From *in vitro* to *in vivo* quantification of antiretroviral drugs effects based on dynamical models of HIV

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Abstract
Population dynamics of HIV and CD4+ T cells can be modeled with Ordinary Differential Equations (ODE). We aim at quantifying the *in vivo* effect of combinations of antiretroviral drugs treatments (cARTs) by a function of the effects of the antiretroviral drugs (ARVs) in the combination. To estimate the ARVs effects we must have a large dataset and it is desirable to add external information to ensure identifiability. An adequate modeling of *in vitro* assays yields such information. Recent single-round infectivity assays allowed quantifying the dose-response curves *in vitro*: the instantaneous inhibitory potential (IIP) has been established as a measure of ARVs activity. The IIPs of cARTs can be viewed as a function of ARV's IIPs based on known interactions. Bliss independence is a convenient assumption to build dynamical models. Random effects account for inter-individual variability of IIPs that may result from host and virus genetics. Finally, more flexibility is provided by estimating an *in vitro* to *in vivo* conversion factor. We used a Bayesian approach for estimating the ARVs effects: cARTs effects follow by computation. We demonstrate that this model has good fit abilities and that our approach opens the perspective to deeper the understanding of treatment action regarding adherence and latent reservoirs and to envisage treatment choice optimization. This analysis is applied to a dataset of 350 patients taking 7 different antiretroviral drugs (AZT, D4T, ddI, 3TC, LPV, APV, DRV) from two stand-alone clinical trial: ALBI, PUZZLE, and two clinical trials nested in the ANRS CO3 Aquitaine Cohort: ZEPHIR and PREDIZISTA.

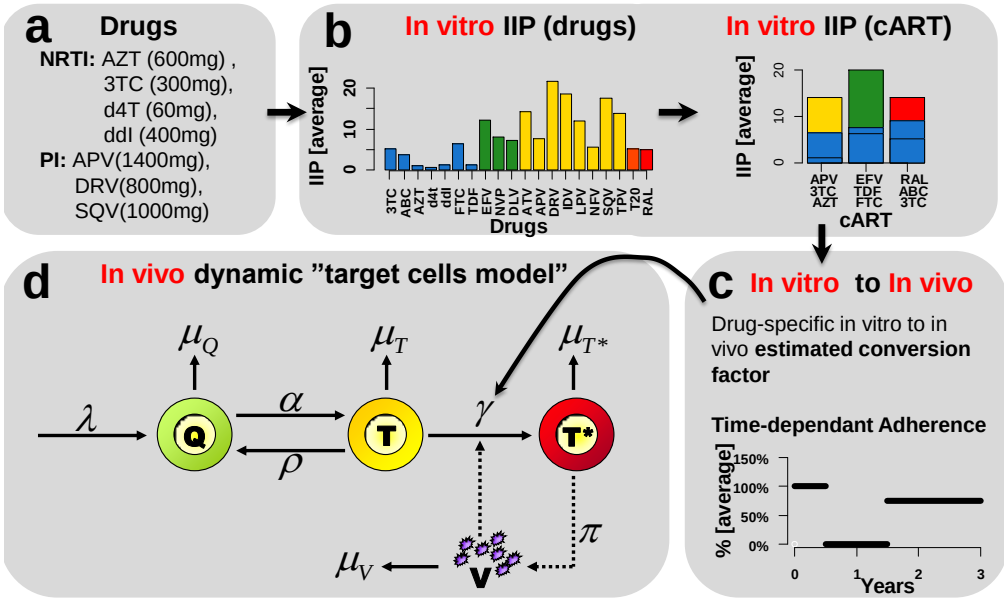


Figure 1. Graphical abstract. (a) the 7 drugs studied in the application (b) have intrinsic antiviral activities called IIPs evaluated *in vitro*. Assuming Bliss independence IIPs of cART can be computed from drugs IIPs by summation. (c) Estimated conversion factor and patients' adherence information allow us to use *in vitro* IIPs in (d) the *in vivo* "target cells model" of the dynamics between HIV and the immune system.

Biological and Pharmacological Model

Mathematical model. We recall the "target cells model" (see Figure 1.d). This is exactly similar to the "activated cells model" but was renamed to avoid over interpretation regarding to the term "activation".

$$\begin{cases} \frac{dQ}{dt} &= \lambda + \rho T - \alpha Q - \mu_Q Q, \\ \frac{dT}{dt} &= \alpha Q - \gamma T V - \rho T - \mu_T T, \\ \frac{dT^*}{dt} &= \gamma T V - \mu_{T^*} T^*, \\ \frac{dV}{dt} &= \pi T^* - \mu_V V, \end{cases}$$

***In vitro* pharmacodynamics.** [?] designed the instantaneous inhibitory potential (IIP) as a measure of antiviral activity. It is the number of logs single-round infection events that are prevented by the treatment. Thus, it is related to the fraction of cells unaffected by an antiretroviral drug X, f_u^X, that we will call fraction of infectible cells. Then, IIP depends on the drug intake $d_X(t)$, the concentration producing 50% inhibition of viral replication (IC_{50X}) and the Hill coefficient (m_X), see [?] for definitions.

$$\begin{aligned} \mathrm{IIP}_X(d_X(t)) &= \log\left(1 + \left(\frac{\mathrm{d_X(t)}}{\mathrm{IC_{50X}}}\right)^{\mathrm{m_X}}\right), \\ &= -\log(\mathrm{f_u^X(t)}). \end{aligned}$$

[?] showed that drug-drug interaction in combination of antiretroviral therapies (cART) obeys most of the time Bliss independence [?]. In other words, drugs act through competitive phenomenon on the same receptors, thus, f_u^{cART} is the product of the fraction of infectible cells for each antiretroviral in the cART.

$$\textit{In vitro}: \ \mathrm{IIP_{cART}}(d_{X_1}(t), \ldots, d_{X_{n_{cART}}}(t)) = -\log(\mathrm{f_u^{cART}(t)}),$$

$$
\begin{aligned}
&= -\log(\mathrm{f_u^{X_1}(t)} \times \ldots \times \mathrm{f_u^{X_{n_{cART}}}(t)}),\\
&= \sum_{i=1}^{n_{cART}} \mathrm{IIP}_{X_i}(d_{X_i}(t)).
\end{aligned}
$$

Finally, we model a drug-specific conversion factor (β's) between *in vitro* and *in vivo* IIPs. This gives the model more flexibility compared to β's common to all drugs [**?**, **?**] or class-specific [**?**]. If *in vitro* and *in vivo* are equivalent $\beta = 1.0$.

$$
\textit{In vivo}: \ \mathrm{IIP_{cART}}(d_{X_1}(t), \ldots, d_{X_{n_{cART}}}(t)) \ = \ \sum_{i=1}^{n_{cART}} \beta_{X_i} \mathrm{IIP}_{X_i}(d_{X_i}(t)).
$$

***In vivo* dynamical modeling of effects of cART.** NRTI, NNRTI, FI and II directly act on the fraction of infectible T cells: under such antiretroviral drug X, only $f_u^X T$ cells can be infected. PI plays on the number of T^* cells producing viruses, thus only $f_u^X V$ viruses can infect T cells. For all cARTs, all happens such as f_u^X modifies γ in a multiplicative way. Figure 2 shows the impact of f_u^{cART} on the law mass action of production of T^* cells Altogether, impact of cART is on infectivity γ. We denote $\tilde{\gamma}$ the log-infectivity under

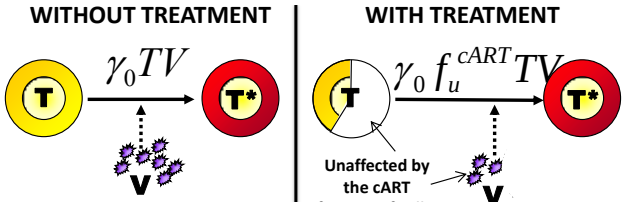


Figure 2. Effect of cART on the infectivity parameter.

treatment and $\tilde{\gamma}_0$ the base infectivity without treatment :

$$
\tilde{\gamma} = \tilde{\gamma}_0 - \sum_{i=1}^{n_{cART}} \beta_{X_i} \mathrm{IIP}_{X_i}(d_{X_i}(t))
$$

Statistical and Observational Model

Statistical Model. We use a mixed effect model, with the structure proposed by [**?**], on the ODE parameters in log-transformation to ensure their positivity. Normally distributed random effects are put on immune system cells input ($\tilde{\lambda}$) and death rate of infected cells ($\tilde{\mu}_{T^*}$) which is consistent with biological mechanism knowledge [**?**, **?**]. An additional random effect is put on γ to model variability in subject's response to treatment potentially due to pharmacogenomics [**?**] or virus mutations [**?**, **?**]. This modeling is consistent with *in vitro* knowledge. Actually, [**?**] showed that for the same concentration of cART, IIPs may change depending on the virus mutations; generally wild-type viruses lead to greater IIPs [**?**]. **Observational Model.** In HIV studies, longitudinal data collected in routine are the total CD4 count in cells/μL and viral load in copies RNA/μL. To model measurement error, we use transformations to achieve normality and homoscedasticity of noises. Thus, we assume that combinations of compartments are observed, to say $(Q + T + T^*)^{0.25}$ and $\log_{10}(V)$. The standard deviation of measurement errors are respectively σ_{CD4} and σ_{VL}.
We define the adherence as a time-dependent drug-specific covariate $A_X(t)$, which is the percentage of the drug taken by the patient compared to what was prescribed by the clinician. Thus, drug intake $d_X(t)$ at time t can be defined as $d_X(t) = A_X(t)D_X$, where D_X is the standard dose given in routine for a drug X.

Descriptive analysis of the dataset : Albi, Puzzle, Zephir and Predizista

Study Population. The ALBI, PUZZLE, ZEPHIR and PREDIZISTA studies have been described elsewhere [**?**, **?**, **?**, **?**]. Briefly, we used the pooled data of these 4 clinical trials with an overall study population of 350 patients. Studies size, number of available data, lengths of follow-up, antiretroviral drugs repartition and patients characteristics are available Table 1.
We considered eight main antiretroviral drugs given in cART, other peripheral drugs were omitted (less than 5% of the patients). We studied zidovudine (AZT), stavudine (D4T), didonasine (ddI) and lamivudine (3TC) as Reverse Transcriptase Inhibitors (RTI), and lopinavir (LPV), amprenavir (APV), darunavir (DRV) as Protease Inhibitors (PI). Ritonavir (RTV) was given as a boost but always in the same cART. Thus, to avoid identifiability problems we avoided estimation of its individual effect. As a consequence, we consider the estimation of boosted DRV/r, APV/r and SQV/r effects. Daily adherence documented by self-reported questionnaire was available for 42.5% of the population (patients from ALBI Study). For patients in other studies we had information about treatment doses, regimen switches and interruptions reported during visits to clinician. **Statistical analysis.** A total of 22 parameters have to be estimated on clinical data : biological parameters, *in vitro* to *in vivo* conversion factors, standard deviation of random effects and measurement error. We used the NIMROD program for estimation (Normal approximation Inference in Models with Random effects based on Ordinary Differential equations) [**?**].
Drug, patient and time specific adherence indicator was built taking standard commercial daily doses (whatever the daily number of intake) for each antiretroviral drugs (see Figure 1 a for details on these values). Concerning the Bayesian analysis, we put informative priors on biological parameters, see [**?**] for a description of the choice of these priors. We put weakly informative priors for the conversion factor, to say wide normal laws with mean 1. Priors

and posteriors are presented Table 2.
Biological parameters estimates values are consistent with the literature. Measurement errors have the expected magnitude, however it remains high for σ_{CD4}. A Wald significance test found that, as expected, conversion factors were negative (to say, the treatment decrease the infectivity γ) except for 3TC (p-value=11.5%).
We have a high variability of β's values between drugs and drug classes (NRTI and PI). Moreover, the conversion factors β's are significantly different from -1. Thus conversion between doses used *in vitro* and *in vivo* is not straightforward. For instance, the *in vivo* effect of ddI is significantly higher than it *in vitro* effect. This may suggest that the dose used *in vitro* were too low compared to standard *in vivo* dose. The reverse reasoning applies for DRV/r. However, data adjustment quality is acceptable, see fits examples (Figure 3, Figure 4 and Figure 5) and visual checks 6 (Figure 6).

Table 1. Studies and patients characteristics of 350 patients from ALBI, PUZZLE, ZEPHIR and PREDIZISTA studies.

	ALBI	PUZZLE	ZEPHIR	PREDIZISTA	ALL
Studies characteristics					
Number of patients	148	22	116	64	350
Average Duration (weeks)	24	26	12	12	19
Drugs characteristics					
Use of AZT (%)	66	23	22	3	37
Use of 3TC (%)	66	86	56	17	55
Use of d4T (%)	68	46	3	3	33
Use of ddI (%)	68	68	11	20	41
Use of LPV (%)	0	86	10	2	9
Use of APV (%)	0	86	100	0	39
Use of DRV (%)	0	0	100	0	18
Patients characteristics					
Age (year)	36	42	44	45	42
Median follow-up (days)	173	200	168	165	171
log10 HIV-RNA baseline (copies/mL)	4.5	4.8	4.3	4.6	4.5
log10 HIV-RNA 4 months (copies/mL)	2.5	2.5	2.9	2.7	2.6
CD4 count baseline (cells/mL)	406	198	294	183	317
CD4 count 4 months (cells/mL)	521	341	369	198	459

Figure 3. Biomarkers adjustment according to adherence for patient 39 in ALBI. Observations (triangle), fits (plain), 95% predictivity intervals (dashed)

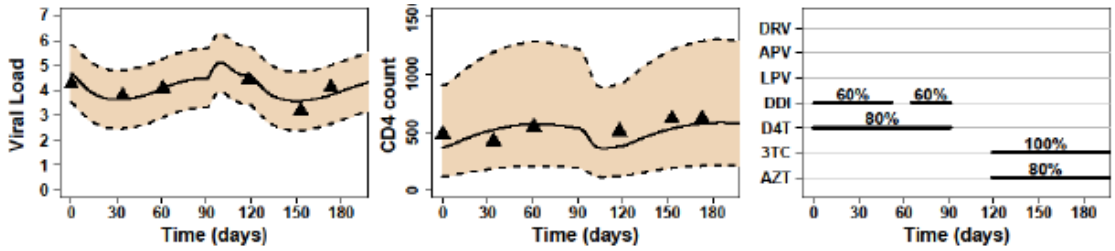


Table 2. Priors and posteriors for the "target cells model" parameters estimated from ALBI, PUZZLE, ZEPHIR and PREDIZISTA trials data

	Priors		Posteriors			Priors		Posteriors	
Parameters	Mean	s.d.	Mean	s.d.	Parameters	Mean	s.d.	Mean	s.d.
Biological Parameters					**Conversion factors**				
λ	12.80	24.3	3.87	0.529	β_{AZT}	1.00	2.00	2.54	0.078
μ_{T^*}	0.95	0.64	0.96	0.170	β_{3TC}	1.00	2.00	-0.01	0.015
γ	0.003	0.013	0.001	0.0001	β_{d4T}	1.00	2.00	4.39	0.215
α	0.018	0.037	0.03	0.005	β_{ddI}	1.00	2.00	0.43	0.153
ρ	0.013	0.018	0.004	0.002	β_{LPV}	1.00	2.00	0.22	0.028
μ_T	0.075	0.026	0.03	0.001	β_{APV}	1.00	2.00	0.20	0.015
μ_Q	0.0001	0.0002	0.0001	0.000001	β_{DRV}	1.00	2.00	0.15	0.009
π	56.8	151.2	1.68	0.379					
μ_V	18.17	12.36	0.305	0.019					
Standard deviations of random effects					**Standard deviations of measurement error**				
σ_λ	-	-	0.68	0.019	σ_{CV}	-	-	0.63	0.013
$\sigma_{\mu_{T^*}}$	-	-	0.72	0.023	σ_{CD4}	-	-	0.44	0.001
σ_γ	-	-	1.34	0.046					

Figure 4. Biomarkers adjustment according to adherence for patient 230 in PUZZLE. Observations (triangle), fits (plain), 95% predictivity intervals (dashed)

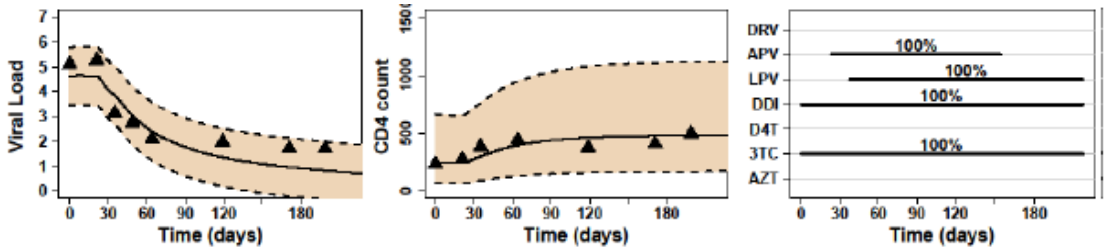


Figure 5. Biomarkers adjustment according to adherence for patient 348 in PREDIZISTA. Observations (uncensored triangle, censored round), fits (plain), 95% predictivity intervals (dashed)

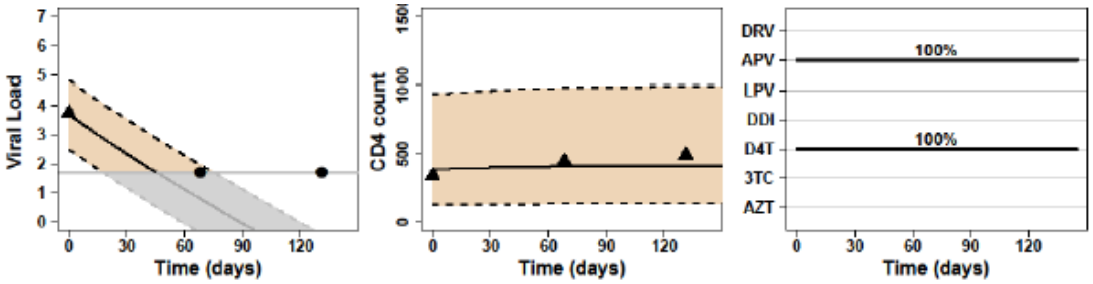
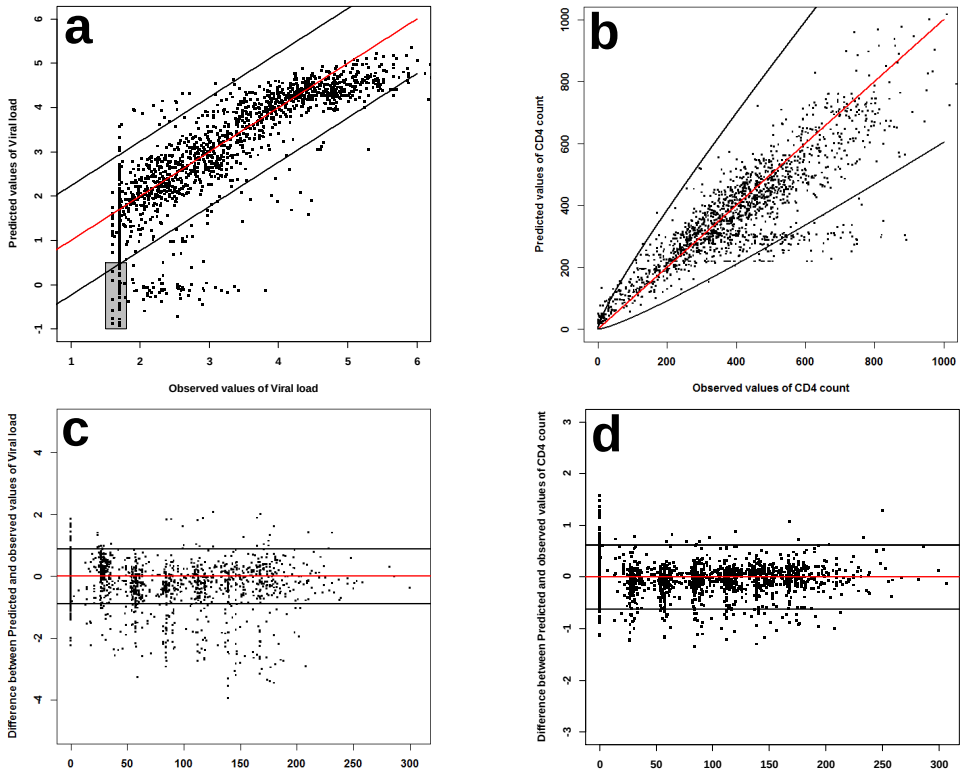


Figure 6. Visual diagnostic plot for data fits of viral load (a,c) and CD4 count (b,d). In scatter plot (a,b), model trajectories values are plotted against observed values. Lines represent the first bisector and acceptable error regarding estimated measurement error for each biomarker. For viral load, shaded square highlight points that are not misclassified since true viral load is unknown because of detection limit complication in data. In (c,d) Differences between model trajectories and observation are plotted against time together with acceptable deviance threshold of 95% confidence interval regarding measurement error.



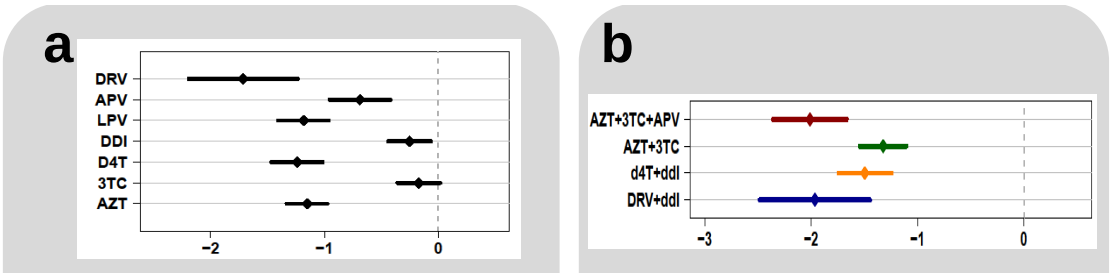
The assessment of treatment effects : *in vivo* quantification and forecast

Individual antiretroviral effect. It is interesting to look at the *in vivo* effect of each antiretroviral ($\beta_X \text{IIP}_X$), presented in Figure 7a. Estimates and 95% credible intervals can be directly computed by multiplying the estimates of conversion factors in Table 2 and drugs IIPs. We observe that drugs may be ranked. DRV/r is the most effective. It effect in mono-therapy is comparable with the *in vivo* effects of some weak cART (let say AZT+3TC Figure 7b), which is in accordance with previous studies [**?**, **?**]. **The cART effect.** As

an illustration, we selected four commonly used cART (AZT+3TC, d4T+ddI, DRV+ddI and AZT+3TC+APV) and computed their effects *in vivo* ($\sum_{i=1}^{n_{cART}} \beta_{X_i} \text{IIP}_{X_i}$) which are displayed on Figure 7b. Credible intervals at 95% are not significantly different, maybe due to lack of power because of small samples ($n_{\text{DRV+ddI}} = 9, n_{\text{d4T+ddI}} = 52, n_{\text{AZT+3TC}} = 47$ and $n_{\text{AZT+3TC+APV}} = 24$). However, AZT+3TC+APV and DRV+ddI seem to have a greater effect than old fashion NRTI bi-therapies. This guess is consistent with knowledge on the benefit to at PI in cART [**?**]. Moreover, this ranking in these cARTs is consistent with the average observed viral load and CD4 count for patient. Regimens AZT+3TC+APV

and DRV+ddI lead to a greater decrease of the viral load (about 2 log10 in 6 months) and increase of CD4 count (respectively 50 and 100 cells in 6 months) compared to AZT+3TC and d4T+ddI. **Limitation and perspectives.** These preliminary results are obtained

Figure 7. *In vivo* estimated treatment effects for antiretroviral drugs and selected cART for standard daily doses (a) $\beta_{X_i}\text{IIP}_{X_i}$ (b) $\sum_{i=1}^{n_{cART}} \beta_{X_i}\text{IIP}_{X_i}$.



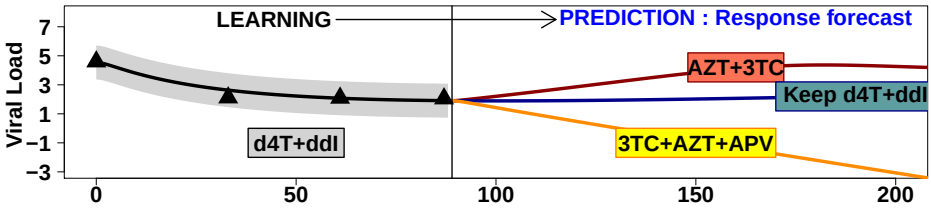
from samples with moderate size regarding the quantification of treatment effects. They are mainly driven by ALBI sub study (60% of our dataset) and cARTs. Most of other cARTs are not given for more than 20 patients.

Then, concerning the validation of our model, it would be interesting to estimate *in vivo* effects of drugs on a learning dataset and test it on a validation dataset. Although cross-validation remains a solution to assess the forecast quality, we expect to do the same analysis on a larger dataset. Moreover, we aim at estimating the *in vivo* effect of a larger number of antiretroviral drugs.

Finally, if forecasts are possible we could consider the possibility of optimizing the treatment change as suggested by Figure 8. We could forecast the response to various cARTs according to individual observation of the patient and take the best cART according to criteria such as to have the highest probability of infection control.

We currently build collaborations to gather more data with a wider variety of antiretroviral

Figure 8. Forecasts of various cARTs responses after change from first cART Patient 50 from ALBI Study.



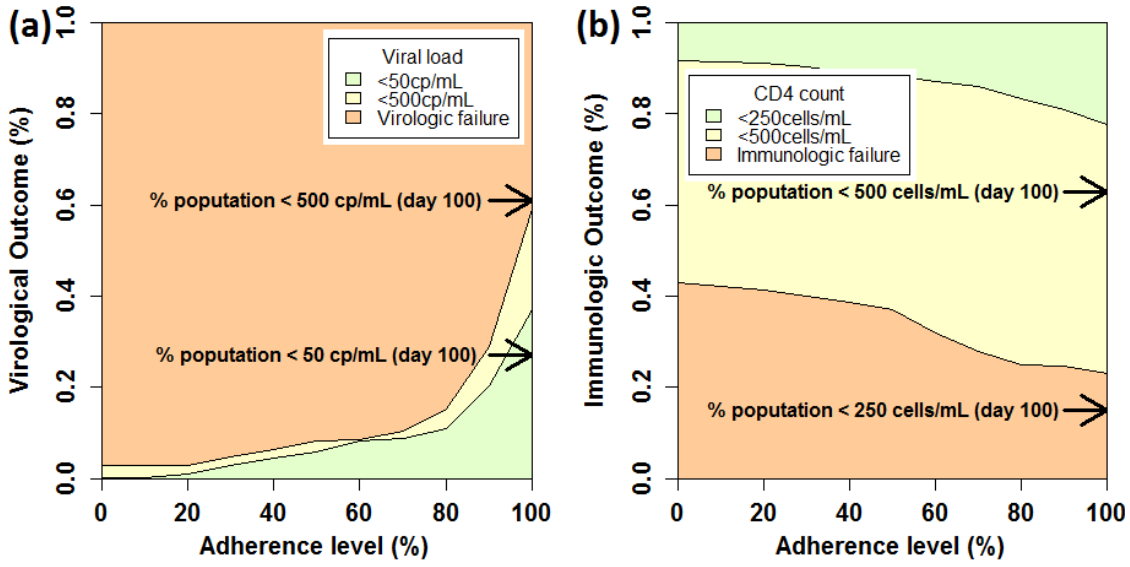
drugs and cARTs.

Dynamic models are valuable to investigate the matter of adherence

Simulation of adherence in dynamical models. Adherence is very difficult to assess accurately but is a main issue in HIV [**?**]. In our model we can account for adherence through $A_X(t)$. Thus, at each time t, we may simulate a stochastic term $A_X(t)$ which equal 100% with the probability p and 0% with the probability $(1-p)$. This is the adherence pattern of a patient who is randomly adherence $100p$% of the time. Then, parametric empirical bayes predictions [**?**] are used to forecast how patients would have responded to cART according to their simulated adherence pattern. **To what extend random adherence may impact**

treatment failures? We tried to investigate the effect of $100p$% random adherence on virologic and immunologic failure after 100 days. Actually this length of study should let the time to the mechanistic model to reach equilibrium state.
Impact of adherence seems to be more perceptible on viral load than on CD4 count (Figure 9). We found that there is a treshold at 80% for adherence below which there is a high probability of treatment failure whatever the real adherence level. Figure 9a shows that the probability of experiencing virologic failure is greater than 90% for all adherence level lower than 70%. For a full adherence 50 copies undetectability (resp. 500 copies) is reached 35% (resp. 60%) of the time. Thus, our model correctly mimics observed data where only 27% (resp. 61%) of the population reached 50 copies/mL (resp. 500 copies/mL) undetectability after 100 days. Figure 9b shows that an adherence greater than 80% should be sufficient to ensure less than 25% of immunologic failure which is pessimistic compared to observed data where only 15% of the patients have less than 250 cells/mL after 100 days. **Limitation**

Figure 9. Probability of virologic (a) and immunologic (b) failure as a function of adherence levels Observed proportions of failures in the studied population are indicated for comparison purpose.



and perspectives. In this investigation, we tried to see if we were able to assess impact of adherence patterns. This could be extended to the analysis of all adherence patterns including intermittent treatments (x days on, $(7-x)$ days off). However, before answering

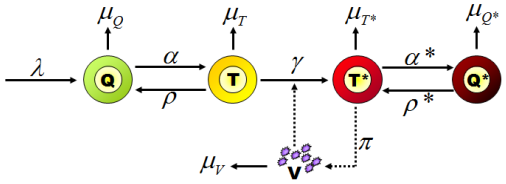
precisely questions on adherence, estimation must be done on proper dataset. Actually the major weakness in this analysis is that adherence information is sparse and number of 100% adherent patients is probably overestimated. During estimation, we think that this poor measurement resulted in an underestimation of drug effectiveness [**?**]. This may explain why we showed that an adherence of more than 80% is requested which is slightly higher than what is realistic, to say around 60-70% [**?**], but not impossible [**?**]. We currently build collaborations to gather studies with good self-reported adherence data and possibly MEMS (Medication Events Monitoring System) information.

Additional discussions and concerns

The case of mutations. We made the choice to account for mutations by including random effects, especially on γ, so that drugs can have an effect on some patients but be less effective for others. This is justified because drug resistance mutations reduce the virus fitness relatively to wild-type virus. There is no trend in time on biomarkers predictions (Figure 6), thus we can guess that our model remains valid on our data. Probably, there is no major resistance occurrence. However, genotypic susceptibility scores (GSS) were available for all the patients except those from ALBI. We showed that high GSS was correlated with high values of random effects on γ. Thus we conclude that the use of resistance testing data (genotypic and phenotypic test) could help in accounting for preexisting mutations and probably significantly improve the results. **Dynamic and treatment effect in latent reservoirs.** One of the major concern in HIV treatment and cART dynamics is to assess their impact on latent reservoirs. For this purpose, we begin to use the " Target cell with latent reservoir model" (Figure 10). First investigation on ALBI data showed that this model improves the log-likelihood (-950 vs -1293) as well as model choice criterion LCV_a (6.3 vs 7.9). Regarding parameters estimates, $\mu_Q \simeq \mu_{Q^*}$, $\alpha \gg \alpha^*$ and $\rho \gg \rho^*$. Order of magnitudes are similar to those found by [**?**]. Nevertheless, two research leads are considered :

- to enhance the identifiability by fixing some parameters (for example $\mu_Q = \mu_{Q^*}$),
- to find data where viral DNA is measured to have a proxy to measure the umber of latent cells ($T^* + Q^*$).

Figure 10. Target cell with latent reservoir model : we extended the "target cell model" by adding a latent infected cell (Q^*) compartment.



Investigation of other pharmacological modeling. The model we used in this preliminary note sheet is highly parameterized, particularly because of the conversion factors. If data are not rich enough and we plan to estimate a greater number od antiretroviral drugs *in vivo* effects, we may often encounter identifiability problems. Instead of using IIPs, we propose to compare our approach with the use of inhibitory factors (IF) that may be computed and consist in time-averaged IIPs over all antiretroviral drugs in the cART. In other words, the pharmacodynamical function will not be drug-specific anymore but cART-specific (Figure 11). In other words, due to the shape of dose-response curves, IF is particularly sensitive to short times when drug concentrations are low.

Acknowledgments

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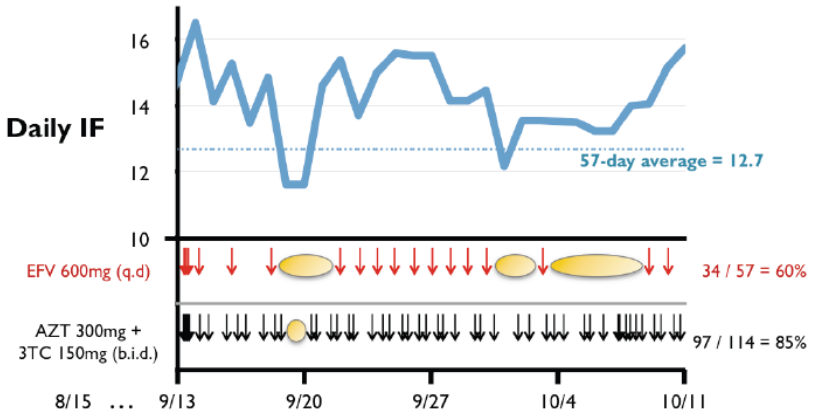
Figure 11. Inhibitory Factors (IF) may be computed according to cART administration and adherence information (source : D. Rosenbloom 8th International Conference on HIV treatment and prevention adherence).



trial particularly G. Raguin and the investigators of the ALBI ANRS 070 trial particularly J.M. Molina. Parallel computing was used thanks to the computing facilities MCIA (Mésocentre de Calcul Intensif Aquitain) of the Université de Bordeaux and of the Université de Pau et des Pays de l'Adour.