months or more in an attempt to "drown out" the recently mutated HIV in a wave of returning WT HIV (49, 50). Therefore, patients 5, 8, and 13 could resume 7 day per week attack combinations comprising drugs previously deemed ineffective against the mutant virus at the time of escape. [The fact that the combinations, now successful on a 7 (patient 8), 4 (patient 5), or 3 (patient 13) day per week regimen, comprised one or more antiviral components genotypically "unfit" against the preinterruption mutant virus supports the notion that the mutant species had functionally been "washed out."] Patient 8, whose emerging virus carried four *de novo* acquired mutations, went off of all antiviral medications for

3 months. He since has been back on a virally effective triple combination comprising 2 antiviral components genotypically ineffective against the escaped mutant.]. In all 18 instances, under proper 7 day per week salvage combinations, HIV loads fell to undetectable levels; 11 of the 18 have since undertaken a second ICCARRE course, down to 4 days per week (patients 1, 2, 6, and 13), 3 days per week (patients 3, 4a, 7, and 9), or 2 days per week (patients 2, 8, 10, and 11a), for an average 82 weeks as of this writing (Table 3, HIV genotype around viral escape column).

Of note, archival-resistant HIV strains, which eventually resurfaced on a 3 day per week schedule (patients 1, 2, 3 4a, and 4b) or on a 2 day per week schedule (patients 5 and 11), had been kept in check on the precedent 4 or 3 day per week schedule, on that same combination that proved ineffective at a lower intermittent schedule. This emphasizes the safety of the 4 days per week treatment schedule on all combination types prescribed, including those turning suboptimal at lower intermittent schedules. (Conversely, in view of the 84/100 failure rate with RAL-based combinations given 3 days per week and until thorough investigations on the topic have been conducted, integrase inhibitor-based combinations cannot be recommended for ICCARRE.)

Not just any antiviral therapy will do for intermittent maintenance therapy

In the super intermittent treatment setting, 20 of 25 patients carrying archival mutant HIV went on antiviral combinations, which effectively confronted the resistant virus with an average 2.9 functionally effective antiviral components, and no viral escape ensued. In contrast, in 5 patients for whom the antiviral drug selection provided <2 functionally effective antivirals, the mutant HIV eventually broke through at a 3 or 2 day per week treatment schedule. [The apparent need for more than a dual antiviral combination echoes with the weakness of mono-drug maintenance on boosted PIs and the failures with induction-alleviated maintenance (referenced).] Whatever the underlying physiological or pharmacological requisites, <3 functionally additive or potentiating drug combinations should not be considered for intermittent maintenance below 4 days per week on therapy.

Hyperintermittent maintenance regimens of 2 and 1 days per week on a NVP-based 4 RT combination turned out to be safe and effective over an average of 140 treatment weeks in 45 of 49 patients. Still, since over the last 4 years 1 inadvertent viral failure per year occurred under that combination, we urged the relevant patients to set back their weekly drug intake from 2 days to no less than 3 days per week until markers predictive of failure or success on hyperintermittent combinations and regimens come to the clinic. Pharmacological quantitation of residual antiviral drugs within mononucleated cells of patients under intermittent treatment might eventually help at resolving such an issue (51).

ICCARRE, a refutation of the dogma never to give less than 7 days per week antiviral treatment

Short, intermittent weekly cycles of antivirals were initiated based on the fact that the patients' lymphoid system under HAART would be altered in the direction of a deactivated quiescent state (52), less favorable to HIV replication (53–55). This and the marked reduction of the infectious load should prolong the lag phase between the interruption of treatment and HIV rebound. Indeed, HIV eclipse times following ARV cessation extended from 1 to 3 days early treatment to 7 days and more during treatment cessation after effective therapy (56–58). These physiopathological alterations provided the basis for the 7 day treatment on/7 day treatment off trial launched by Dybul et al. (13). That HIV did not rebound during the 24 off-treatment periods in 8 patients led the investigators to repeat their feat successfully in 2004 with other triple ARV combinations (59). Yet, following less-favorable reports from various sources (60–62), the flagship team retreated, stressing the significantly increased risk of failure with treatment interruptions beyond 2 days (63).

In defiance of these revolving conclusions, our 94 participants selected from a small cohort of ambulatory patients benefitted from discontinuous treatment 4 days per week and less, keeping HIV under control over an average 4.2 intermittent treatment years per patient. Aside from confirmed viral escapes, there was no sign of *de novo* HIV activity under ICCARRE, as judged from sequential plasma viral counts, perennially stable or declining cell activation markers and proviral DNA counts in mononucleated blood cells, not sensibly different from patients with continuous treatment over similar treatment durations (Table 5). Blood CD4 T cell counts continued on their preintermittence increases, rising from a mean of 513 to 646/μl. Lymphoid tissue also improved, as seen in the rising proportions of blood CD4/CD8 ratios ≥ 1, from 7% under continuous therapy to 27% under ICCARRE. Three patients with hepatitis virus C receiving discontinuous anti-HIV regimens, 4 days per week for 1 patient or 2 days per week for 2 patients, were cured of their liver infections (on a ribavirine/pegylated IFN-α dual therapy, occasionally reinforced by recombinant erythropoietin). On an especially happy note, 6 different HIV couples in whom the HIV-positive partner was on discontinuous therapy, 4 days per week or less, conceived and gave birth to 10 HIV-free babies, the unprotected HIV-negative parents having remained free of HIV contamination.

At a time when only 25% of HIV-infected people in the United States has successfully navigated the present care continuum to achieve undetectable viral load (64), making ARV treatment more attractive and more binding, with decreasing toxicity to enhance acceptability while maintaining antiviral efficacy, is just what prescribers hoped they could accomplish since the early years of triple-combination therapy. Yet, not just any medicinal treatment simplification will do; the alleviation of triple-combination therapy with 2 drugs as maintenance therapy, 7 days per week, once failed radically (65–68), and ritonavir-boosted PI monotherapies, 7 days per week, have yet to match (45–48) current triple combination standards virologically (34–44).

Conclusions

By cutting into overmedication by 40–85%, ICCARRE offered an average 3 drug-free/virus-free remission years per

patient, saving approximately €3 million for just 94 patients at the cost of 2.2 intrinsic antiviral failures per 100 1 and 2 day intermittent treatment years. Furthermore, to emphasize again, on the 4 days per week schedule, there was not one HIV viral escape in the 94 patients. Over 10 years, this would amount to a 4 year drug-free/virus-free exemption from unnecessary overmedication. FJ

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