Four Days a Week or Less on Appropriate Anti HIV Drug Combinations Provided Long Term Optimal Maintenance in 94 Patients. The ICCARRE* PROJECT

***I**ntermittents, en **C**ycles **C**ourts, les **A**nti **R**étroviraux **R**estent **E**fficaces
Intermittent, in **Careful short **C**ycles, **A**nti **R**etrovirals may **R**etain **E**fficacy*

Jacques Leibowitch[1], Dominique Mathez[1], Pierre de Truchis[1], Damien Ledu[1], Jean Claude melchior[1], Guislaine Carcelain[2], Jacques Izopet[3], Christian Perronne[1], John R. David[4]

[1]Raymond Poincaré Hosp, Garches; [2]Pitié-salpétrière Hosp, Paris; [3]Purpan Hosp, Toulouse; USA: [4]Harvard School of Public Health, Harvard Medical School, Boston

ABSTRACT

Background: Short intra-weekly cycles of anti-HIV combinations have provided intermittent effective therapy (JL et al, Faseb J, 2010, on 48 patients). The concept is now extended to 94 patients on treatment (Rx) 4 days a week (d/wk) or less over a median of 2.7 discontinuous treatment-years per patient

Patients and Combinations: on suppressive combinations, 94 patients volunteered to treatment 5 and 4 d/wk, or reduced stepwise to 4, 3, 2, 1 d/wk in respectively 94, 84, 66, 12 pts, on various triple, standard anti-viral combinations, or non-registered quadruple antiviral combinations.

Results: 94 patients on 4 d/wk Rx aggregated 165 intermittent treatment-years ; no viral breakthrough was observed over 87 average Rx-weeks per patient, 63/94 having passed 2,5 intermittent treatment years on any of the antiviral combinations prescribed. On the hyper intermittent Rx of 3 d/wk, 2 d/wk,1 d/wk, HIV RNA surged >50 copies 4 weeks apart in 18 instances (6.8 viral escapes/100 hyper discontinuous maintenance-years. Viral escapes could have been due to erratic adherence to regimen or follow-up (3 patients); drug taken at ½ the daily recommended dosage (8 pts), and/or overlooked archival resistant HIVs from antecedent treatment failures (6 pts). Aside from the above circumstances, HIV unexpectedly rebounded in 3 patients on 2 d/wk Rx and 1 patients on 1 d/wk Rx, posting 2.2 intrinsic viral escapes per 100 highly discontinuous treatment-years. All 18 escapes were eventually reversed by 7 day-a-wk salvage combinations and 11/18 patients have been back to a second course of intermittent therapy 4 days a 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, while CD4 counts went ≥ 500 /µl in 75 %. These values previously were 7 % and 40 % respectively on 7 d/wk therapy.

Conclusion: in our aging, long-HIV enduring, multi-treated patient cohort, on treatment 4 days a week and less over 421 intermittent treatment years reduced prescription medicines by 60 % - equivalent to 3 drug-free/ 3 virus-free remission years per patient- actually sparing 3 million euros on just 94 patients, at the cost of 2.2 intrinsic viral failure per 100 hyper intermittent treatment-years. At no risk of viral escape, maintenance therapy 4 days a week would quasi universally offer 40 % cuts off current over-prescriptions.

INTRODUCTION

Combined antiretroviral therapy 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[1]. Yet under effective antiretroviral therapy (ART or HAART), HIV persists as proviral DNA, forming a latent reservoir within resting memory CD4+Tcells ever since the time of primary HIV infections[2], recalcitrant to current antiviral therapy. As long as a fraction of such latently infected cells can carry inducible replication-competent proviruses **-** maintained throughout patient's life time by the proliferation of infected memory T cells or by their intermittent antigen-driven clonal expansion[3]**,-** 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 day a week antiviral therapy, as more relaxed adherence have in the past predicted poorer outcomes[4]. Yet the unremitting treatment doctrine was somewhat refuted by a landmark study in which 8 patients maintained optimal control on their HIV for a year on a 7 days Off / 7 days On weekly antiviral regimen[5.] Spurred by such results, which also highlighted the physicians' obligation to adjust prescriptions so as to not overtreat patient[6], in 2003 we asked our patient volunteers if they were willing to take their antiviral treatment 6 days, 5 days and 4 days each week while having their HIV viral load carefully monitored. Having considered the effectiveness of these intermittent regimens, we went to 3 days on treatment each week, eventually reduced to 2 days and 1 day a week. The present report consolidates and adds to our report on 48 patients[7], extended here to 94 patients on intermittent antiretroviral treatment 4 days a week or less for 6 months to 9 years, aggregating 420 intermittent treatment-years for an average 173 treatment-weeks (median 142 treatment weeks) per patient.

PATIENTS AND METHODS

Patients and weekly treatment regimens

Prescriptions for Intermittent maintenance therapy were written by one of us (JL) 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. Before entering the discontinuous maintenance period, all patients had been on continuous suppressive therapy with triple or quadruple antiretroviral combinations for the classic 7 days-a-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[I]. On 111 treatment episodes, 84 patients experienced treatment 3 days a week. On 87 treatment episodes, 66 patients experienced treatment 2 consecutive days a week. On 14 treatment episodes,12 patients experienced treatment on 1 day a week. At each intermittent treatment station, 2 or more plasma viremia (typically 2 to 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 a week treatment schedule, plasma viremias were checked every 8 to 10 weeks. For those on a 1 day a 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). Plasma HIV spikes over 50 copies/ml at 2 consecutive dosages within 1 month defined viral escape, prompting treatment re-adjustments. Blood lymphocyte counts and subsets were assayed on fresh EDTA blood, before treatment and at distant times under therapy, using an automated cyto-fluorometric 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 on-treatment per week - added one pair (out of 6) of NRTIs[II] either to one PI[III] or to one NNRTI backbone[IV]

I 47 of the 94 had had an intermediary 5 days on / 2 days off treatment period over 2626 cumulated weeks as reported (JL et al ***Faseb J*** **2010**); 47 patients reduced their weekly medicine intakes directly from 7 to 4 days.

II NRTI (Nucleoside analogue Reverse Transcriptase Inhibitor) preferred pair order - exclusive of thymidine analogues: emtricitabine+ tenofovir; or emtricitabine +didanosine; or emtricitabine +abacavir; or abacavir+ tenofovir; or didanosine + abacavir , or didanosine +tenofovir.

III PI (Protease Inhibitor) ritonavir-boosted : lopinavir 800 mg, or atazanavir 300 mg, or amprenavir 1200 mg, or darunavir 600-800 mg; or unboosted atazanavir 400 mg

Un-registered quadruple anti-reverse transcriptase compositions - prescribed for 4, 3 , 2 and 1 day on-treatment per week - combined 1 (out of 3) NNRTI[IV] with 1 of 3 NRTI triads[VI].

HIV-1 integrase inhibitor (raltegravir) -based compositions - for 4 and 3 days on-treatment per week – added : etravirine (NNRTI) + one ritonavir-boosted PI[8] in 5 patients ; or etravirine + 2 or 3 NRTIs in 4 patients; or emtricitabine +tenofovir (NRTIs) in 3 patients ; or emtricitabine +tenofovir +didanosine (NRTIs) in 4; or ritonavir-boosted lopinavir (PI) +didanosine (NRTI) in 1.

Prescriptions recommended taking the medication**s** at one time per day, preferably at bed time. Antiviral compositions in the individual patient might have changed on one or more occasions, because of unwanted side effects, or of a promising new drug combination, or of patients' preferences[9].

HIV-1 genotypes

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

Archival HIVs acquired through antecedent treatment failures. Genotypes from pre-intermittent treatments were available for 70 patients. In 25, 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); nevirapine / efavirenz (n=12); tenofovir (n=9); didanosine (n=8); zidovudine /stavudine (n=7).

Lymphocyte surface phenotypes

Freshly EDTA-collected blood was placed on Ficoll-Hypaque gradient and the mononucleated fraction was isolated, washed, and stored frozen in liquid nitrogen as part of our regular clinical repository. Monoclonal antibodies conjugated to either of five chromophores were obtained from Becton Dickinson. Frozen cell samples were thawed, washed and stained with the relevant pre-mixed 5-color antibody combinations[VII] for 15 min, washed in PBS containing 3% fetal bovine serum, fixed in 2% paraformaldehyde, and analyzed within 24 h on a Beckman Coulter CXP™ Cytometer under a Cell Quest Software (Becton Dickinson). Cell activation markers (CD38, HLA DR, PD-1 molecules on the surface of live T cells) were numbered after calibration with

---

IV NNRTI (Non-nucleoside Reverse Transcriptase Inhibitor): efavirenz preferably to etravirine, nevirapine being excluded as a base drug as standard triple combination

V preferred NNRTI order: nevirapine (400 mg), or efavirenz (600mg or less), or etravirine (400 mg)

VI preferred NRTI triad order : emtricitabine +tenofovir +didanosine ; or emtricitabine +tenofovir +abacavir; or didanosine +abacavir +tenofovir

VII *ECD* tagged CD3 ; *PC5* tagged CD4; *FITC* tagged CD45RA; *PC7* tagged CD197 (CCR7) PE-CyTM7 ; *PE* tagged antibodies marked cell-surface PD1 or CD38 or HLA-DR proteins. The relevant lymphocyte populations were characterized as follows : total CD4 T cells: *CD3+CD4+;* total CD8 T cells: *CD3+CD4-;* CD4 naïve: *CD3+CD4+CD45RA+CCR7+;* CD8 naïve: *CD3+CD4-CD45RA+CCR7+;* effector memory CD4: *CD3+CD4+CD45RA-CCR7-;* effector memory CD8: *CD3+;CD4-; CD45RA-; CCR7-;* central memory CD4: *CD3+CD4+CD45RA-CCR7+;* central memory CD8: *CD3+CD4-CD45RA+CCR7+;* terminally differentiated effector memory CD4 T cells: *CD3+CD4+ CD45RA+CCR7-;* terminally differentiated effector memory CD8 T cells: *CD3+CD4-CD45RA+CCR7- ;*

QuantiBRITE Beads[10]

Quantifying cell-associated HIV-1 DNA

In brief[11], quantitation was performed by real-time PCR using TaqMan probes on a Light Cycler 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 using primers that annealed in the U5 region of the LTR and in the 5' end of the gag gene. Copy numbers of total HIV-1 DNA was determined in reference to a standard curve prepared by amplification of quantities ranging 10 to 105 copies of cloned HIV DNA.

RESULTS

Patient characteristics at baseline, Table 1

The 94 volunteers were part of an ambulatory cohort followed for an HIV-1 infection over an average 17 years. One or more clinical or immunological 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 patients (58 %) and 26 patients (27%) respectively. The zenith of the plasma retroviral copies/mL before therapy was ≥ 100 000 in 75 patients (80%). Previous conventional antiviral treatments (1 to 9 episodes) were seen in 71 patients (75%), which was interspersed in many patients with momentary treatment-free intervals. Only 17 patients had their first 7 days-a-week attack treatment once before discontinuous maintenance therapy. All 94 went through a continuous 7 days a week treatment for 5 to 75 months on diverse triple or quadruple drug combinations before commencing discontinuous maintenance therapy.
Examples of patients under ICCARRE can be seen in Figure 1 A, B, C, D

On intermittent treatment 4 days a week : antiviral performance, all drug combinations, Figure 2.

HIV was optimally controlled without any failures in 160 attempts extended over 8164 intermittent treatment-weeks in 94 patients on either standard triple combinations (63 % of the 4-d/wk Rx), or novel anti RT quadruple compositions (25 %), or raltegravir-based triple or quadruple combinations (11 %). Standard triple combinations combined 2 NRTIs to a protease inhibitor in 50 % of prescriptions at that level of intermittence, or to a NNRTI, mostly efavirenz (36% of all standard triple combinations prescribed at that level). Tetra RT inhibitor compositions associated 3 NRTIs + 1 NRTI base, 72 % nevirapine-based. Raltegravir was combined to a boosted protease inhibitor + one NNRTI, mostly etravirine (as in TRIO[8]) in 59 % of prescriptions with that HIV integrase inhibitor.

On super short cycles of anti retrovirals 3 or 2 or 1 day a week: antiviral performances, all combinations, Table II, Table III.

Three days-a-week super intermittent therapy was carried out over 115 treatment episodes, optimally maintaining antiviral effectiveness for 5694 treatment-weeks in 88 patients. HIV escaped control in 10 instances at a rate of 9.2 per 100 highly intermittent treatment-years. Two days-a-week on super intermittent therapy over 74 treatment episodes maintained effectiveness in 66 patients over 7176 treatment-weeks, control failing on 7 instances at a rate of 5 escapes per 100 super intermittent treatment-years. One day a week on super intermittent therapy over 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 super intermittent treatment-years, however, leading to our termination of the 1 day-a-week regimen[VIII.] 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 anti retrovirals 3 or 2 or 1 day a week: antiviral performances depending on the medicinal combination (Table II, Table III).

On standard triple combinations given 3 and 2 days a 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. Further, on tetra anti RT combinations given 3, 2, and 1 day a week to respectively 60, 60 and 12 patients on 57, 76, 14 occasions, HIV was fully controlled over 10192 treatment-weeks , interrupted by 6+7+1 HIV escapes, respectively on 3, 2, and 1 day a week treatments, for an overall failure rate of 7 per

VIII except for 5 self-determined volunteers

100 super intermittent treatment-years under such combinations. In contrast, on anti-integrase-based combinations given 3 days a week to 9 patients for 185 treatment-weeks, HIV eventually rebounded in 3 of 3 patients under raltegravir + 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 MaintenanceTherapy : Contextual Circumstances and Outcomes (See Tables II and III)

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

Of the 18 viral escape episodes encountered at 3, 2 1 day-a-week treatment, 14 occurred in the context of 3 plausibly etiological circumstances: overlooked resistant viruses from past treatment failures (in patients 1, 2, 3, 4a, 4b, 6); a drug-base given below the recommended daily dose[IX]: raltegravir 400 mg once a day instead of 400 mg twice a day (patients 4a, 10, 11a); nevirapine (pt 6), or etravirine (pt 7) took 200 mg daily instead of 400; lopinavir (pt 8) took 400 mg in one daily take instead of 800 mg in two takings; patient 9 failed a full dose of her etravirine-based combination after she developed clinical steatorrhea from an insoluble carbohydrate fiber diet, altogether entailing the malabsorption of the lipid-soluble NNRTI[12]. Acute erratic adherence to treatment and/or follow-up was noted in pts 11b, 13, 14.

B. Viral escapes with no particular etiological context

Of 49 patients on our preferred nevirapine-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 15,16,17 were on a 2 days a weekRx and patient 18 was on a 1 day a week Rx. This was a rate of 2.2 intrinsic failures per 100 super intermittent treatment-years.

Viral escapes under intermittent therapy: outcomes (table III)

Of the 18 viral escapes, HIVs bearing newly acquired resistance mutations emerged in 12 instances with one mutation to NTRIs (pts 4a, 8, 10, 11a) or to NNRTIs (pts18, 19), two mutations to NRTI and NNRTI (pts 11b, 16, 17), three mutations (pts 9, 18) and five mutations (pt 15). All escaped mutants were effectively countered by adjusting the antiviral regimens to 7 days a week triple or quadruple salvage combinations, the composition of which was chosen to circumvent the emerged mutations. Six months after a 7 days a week re-induction period, intermittent maintenance schedules were successfully re-instated (Column J Table III), eventually down to discontinuous treatment : 5 days a week (pt 14), 4 days a week (pts 1, 2, 6, 13), 3 days a week (pts 4a, 7), or 2 days a week (pts 8, 9, 10, 11a). As further taken up in our discussion, viral escape rates on 3, 2, or 1 day a week therapy matched those reported in most studies with 7 days a week maintenance treatment[19-21].

Lymphocyte Activation Markers and Discontinuous Maintenance Therapy (Table IV)

Fluorescent monoclonal antibodies to CD3, CD4, CD8, CD38, HLA-DR and PD1 surface proteins, evocative of an "on-going activation" process, were applied to sequential live frozen blood mononucleated cell specimens. These were obtained before treatment, on treatment 7 days a week, on intermittent treatment 4 days a week, and on 3 or 2 or 1 day a week, and had been stored in our clinical specimen repository. The cyto-fluorometric evaluation of the 5 most representative T lymphocyte subpopulations are presented in table IV. Before antiretroviral treatment, cell-surface expression levels ranged from 2 to 10 times higher than those of HIV

IX In treatment episodes 4a and 6, patients 4 and 6 cumulated both an archival resistant virus and sub-optimal base-drug dosage when HIV escaped on a 3-days-a week and 2 days-a-week regimen.

negative controls; under continuous treatment in the patients, the levels plummeted 0.6 to 5 folds 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) as compared to 56 patients (from an independent local patient cohort) on unremittent 7 day-a-week treatment for a matched median treatment time (see legend table IV).

Cell-associated HIV DNA and Discontinuous Maintenance Therapy (table V)
Pro-viral 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. Before antiviral treatment, cell-associated HIV DNA had ranged at average high levels relatively to other published cohort[13] ; under continuous 7 day-a-week treatment, levels plummeted by 70 to 75 %, subsiding further or remaining low and stable under discontinuous treatment at 20 % of pre-ART values.

DISCUSSION

High levels of adherence to anti-retroviral treatment have generally been deemed necessary for optimal viral responses as the probability of long-term viral suppression statistically followed (close to) perfect adherence to 7 day-a-week ARV regimens[14]. However, unrelenting life-time HAART, with its physiological and psychological constraints, pending toxicities, soaring costs, and the challenges with unremitting long-term adherence[15], have been calling for treatment alleviation.

ICCARRE 4 days treatment a week : not a single HIV escape.

Our study of short intermittent anti-viral treatment cycles of 4 days a week (one step down from the previously tried 5 days a week regimen[16]) 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 taking of half the daily recommended dose of a drug base in 11 patients, there was not one viral escape over 8164 weeks. The average patient benefited from 262 *virus-free / drug free days*. Treatment 4 days a week removed 40 % off 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 a week should benefit from ICCARRE 4 days a week

Our long term virus carriers - infected with HIV for an average 17 years - came to intermittent maintenance therapy following long medical histories encompassing AIDS-defining conditions, low CD4, high pre-treatment viral loads, past treatment failures, archived resistant HIVs, dense cell-activation markers and abundant cell-borne pro-viral DNA[X], the latter purportedly predictive of treatment failure[17]. The very patchiness and overall vulnerability of this uncontrolled selection of patients would support a general applicability of the 4 days a week regimen to many nay 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[18].

Super Intermittent Treatment *3,2,1 day a week*: work in progress still requiring caution (Table II and III).

In 84 patients, ARVs were reduced to 3 days week over 70 average treatment-weeks met with 10 viral escapes; in 66 patients, treatment was reduced to 2 days a week over an extended 109 average treatment-weeks, encountering 7 viral escapes; 12 patients went on 1 day a week schedule over 71 treatment-weeks, ending in 1 viral escape. 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-a-week combinations[19, 20], yet lower than on unremitting protease inhibitor monotherapy[21]. Congruent with the tentative exploratory nature of our prescriptions, 14 of the 18 escapes could reasonably be linked to either unfitting prescriptions or erratic adherence (table V). 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

X In 6 patients who successfully endured 2 days or 1 day a wk RX over 825 treatment-weeks, proviral DNA at the onset of ART had totalled an average high 6500 copies per million cells

treatment years, in line with 7 day treatment standards, and way upbeat from PI monotherapies (see references[19, 20, 21]).

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, or 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, 13 for 6 months or more, in an attempt to "drown out" the recently mutated HIV in a wave of returning wild-type HIV[22]. And so, patients 5, 8,13 could resume 7 day a week attack combinations comprising drugs previously deemed ineffective against the mutant virus at the time of escape[XI,XII]. In all 18 instances, under proper 7 days a week salvage combinations, HIV loads fell to undetectable levels; 11 of the 18 have since undertaken a second ICCARRE course, down to 4 days a week (patients 1, 2, 6, 13), 3 days a week (patients 3, 4a, 7, 9), or 2 days a week (patients 2, 8, 10, 11a), for an average 82 weeks as of this writing (table V column j).

Of note, archival resistant HIV strains, which eventually resurfaced on a 3 days a week schedule (patients 1, 2, 3 4a, 4b), or on a 2 days a week schedule (patients 5,11), had been kept in check on the precedent 4 days or 3 days week schedule, on that same combination which proved ineffective at a lower intermittent schedule. This emphasizes the safety of the 4 days a week treatment schedule on all combination types prescribed, including those turning sub-optimal at lower intermittent schedules[XIII].

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 less than 2 functionally effective antivirals, the mutant HIV eventually broke through at 3 or 2 days a week treatment schedule[XIV]. Whatever the underlying physiological or pharmacological requisites, less than three functionally additive or potentiating drug combinations should not be considered for intermittent maintenance below 4 days a week on therapy.

Hyper intermittent maintenance regimens 2 days and 1 day a week on a nevirapine-based tetra RT combination turned out safe and effective over an average 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 a week, until markers predictive of failure or success on hyper intermittent combinations and

---

XI The fact that the combinations, now successful on a 7 days (patient 8), 4 days (patients 5) or 3 days a week regimen (patient 13), comprised one or more antiviral components genotypically "unfit" against the pre-interruption mutant virus supports the notion that the mutant species had functionally been "washed out".

XII Patient 8 whose emerging virus carried four *de novo*-acquired mutations went *off* all antivirals for 3 months. He since has been back on a virally effective triple combination comprising 2 antiviral components genotypically ineffective against the escaped mutant.

XIII Conversely, in view of the 84 per 100 failure rate with raltegravir-based combinations given 3 days a week, and until thorough investigations on the topic have been conducted, integrase inhibitor-based combinations cannot be recommended for ICCARRE

XIV The apparent need for more than a dual antiviral combination echoes with both the weakness of mono-drug maintenance on boosted protease inhibitors and the failures with induction-alleviated maintenance (referenced)

regimens come to the clinic. Pharmacological quantitation of residual anti viral drugs within mononucleated cells of patients under intermittent treatment might eventually help at resolving such issue[23].

ICCARRE, a refutation of the dogma never to give less than 7 days a week anti-viral treatment.

Short intermittent weekly cycles of antivirals were initiated based on the fact that patients' lymphoid system under HAART would be altered in the direction of a de-activated quiescent state[24], less favourable to HIV replication[25]. 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 on treatment, to 7 days and more during treatment cessation after effective therapy[26]. These physio-pathological alterations provided the basis for the ~~trial of~~ *7-day-on* treatment */ 7-day-off* trial launched by Dybul et al, 2001 (reference[5]). That HIV did not rebound during the 24 off treatment periods in 8 patients led the investigators to successfully repeat their feat in 2004 with other triple ARV combinations[27]. Yet, following less favourable reports from various sources[28], the flagship team retreated, stressing the significantly increased risk of failure with treatment interruptions beyond 2 days[29].

In defiance of these revolving conclusions, our 94 participants selected from a small cohort of ambulatory patients benefitted from discontinuous treatment 4 days a 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 both cell activation markers and proviral DNA counts in mononucleated blood cells, not sensibly different from patients with continuous treatment over similar treatment durations (table V). Blood CD4 T cell counts continued on their pre-intermittence 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 hepatitis virus C co-infected patients on discontinuous anti HIV regimens 4 days a week for 1 patient, or 2 days a week for 2 patients, were cured of their liver infections[XV]. Of especially happy note, 6 different HIV couples in whom the HIV-positive partner was on discontinuous therapy 4 days a week or less, conceived and gave birth to 10 HIV-free babies, the un-protected HIV negative parents having remained free of HIV contamination.

At a time when only one quarter of HIV-infected people in the United States have successfully navigated the present care continuum to achieve undetectable viral load[30], making ARV treatment more attractive, more binding, with decreasing toxicity to enhance acceptability while maintaining antiviral efficacy, are just what prescribers hoped they could accomplish since the early years of triple combination therapy. Yet not just any medicinal treatment simplification will do : alleviating triple combination therapy with two drugs as maintenance therapy 7 days a week once radically failed[31]; ritonavir-boosted protease inhibitor monotherapies 7 days a week have produced viral escape rates above current triple combination standards (reference[21]).

In conclusion: By cutting into overmedication by 40 to 85 %, ICCARRE offered an average 3 drug-free/ virus-free remission years per patient, saving some 3 million euros for just 94 patients, at the cost of 2.2 intrinsic antiviral failures per 100 1day/ 2days intermittent treatment-years. And to emphasize again, on the 4 days a week schedule, there was not one HIV viral escape in the 94 patient. Over 10 years this would amount to a 4-year drug-free / virus-free exemption from unnecessary over medication.

XV on a ribavirine / pegylated alpha interferon dual therapy, occasionally re-inforced by recombinant erythropoietin

1 Ledergerber B, Cavassini M, Battegay M et al.Trends over time of virological and immunological characteristics in the Swiss HIV Cohort Study. ***HIV Med*** **2011**;12(5):279-88 ; Kaulich-Bartz J, Dam W, May MT, et al. Insurability of HIV-positive people treated with antiretroviral therapy in Europe. ***AIDS*** **2013** ;27(10):1641-55.

2 Finzi D, Hermankova M, Pierson T et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. ***Science*** **1997** 278, 1295–1300 ; Chun TW, Engel D, Berrey MM, et al. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. ***PNAS*** **1998**;95:8869-73; Siliciano JD, Kajdas J, Finzi D, et al : Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. ***Nat Med*** **2003**,9(6):727-728

3 Joos B, Fischer M, Kuster H et al. HIV rebounds from latently infected cells, rather than from continuing low-level replication. ***PNAS 2008***, 105:16725-30 ; Ho YC, Shan L, Hosmane NN, et al. Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure. ***Cell*** **2013**, 155(3):540-51; Siliciano JD, Siliciano RF. HIV-1 eradication strategies: design and assessment. ***Curr Opin HIV AIDS*** **2013** 8: 318–325.

4 Paterson DL, Swindells S, Mohr J, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. ***Ann Intern Med*** **2000**, 133(1):21-30 ; Dybul M, Fauci AS, Bartlett JG, et al. Panel on Clinical Practices for Treatment of HIV. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. ***Ann Intern Med*** **2002**, 137(5 Pt 2):381-433 ; Ferguson NM, Donnelly CA, Hooper J, et al. Adherence to antiretroviral therapy and its impact on clinical outcome in HIV-infected patients. ***J R Soc Interface*** **2005**;2:349–363

5 Dybul M, Chun TW, Yoder C, et al. Short-cycle structured intermittent treatment of chronic HIV infection with highly active antiretroviral therapy: effects on virologic, immunologic, and toxicity parameters. ***PNAS*** **2001**; 98(26):15161-6

6 "*Physicians have a responsibility to practice efficient health care and use health care resources responsibly. Parsimonious care utilizes the most efficient means to effectively treat a patient*", ***American College of Physicians*** **2012** Manual Code of Ethics, *Sixth Edition*

7 Leibowitch J, Mathez D, de Truchis P, et al. Short cycles of antiretroviral drugs provide intermittent yet effective therapy: a pilot study in 48 patients with chronic HIV infection. **FASEB J 2010,** 24(6):1649-55

8 Yazdanpanah Y, Fagard C, Descamps D, et al.High rate of virologic suppression with raltegravir plus etravirine and darunavir/ritonavir among treatment-experienced patients infected with multidrug-resistant HIV: ANRS 139 **TRIO** trial. ***Clin Infect Dis*** **2009**, 49(9):1441-9

9 Boyle A, Sonecha S, Mandalia S, Nelson M. An investigation into frequency and reasons why patients switch antiretroviral therapy and which antiretrovirals are commonly implicated in toxicity. ***J Int AIDS Soc*** **2012,** 15(6):18121

10 Sauce D, Almeida JR, Larsen M, et al. PD-1 expression on human CD8 T cells depends on both state of differentiation and activation status ***AIDS*** **2007**, 21(15):2005-13

11 Izopet J, Cazabat M, Pasquier C, et al. Evolution of total and integrated HIV-1 DNA and change in DNA sequences in patients with sustained plasma virus suppression. ***Virology*** **2002**, 302(2):393-404

12 Kesäniemi YA, Tarpila S, Miettinen TA. Low versus high dietary fiber and serum, biliary, and fecal lipids in middle-aged men. *Am J Clin Nutr*. 1990, 51(6):1007-12; Kent SJ. Loss of control of HIV viremia associated with the fat malabsorption drug orlistat. ***AIDS Res Hum Retroviruses*****. 2012;** 28(9):961-2; Tseng AL, la Porte C, Salit IE. Significant interaction between activated charcoal and antiretroviral therapy leading to subtherapeutic drug concentrations, virological breakthrough and development of resistance. ***Antivir Ther*** **2013**;18(5):735-8

13 Lambert-Niclot S, Flandre P, Valantin MA, Soulie C, et al. Similar evolution of cellular HIV-1 DNA level in darunavir/ritonavir monotherapy versus triple therapy in MONOI-ANRS136 trial over 96 weeks. ***PLoS One*** **2012**;7(7):e41390

14 Lucas GM, Chaisson RE, Moore RD. Highly active antiretroviral therapy in a large urban clinic: risk factors for virologic failure and adverse drug reactions. ***Ann Intern Med*** **1999**, 131(2):81-87; Glass TR, Rotger M, Telenti A, et al. Determinants of sustained viral suppression in HIV-infected patients with self-reported poor adherence to antiretroviral therapy. ***PLoS One***. **2012**;7(1):e29186 ; Wilson IB, Bangsberg DR, Shen J, et al. Rates of Decline of Antiretroviral Therapy Adherence Over Time. ***JAIDS*** **2013**, 64 : 48–454

15 Thompson MA, Aberg JA, Cahn P, et al. Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society-USA panel. ***JAMA*** **2010**; 304:321–333 ; EACS Guidelines: clinical management and treatment of HIV-infected adults in Europe **2011** http://www.europeanaidsclinicalsociety.org; Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents/Adherence to Antiretroviral Therapy, **2014**, ***HIV/AIDS Treatment Guidelines*** | ***AIDS info***

16 Cohen CJ, Colson AE, Sheble-Hall AG, et al. Pilot study of a novel short-cycle antiretroviral treatment interruption strategy: 48-week results of the five-days-on, two-days-off (FOTO) study. ***HIV Clin Trials*** **2007**, 8(1):19-23.

17 Piketty C, Weiss L, Assoumou L, et al, ANRS 116 SALTO Study. A high HIV DNA level in PBMCs at antiretroviral treatment interruption predicts a shorter time to treatment resumption ***J Med Virol*** **2010,** 82(11):1819-28; Boulassel MR, Chomont N, Pai NP, et al. CD4 T cell nadir independently predicts the magnitude of the HIV reservoir after prolonged suppressive antiretroviral therapy. ***J Clin Virol*** **2012**;53(1):29-32 ; Fourati S, Flandre P, Calin R, et al. Factors associated with a low HIV reservoir in patients with prolonged suppressive antiretroviral therapy. ***J Antimicrob Chemother*** **2014**, 69(3):753-6

18 Mugavero M, Lin H, Willig J, et al. Missed visits and mortality among patients establishing initial outpatient HIV treatment. ***Clin Infect Dis*** **2009***;48(2):248-256*

19 Ledergerber B, Egger M, Opravil M, et al , for the Swiss HIV Cohort Study. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. ***The Lancet*** **1999,** 353(9156) 863-868 ; Ianotti N, Galli L, Racca S, et al. Residual viraemia does not influence 1 year virological rebound in HIV-infected patients with HIV RNA persistently below 50 copies/mL. ***J Antimicrob Chemother*** **2012**;67:213-7; Charpentier C, Landman R, Laouénan C,et al. Persistent low-level HIV-1 RNA between 20 and 50 copies/mL in antiretroviral-treated patients: associated factors and virological outcome. ***J Antimicrob Chemother*** **2012,** 67(9):2231-5;

20 Gatell J, Salmon-Ceron D, Lazzarin A et al. Efficacy / safety of atazanavir-based HAART in patients with virologic suppression switched from a boosted /unboosted protease inhibitor regimen. ***Clin Infect Dis*** **2007**; 44: 1484–92; Ghosn J, Carosi G, Moreno S et al. Unboosted atazanavir-based therapy maintains control of HIV type-1 replication as effectively as a ritonavir-boosted regimen. ***Antivir Ther*** **2010**; 15: 993–1002; Giuntini R, Martinelli C, Ricci E, et al. Efficacy and safety of boosted and unboosted atazanavir-containing antiretroviral regimens in real life: The SCOLTA multicenter study. ***HIV Med*** **2010, 11: 40–45 ;** Hocqueloux P, Choisy g, Le moal et al. pharmacologic boosting of atazanavir in maintenance hiv-1 therapy : the coreya study. ***Plos one*** **2012**, **7(11):** e49289 ; Molina JM, Clumeck N, Orkin C, et al. Week 96 analysis of rilpivirine or efavirenz in HIV-1-infected patients with baseline viral load ≤ 100 000 copies/mL in the pooled ECHO and THRIVE phase 3, randomized, double-blind trials. ***HIV Med*** **2014**, 15(1):57-62; Rokx C, Fibriani A, van de Vijver DA,et al. Increased Virological Failure in Naive HIV-1-Infected Patients Taking Lamivudine Compared With Emtricitabine in Combination With Tenofovir and Efavirenz or Nevirapine in the Dutch Nationwide ATHENA Cohort. ***Clin Infect Dis*** **2014,** doi: 10.1093/cid/ciu763**;** Gantner P, Koeppel C, Partisani M, et al. Efficacy and safety of switching to raltegravir plus atazanavir dual therapy in pretreated HIV-1-infected patients over 144 weeks: a cohort study. ***Scand J Infect Dis*** **2014**, 17:1-8 ; Baril J, Conway B, Giguère P, et al. A meta-analysis of the efficacy and safety of unboosted atazanavir compared with ritonavir-boosted protease inhibitor maintenance therapy in HIV-infected adults with established virological suppression after induction. ***HIV Med*** **2014,** 15(5):301-10

21 Bierman WF, van Agtmael MA, Nijhuis M, et al. HIV monotherapy with ritonavir-boosted protease inhibitors: a systematic review. ***AIDS*** **2009**, 23(3):279–291; Arribas JR, Horban A, Gerstoft J, et al. The MONET trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV RNA below 50 copies/ml. ***AIDS*** **2010**, 24(2):223–230; Mathis S, Khanlari B, Pulido F, et al. Effectiveness of protease inhibitor monotherapy versus combination antiretroviral maintenance therapy: a meta-analysis. ***PLoS One*** **2011**, doi 6:e22003; Guiguet M, Ghosn J, Duvivier C et al. Boosted protease inhibitor monotherapy as a maintenance strategy: an observational study. ***AIDS*** **2012**, 26(18):2345-2350

22 Devereux HL, Youle M, Johnson MA, et al. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. ***AIDS*** **1999**;13(18):F123-127; Tseng A, Foisy M. Handbook and website on HIV drug therapy. ***Am J Health Syst Pharm*** **2012**, 69(15):1284-86

23 Zehnacker L, Abe E, Mathez D, et al. Plasma and Intracellular Antiretroviral Concentrations in HIV-Infected Patients under Short Cycles of Antiretroviral Therapy, ***AIDS Research and Treatment*****, 2014**, ID 724958

24 Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. ***Science*** **1997**, 277(5322):112-

25 Stevenson M, Stanwick TL, Dempsey MP, et al. HIV-1 replication is controlled at the level of T cell activation and proviral integration. **EMBO J 1990**; 9:1551–1560; Zhang ZQ, Wietgrefe SW, Li Q, et al. Roles of substrate availability and infection of resting and activated CD4+ T cells in transmission and acute simian immunodeficiency virus infection. ***PNAS*** **2004** 101: 5640–5645; Pan X, Baldauf H, Keppler OT, et al. Restrictions to HIV-1 replication in resting CD4(+)T lymphocytes ***Cell Res*** **2013,** 23: 876–885

26 Davey RT Jr, Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. ***PNAS*** **1999**, 96(26):15109-14; Garcia F, Plana M, Vidal C, et al. Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy. ***AIDS*** **1999;**13(6):677-83; Neumann AU, Tubiana R, Calvez V, et al. HIV-1 rebound during interruption of highly active antiretroviral therapy has no deleterious effect on reinitiated treatment. Comet Study Group. ***AIDS*** **1999**, 13:677–683

27 Dybul M, Nies-Kraske E, Dewar R, et al. A proof-of-concept study of short-cycle intermittent antiretroviral therapy with a once-daily regimen of didanosine, lamivudine, and efavirenz for the treatment of chronic HIV infection. ***J Infect Dis*** **2004**, 189(11):1974-82

28 Ananworanich J, Nuesch R, Le BM, et al. Failures of 1 week on, 1 week off antiretroviral therapies in a randomized trial. ***AIDS*** **2003** 17: F33–F37 **;** Parienti JJ, Das-Douglas M, Massari V, et al. Not all missed doses are the same: sustained NNRTI treatment interruptions predict HIV rebound at low-to-moderate adherence levels. ***PLoS ONE*** **2008**. 3: e2783; Rudy BJ, Sleasman J , Kapogiannis R, et al The Adolescent Trials Network for HIV/AIDS Interventions. Short-Cycle Therapy in Adolescents after Continuous Therapy with Established Viral Suppression: The Impact on Viral Load Suppression ***AIDS Res Hum Retroviruses*** **2009**, 25(6): 555–561

29 Reynolds SJ, Kityo C, Hallahan CW, et al. A randomized, controlled, trial of short cycle intermittent compared to continuous antiretroviral therapy for the treatment of HIV infection in Uganda. ***PLoS One*** **2010**, 5(4):e10307

30 Fauci A, Marston HD. Ending AIDS—is an HIV vaccine necessary ? ***N Engl J Med*** **2014**, 370:495

31 Reijers MH, Weverling GJ, Jurriaans S, et al. Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: ADAM study. ***Lancet*** 1998, 352:185–190; Havlir DV, Marschner IC, Hirsch MS, et al. Maintenance antiretroviral therapies in HIV infected patients with undetectable plasma HIV RNA after triple-drug therapy. ACTG 343 Team. ***N Engl J Med*** 1998, 339:1261–1268; Pialoux G, Raffi F, Brun-Vezinet F, et al. A randomized trial of three maintenance regimens given after three months of induction therapy with zidovudine, lamivudine, and indinavir in previously untreated HIV-1-infected patients. Trilege ANRS 072. ***N Engl J Med*** 1998, 339:1269–1276; Descamps D, Flandre P, Calvez V, et al. Mechanisms of virologic failure in previously untreated HIV-infected patients from a trial of induction-maintenance therapy. ***JAMA*** 2000, 283:205–211