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Suhaib Hattab

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Suhaib Hattab. Impact of different antiretroviral therapy (ART) regimens on the evolution of soluble markers of inflammation and immune activation in HIV-infected patients. Immunology. Université Pierre et Marie Curie - Paris VI, 2014. English. NNT : 2014PA066126 . tel-01069303

HAL Id: tel-01069303
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THESE DE DOCTORAT DE L'UNIVERSITE PIERRE ET MARIE CURIE

Spécialité

Epidémiologie

Ecole doctorale Pierre Louis de santé publique
Epidémiologie et Sciences de l'Information Biomédicale

Présenté par

M. Suhaib HATTAB

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITÉ PIERRE ET MARIE CURIE

Sujet de thèse

Impact de différents traitements antirétroviraux (ARV) sur l'évolution des marqueurs d'inflammation et d'activation immunitaire plasmatiques chez les patients infectés par le VIH

Le 29 juillet 2014 devant le jury composé de:

Pr. Jacqueline CAPEAU	Examinateur
Pr. Laurence WEISS	Rapporteur
Mme. Carole DUFOUIL	Rapporteur
Pr. Christine KATLAMA	Directrice de thèse

I dedicate this work to my parents, brothers, sisters and to my lovely wife.

ACKNOWLEDGMENTS

This PhD work could not have been realized without the permanent and kind support of different people around me. Hereby, I would like to express my deepest thanks to them.

My thanks to,

Madam Dominique Costagliola for accepting my integration in her research team. Her immense knowledge and experience in epidemiology and in statistics offered me a new field of cognition. Her guidance helped me in all the time of PhD study. It was my honor to work with her.

Pr. Christine Katlama who agreed to direct my thesis despite the huge responsibilities she has. Her experience in the clinical research widened my angle of thinking concerning the research problem, the establishment of research hypothesis and study design.

Madam Marguerite Guiget, the co-director of my thesis for enlightening me the first glance of research, who guided me in the first step of my thesis, who learned me the principals of statistics, who helped me realize all the necessary analyses. Her remarks and our discussions were always pertinent and helped me better do my job. Her encouragement and optimism relieved my desperation in the tough moments.

Pr. Laurence Weiss and *madam Carole Dufouil,* who accepted to participate in my thesis jury and to review my manuscript. My thanks for their insightful comments.

Pr. Jacqueline Capeau not only for accepting to be examiner member in the jury, but also for accepting my integration in her team to perform my Master training in 2009-2010 and then to orient me to join the research team of Pr. Christine Katlama and Madam Dominique Costagliola to perform my PhD. My experience in her team was the overture of the research field.

Pr. Rose Katz, for all the efforts she has made since 10 years to create and follow the cooperation program between the UPMC and the Palestinian faculty of medicine. My sincere gratitude for all the logistical and human support and for the time she spent to accompany us during our stay in France.

My thanks to,

My colleges in the clinical research unit at the infectious diseases department of the Pitié Salpêtrière hospital, all the physicians, the assistants of clinical research and the nurses.

My colleges in the INSERM U_1136 unit, PhD and master students, statisticians and assistants of clinical research.

My colleges in the immunology and virology laboratories. The collaboration with them was indispensable to realize this work.

Rachid Agher, the informatics specialist in our service, his kind help, patience, smile and jokes made us laugh in the difficult moments.

An-Najah University, the university to which I am going, and the French consulate at Jerusalem for offering me the opportunity to get my PhD in one of the developed countries; France. Special thanks to our former dean Dr. Anwar Dudin.

The Palestinian community and my friends in France with whom I spent the wonderful moments.

My sincere thanks to my father, my mother, my wife, my brothers and sisters and all the members of my grand family. Their logistic, spiritual and psychological support alleviated the difficulties of being far from them during my stay in France.

Above all, I owe it all to ALLAH for granting me the health, the ability and the strength to undertake this research task and enabling me to its completion.

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I. RESUME DES TRAVAUX DE THESE

Introduction

Les traitements antirétroviraux combinés contrôlent la charge virale plasmatique du VIH chez la plupart des patients qui y ont accès. Cependant, il ne permet pas de restaurer complètement la santé et les patients sont encore plus à risque de comorbidités par rapport à la population générale (Shiels MS *et al*, 2009; Lang S *et al*, 2010). En outre, des niveaux élevés de l'activation immunitaire et de l'inflammation persistent malgré le contrôle de la virémie VIH par rapport à la population non infectée par le VIH (Reingold J *et al*, 2008; Neuhaus J *et al*, 2010; Alcaide ML *et al*, 2013).

L'activation immunitaire et l'inflammation a retenu l'attention dans les dernières années après l'observation de l'association entre les marqueurs de l'inflammation et de la coagulation et la mortalité dans l'étude SMART; une étude qui a modifié les concepts sur la pathogenèse du VIH (Kuller LH *et al*, 2008). Suite à cette observation, plusieurs études ont montré une association entre les niveaux élevés des marqueurs inflammatoires et un risque accru de mortalité (Tien PC *et al*, 2010; Sandler NG *et al*, 2011) ainsi que des morbidités non-classant SIDA, notamment les maladies cardio-vasculaires et les cancers non-classant SIDA (Duprez DA *et al*, 2012; Borges AH *et al*, 2013; Tenorio AR *et al*, 2014). En outre, des études ont montré que les patients qui ont des niveaux élevés de l'activation immunitaire sont moins capables de restaurer les taux de cellules CD4+ (Lederman MM *et al*, 2011; Zhang X *et al*, 2013). Dans ce contexte, l'activation immunitaire persistante peut être liée à la virémie résiduelle entraînée par les réservoirs cellulaires du VIH ou à la faible réplication (Mavigner M *et al*, 2009), la réactivation d'autres infections virales chroniques telles que le cytomégalovirus et le virus d'Epstein-Barr (Petrara M *et al*, 2012; Wittkop L *et al*, 2013). En outre, la profonde déplétion des lymphocytes T CD4+ au cours de la primo-infection à VIH peut conduire à une perte progressive de la fonction de la barrière intestinale, ce qui permet la translocation de la flore intestinale dans la circulation systémique (Brenchley JM *et al*, 2006).

Peu d'études ont été menées sur l'impact du traitement antirétroviral sur l'évolution des marqueurs d'activation immunitaire et d'inflammation avec des résultats variables (Smith KY *et al*, 2009 ; Funderburg N *et al*, 2010 ; McComsey GA *et al*, 2012). Ces études ont inclus des personnes ayant des niveaux et des durées variables du contrôle virologique, ce qui explique en partie leurs résultats discordants. Ces différences pourraient être aussi liées à des

différences du statut de maladie, et les différents régimes de traitement. Dans ce travail de thèse, j'ai étudié l'évolution des marqueurs d'activation immunitaire et d'inflammation solubles, chez les patients infectés par le VIH, initiant une première ligne de traitement antirétroviral avec un succès virologique rapide et persistant au cours de deux ans.

Objectifs

1. Identifier les relations entre les caractéristiques des patients et les niveaux des marqueurs de l'activation immunitaire et de l'inflammation avant l'initiation de traitement antirétroviral.
2. Evaluer l'évolution des marqueurs de l'activation immunitaire et de l'inflammation au cours de 2 ans de traitement antirétroviral efficace, en comparant les niveaux des marqueurs avant et après traitements avec les niveaux observés chez les témoins non-infectées.
3. Identifier les facteurs associés à des niveaux anormaux de ces marqueurs après 2 ans de traitement efficace.
4. Évaluer l'impact des différents traitements antirétroviraux sur ces marqueurs dans un groupe des patients qui ont conservé leur traitement initial sur les deux ans.

Méthodes

Population de l'étude

Dans cette étude observationnelle, nous avons évalué l'éligibilité de tous les patients infectés par le VIH–1 qui ont commencé une première ligne de traitement antirétroviral combinée entre Janvier 2006 et Décembre 2009, en utilisant la base de données NADIS. NADIS est un dossier médical informatisé conçu par des professionnels de santé pour améliorer le suivi des patients infectés par le VIH ou les virus de l'hépatite B et C. Les patients étaient éligibles s'ils avaient reçu un des deux 'backbones' (ténofovir-emtricitabine (TDF-FTC) ou abacavir-lamivudine (ABC-3TC), combiné avec un troisième agent (efavirenz ou un inhibiteur de protéase boosté par le ritonavir (atazanavir (ATV/r), lopinavir (LPV/r) ou fosamprénavir (FPV/r)). Ce sont les combinaisons de première ligne recommandés par les recommandations françaises à l'époque. Pour contrôler l'effet possible de la réplication virale

résiduelle sur l'activation immunitaire et l'inflammation, l'analyse a été limitée aux patients qui ont eu une réponse virologique rapide et persistante, définie par une charge virale VIH-1 plasmatique inférieur à 400 copies/mL à 6 mois (M6) et inférieure à 50 copies/mL à 24 mois (M24), sans valeurs supérieures à 1000 copies/mL entre M6 et M24. Les patients éligibles ont été inclus si les échantillons de plasma congelés obtenus au moment de l'initiation du traitement antirétroviral (J0) et à M24 étaient disponibles. Dans l'analyse de comparaison de l'impact des différents traitements antirétroviraux sur l'évolution des marqueurs, seuls les patients qui ont conservé le même régime sur les deux ans de l'étude ont été analysés pour contrôler l'impact de changement de traitement sur l'interprétation des résultats. La population témoin était représentée par 20 donneurs de sang VIH-séronégatifs. Les niveaux des marqueurs chez les témoins ont été évalués dans le cadre de l'étude ACTIVIR qui a été réalisée au laboratoire d'immunologie (INSERM, UMR_S 1135, CIMI, F-75013, Paris, France) en 2010. L'étude a été approuvée par les comités de protection des personnes et les patients ont signé un consentement concernant l'utilisation de leurs plasmas et le recueil de leurs données médicales tel que requis par la loi française.

Recueil des données cliniques

Apres avoir sélectionné la population d'étude, les données des patients ont été extraites de la base NADIS. En plus de NADIS, les dossiers médicaux papiers ont été utilisés pour compléter les informations manquantes ainsi que valider les informations extraites de NADIS. Les variables collectées comprenaient les caractéristiques, l'état clinique, biologique et thérapeutique des patients: sexe, âge, indice de masse corporelle (IMC), tabagisme, co-infections par l'hépatite B (AgHBs) et C (AcVHC), groupe de transmission, événements classant SIDA, taux de cellules CD4+ et de CD8+ pré-thérapeutique, charge virale VIH-1 pré-thérapeutique, combinaison antirétrovirale initiale et toutes les modifications de traitement antirétroviral pendant les deux années. Ces données ont été recueillies dans un questionnaire papier (voir annexes) et ont ensuite été saisies sur Epi-info.

La préparation de plasma et les mesures des marqueurs

La sélection des marqueurs était basée sur deux critères :

1. la fiabilité de la mesure de ces marqueurs sur du plasma congelé.
2. la valeur prédictive de ces marqueurs.

Nous avons évalué l'IL-6 et la CRP-us en tant que marqueurs d'inflammation, IP-10 et MIG comme marqueurs d'activation des lymphocytes T, des monocytes et des macrophages, et CD14 soluble (sCD14) en tant que marqueur d'activation monocytaire. La méthode ELISA a été utilisée pour quantifier l'IL-6, la CRP-us et le sCD14, selon les instructions du fabricant. La cytométrie en flux a été utilisé pour déterminer les niveaux d'IP-10 et MIG sur un dispositif BD FACS Canto I. Ces techniques ont été choisies en tenant compte leur sensibilité pour mesurer les niveaux des marqueurs (la capacité de détecter le marqueur à un seuil bas). Les échantillons de J0 et M24 ont été testés dans la même série. Les mêmes marqueurs biologiques ont été mesurés avec les mêmes kits chez les témoins dans l'étude ACTIVIR.

Les analyses statistiques:

Pour étudier les relations entre les niveaux des marqueurs et les caractéristiques des patients avant l'initiation de traitement, les niveaux des marqueurs ont été comparés chez les patients infectés par le VIH en fonction de leur sexe, âge, IMC, le tabagisme, l'hépatite B, les événements classant SIDA, le taux de CD4+, le rapport CD4/CD8 et la charge virale, en utilisant des tests de Wilcoxon. Lorsqu'en univarié plus d'un facteur avait un $p<0,15$, des modèles de régression pas-à-pas descendante ont été utilisés pour déterminer les facteurs associés avec des taux élevés. L'évolution des marqueurs après 2 ans de traitement antirétroviral (la différence entre les valeurs des marqueurs à J0 et M24) a été testée utilisant le test de Wilcoxon apparié.

Pour identifier les facteurs associés aux niveaux élevés des marqueurs après 2 ans de traitement, les modèles de régression logistique ont été utilisés. Le niveau d'un marqueur a été considéré comme "élevé" si sa valeur était supérieure à la valeur moyenne plus deux écarts-types dans le groupe de témoin. Ces facteurs sont les suivants: sexe, âge, IMC, le tabagisme, l'hépatite B, les événements classant SIDA, le taux de CD4+ et la charge virale pré-thérapeutique, les blips virals entre M6 et M24 (charge virale >50 copies/mL et <1000 copies/mL entre M6 et M24), le changement de taux de CD4, et le rapport CD4/CD8 à M24. Les facteurs associés à des valeurs élevées de marqueur dans l'analyse univariée ($p <0,15$) ont été inclus dans l'analyse multivariée.

Pour comparer l'impact des différentes composantes de traitement antirétroviral (TDF-FTC vs. ABC-3TC et ATV/r, LPV/r vs. EFV) sur l'évolution des marqueurs, des modèles de régression linéaire ont été utilisés. Les groupes de traitement ont été comparés en utilisant un

plan factoriel. Le groupe des patients sous FPV/r n'a pas été inclus dans cette analyse en raison de sa petite taille (n=12) Les résultats sont exprimés comme la différence en pourcentage entre le rapport moyen observé entre M24 et J0 avec un traitement donné et le rapport moyen observé avec TDF/FTC et EFV, pris comme référence pour les comparaisons. Les relations entre les variables à J0 et les changements dans chaque marqueur ont été examinées dans des modèles de régression linéaires univariées. Ces variables étaient le sexe, l'âge, l'IMC, le tabagisme, la co-infection avec l'hépatite B ou C, les événements classant SIDA, et le taux de CD4 et la charge virale pré-thérapeutique. Les variables associées aux changements pour au moins un des marqueurs ($p<0,10$) et les blips viraux entre M6 et M24 ont été retenus dans les modèles de régression linéaire multivariée afin de contrôler les facteurs qui pourraient avoir influencé le choix du traitement ou affecté les changements des marqueurs. L'âge et le tabagisme ont été inclus dans les modèles multivariés car ces variables sont connus pour influencer les niveaux des marqueurs (Deeks SG *et al*, 2013 ; Pine SR *et al*, 2011). Les termes d'interaction entre 'backbones' et troisième agent ont été testés pour tous les marqueurs.

Résultats

Les caractéristiques des patients et les niveaux des marqueurs à l'initiation du traitement (Tableau 1)

Entre janvier 2006 et décembre 2009, un total de 539 patients ont commencé un traitement antirétroviral et ont continué à être suivi à la Pitié-Salpètrière pendant au moins deux ans. Parmi eux, 370 patients ont eu une réponse virologique rapide et persistante au cours des deux ans et 280 patients ont reçu l'un des traitements retenus pour l'étude. Les 90 autres patients avaient reçu un traitement antirétroviral qui n'est plus recommandé ou avaient été inclus dans un essai clinique évaluant de nouveaux antirétroviraux comme le darunavir ou la rilpivirine. Des échantillons de plasma congelés à J0 et à M24 étaient disponibles pour 147 patients. Les caractéristiques des patients évaluables n'étaient pas différents de ceux pour lesquels il n'y avait pas de plasmas congelés en termes d'âge, de taux de CD4, de charge virale, d'évènements classant SIDA ni pour le traitement antirétroviral prescrit. Six patients co-infectés par le VHC et 2 patients ayant refusé de participer à l'étude ont été exclus. Au total, 139 patients sont donc inclus dans l'étude. Soixante-quatre patients (46%) ont eu des

modifications de traitement au cours de la période de l'étude, tous pour des raisons autres que l'échec virologique.

Tableau 1. Caractéristiques des patients à l'initiation du traitement antirétroviral

N=139	N (%) ou médiane (IQR 25- 75%)
Sexe (homme)	112 (81%)
Age, ans	40 (34-47)
IMC, kg/m²	23 (21-25)
Fumeur	50 (36%)
Hépatite B (HBsAg +)	7 (5%)
Groupe de transmission Homme ayant des rapports sexuels avec des hommes Hétérosexuel (homme ou femme) Autres	 55 (42%) 59 (40%) 25 (18%)
Délai depuis le diagnostic du VIH-1, moins	6 (1.3-43)
Evénements classant SIDA	19 (14%)
CD4 /mm^3	294 (190-384)
CD8 /mm^3	900 (571-1240)
Rapport CD4/CD8	0.29 (0.19-0.45)
Charge virale VIH-1 (log_{10} copies/mL)	4.8 (4.3-5.3)
Traitement TDF/FTC/LPV/r TDF/FTC/ATV/r TDF/FTC/FPV/r TDF/FTC/EFV ABC/3TC/LPV/r ABC/3TC/ATV/r ABC/3TC/FPV/r ABC/3TC/EFV	 26 (19%) 25 (18%) 10 (7%) 52 (37%) 8 (6%) 12 (9%) 2 (1%) 5 (3%)

A l'initiation du traitement antirétroviral, les niveaux médians d'IL-6, IP-10, MIG et sCD14 étaient significativement plus élevés chez les patients que chez les témoins (Tableau 2). Dans les analyses de régression pas à pas, les niveaux élevés d'IL-6 ont été associées aux événements classant SIDA ($p<0,001$). Les niveaux élevés d'IP-10 ont été associés à un rapport CD4/CD8 faible ($p=0,007$) et à une charge virale élevée ($p=0,001$), tandis que les niveaux plus élevés de MIG ont été associés à une charge virale élevée ($p=0,024$). Les niveaux élevés de sCD14 ont été associées aux événements classant SIDA ($p=0,01$).

Evolution immune-virologiques et changements des marqueurs de l'activation immunitaire et l'inflammation

Selon les critères d'inclusion, la charge virale du VIH était inférieure à 400 copies/mL à M6 et inférieure à 50 copies/mL à M24 chez tous les patients. Le délai médian jusqu'à la suppression virologique (<400 copies/mL) était de 2 mois. Entre M6 et M24, 29 patients ont eu un blip viral (médiane 77 copies/mL; range 51 à 804). Le taux de CD4+ a augmenté en médiane de 224/mm^3 entre J0 et M24, et la valeur médiane à M24 était 523/mm^3 (IQR 357-676). Le rapport médian CD4/CD8 est passé de 0,29 à l'initiation de traitement à 0,76 (IQR de 0,48 à 1,05) à M24. Soixante-onze patients (51%) avaient un taux de CD4 au-dessus de 500/mm^3 à M24, et 52 patients (37%) avaient un rapport de CD4/CD8 supérieur à 0,9.

Comme le montre le tableau 2, après deux ans de traitement efficace, les niveaux d'IL-6, IP-10 et MIG ont baissés significativement, tandis que les niveaux de sCD14 n'ont pas changé de façon significative. Aucune différence dans les niveaux d'IL-6, IP-10 et MIG n'a été observée entre les patients et les témoins à M24, tandis que les niveaux de sCD14 sont restés plus élevés chez les patients, même après l'exclusion de ceux avec des blips de réplication virale.

Les facteurs associés aux niveaux élevés des marqueurs après 2 ans de traitement

Seuls 4 patients (3%) avaient des niveaux élevés d'IL-6 à M24, comparativement à 11% à J0 (p=0,008), de sorte que nous n'avons pas pu étudier les facteurs associés à la persistance de ce marqueur. Vingt-neuf patients (21%) avaient des niveaux élevés d'IP-10 à M24 par rapport à 86% à J0 (p<0,001). L'âge était associé à une élévation persistante des niveaux d'IP-10 (OR, 1,60 pour 10 ans; p=0.047). Vingt-deux patients (16%) avaient des niveaux élevés de MIG à M24, comparé à 81% à J0 (p<0,001), et l'âge était encore le seul facteur associé à la persistance des niveaux élevés de ce marqueur (OR, 1,92 pour 10 ans; p=0.007). Les niveaux de sCD14 sont restés stables au cours des deux ans du traitement: 24% des patients avaient des niveaux élevés à M24, comparativement à 32% à J0 (p=0,109). Dans une analyse supplémentaire, aucun facteur étudié n'était associé aux niveaux élevés de sCD14 à M24. Dans une analyse de sensibilité excluant les patients avec les blips virals, les proportions des patients qui avaient des niveaux élevés des marqueurs à M24 étaient similaires: 4 % pour l'IL-6, 18 % pour IP-10, 13 % pour MIG, et 25 % pour sCD14.

L'impact des différents traitements antirétroviraux sur l'évolution des marqueurs

Au cours des 2 ans, 78 patients ont conservé leur traitement initial. Si la nature des antirétroviraux prescrits n'est pas associée significativement à l'évolution d'IL-6, de CRP-us ou de sCD14, le type d'antirétroviraux a influencé l'évolution d'IP-10 et de MIG ; la baisse d'IP-10 et MIG était significativement plus faible avec ATV/r qu'avec EFV, alors qu'aucune différence significative n'a été trouvée entre le LPV/r et EFV ou entre ABC-3TC et TDF-FTC.

Tableau 2 : Niveaux des marqueurs chez les témoins (VIH-) et chez les patients infectés par le VIH à J0 et à M24; comparaisons avec les témoins et changements des marqueurs au cours de traitement.

	VIH- (N= 20)	VIH+ J0 (N= 139)	HIV+ J0 vs. VIH-	VIH+ M24 (N= 139)	VIH+ M24 vs. VIH-	Changement entre M24 et J0 (N= 139)
IL-6 (pg/mL) Valeur élevée* ≥ 5.7 pg/mL P-value**	0.45 (0.04-2.62)	1.64 (1.06-2.80) 11%	p=0.005	1.14 (0.68-1.82) 3%	p=0.074	-0.54 (-1.63 to 0.14) < 0.001
IP-10 (pg/mL) Valeur élevée ≥378 pg/mL P-value	246 (185-258)	717 (471-1065) 86%	p<.0001	263 (187-346) 21%	p=0.152	-420 (-723 to -212) < 0.001
MIG (pg/mL) Valeur élevée ≥955 pg/mL P-value	447 (353-525)	1660 (1091-2831) 81%	p<.0001	473 (332-664) 16%	p=0.385	-1107 (-2167 to -594) < 0.001
sCD14 (10^6pg/mL) Valeur élevée ≥$2.98*10^6$ pg/mL P-value	1.34 (0.56-1.87)	2.60 (2.09-3.10) 32%	p<.0001	2.35 (1.97-2.95) 24%	p<.0001	-0.18 (-0.75 to 0.55) 0.102

*, Calculé chez les témoins VIH séronégatifs (moyenne +2 SD) ; **, Wilcoxon signed rank.

Valeurs présentés comme médiane (IQR 25-75%).

Discussion et perspectives

Mon travail de thèse a permis d’évaluer l'évolution des marqueurs d'activation immunitaire et d'inflammation solubles, chez les patients infectés par le VIH, initiant une première ligne de traitement antirétroviral avec un succès virologique rapide et persistant au cours de deux ans, afin de contrôler l’impact de la réplication virale résiduelle sur les changements des marqueurs. J'ai aussi évalué l'impact de différents régimes de traitement antirétroviral chez les patients ayant continué leur traitement initial au cours des 2 ans, contrôlant l’impact éventuel de changement thérapeutique. Dans cette étude, j’ai montré que :

1- Avant l’initiation du traitement antirétroviral, les niveaux des marqueurs d’activation et d’inflammation solubles sont plus élevés chez les patients que chez les témoins VIH-séronégatifs. Cette observation est concordante avec des études précédentes comme l’étude SMART qui a montré une corrélation entre la virémie VIH et les niveaux élevés des marqueurs d’inflammation en comparant avec un group témoin négatif pour le VIH (Neuhaus J *et al*, 2010). En outre, les niveaux élevés de sCD14 et d'IL-6 ont été associés à des événements classant SIDA. Cette association pourrait refléter le rôle de l'inflammation dans la pathogenèse de l'infection à VIH. Une étude cas-témoin a montré que les niveaux élevés de l'inflammation (CRP, IL-6), la coagulation (D-dimère), et la fibrose tissulaire (acide hyaluronique) mesurée chez les patients avant l'initiation du traitement antirétroviral étaient associés à un risque élevé des événements classant SIDA et à des décès (Boulware DR *et al*, 2011). Le fait que la réplication de VIH induit des fortes augmentations de l'IFN-γ (Stacey AR *et al*, 2009) explique l'association entre la charge virale élevée et les niveaux élevés d’IP-10 et MIG, des chimiokines induites par l'IFN-γ.

2- Après deux ans de traitement efficace, IL-6, IP-10 et MIG ont baissé de façon significative alors qu’aucun changement des niveaux de CRP-us et de sCD14 n’a été observé. Alors que les niveaux d'IL-6 sont resté élevé dans seulement 3% des patients, les niveaux d’IP-10 et MIG sont restés élevés chez un cinquième des patients après 2 ans de traitement. La différence dans la proportion des patients présentant un taux élevé d'IL-6, et la proportion des patients présentant un taux élevé d’IP-10 et MIG peut s'expliquer de différentes manières. Premièrement, la majorité des patients avaint eu un niveau dans les limites hautes de la normale d’IL-6 à l’initiation de traitement. En second lieu, la variabilité d'IL-6 dans le groupe témoin était plus importante que la variabilité d’IP-10 et MIG. La stabilité du taux de sCD14 peut s'expliquer par plusieurs façons. Tout d'abord, des facteurs autres que la virémie VIH

peuvent être responsables de l'élévation persistante de sCD14. Ainsi, deux ans de suppression virologique peuvent ne pas suffire à restaurer la fonction de la barrière intestinale permettant que la translocation bactérienne persiste (Cassol E *et al*, 2010). Deuxièmement, les régimes de traitement utilisé dans notre étude peuvent ne pas être efficaces pour atténuer l'activation monocytaire, alors que les régimes comportant du raltégravir ont été montré capable de réduire les niveaux de sCD14 (Taiwo B *et al*, 2013; Pallikkuth S *et al*, 2013). Dans notre étude, nous n'avons pas pu tester cette hypothèse car le nombre des patients qui ont changé leur régime vers raltégravir était très petit. La stabilité de CRP-us malgré la baisse de l'IL-6, l'une des cytokines qui déclenchent sa production par le foie, peut être induite par autres cytokines pro-inflammatoires telles que l'IL-1 (Pepys MB *et al*, 2003). De plus, la variabilité interindividuelle de ce marqueur observée dans cette étude pourrait expliquer en partie sa stabilité.

3- Même si l'âge n'a pas été associé aux niveaux élevés d'IP-10 et de MIG à l'initiation de traitement, une fois que la virémie VIH a été contrôlée, les patients plus âgés étaient plus susceptibles de conserver des niveaux élevés de ces marqueurs après 2 ans de traitement. Chaque augmentation de l'âge de 10 ans étaient associée à une augmentation d'au moins 60% du risque de maintenir des niveaux élevés des marqueurs d'activation immunitaire après traitement. Ces résultats sont compatibles avec le lien proposé entre l'activation immunitaire et le vieillissement prématuré chez les patients infectés par le VIH (Appay V *et al*, 2011) et peuvent expliquer en partie l'augmentation plus lente de cellules CD4+ chez les patients âgés observés dans un travail précédent de notre équipe (Grabar S *et al*, 2004). De la même façon, une étude a évalué les niveaux des marqueurs de l'activation immunitaire, la translocation microbienne et des biomarqueurs des maladies cardiovasculaires chez les femmes en post-ménopause, infectées par le VIH sous traitement antirétroviral avec une suppression virologique. Les niveaux de tous les marqueurs étaient significativement plus élevés chez les femmes infectées par le VIH suggérant que les femmes âgées sous traitement antirétroviral efficace sont dans un état d'activation immunitaire (Alcaide ML *et al*, 2013). Si l'âge était la seule variable associée à des niveaux d'activation immunitaire élevées post traitement, il est possible que le petit nombre des patients avec des niveaux d'activation immunitaire élevée ait empêché la détection d'une association avec d'autres variables telles que le taux des cellules CD4+ ou les blips de réplication virale. Enfin, du fait que l'échantillon a été sélectionné de manière rétrospective, le rôle d'autres co-infections comme le cytomégalovirus et les maladies sexuellement transmissibles, ou de comportements tel que l'usage de drogues ou une

consommation élevée d'alcool ou d'autres états inflammatoires non infectieux n'a pas pu être évalué.

4- Si les changements des marqueurs n'étaient pas différents entre les INTIs (TDF-FTC et ABC-3TC), le type de troisième agent a été associé aux changements des marqueurs d'activation immunitaires IP-10 et MIG. La baisse d'IP-10 et de MIG était plus faible chez les patients qui ont reçu l'ATV/r par rapport aux patients qui ont reçus l'EFV alors qu'aucune différence n'a été observée entre les patients qui ont reçu le LPV/r par rapport aux patients qui ont reçu l'EFV. Ces résultats suggèrent que l'évaluation des marqueurs d'activation immunitaire pourrait être un critère utile lors de l'évaluation des nouvelles molécules antirétrovirales à cote de la mesure de charge virale et le taux des cellules CD4+.

A la fin de ce travail de thèse, plusieurs perspectives de recherche semblent pertinentes. Tout d'abord, si deux années de traitement antirétroviral virologiquement efficace n'est pas suffisante pour entraîner l'activation immunitaire à des niveaux normaux, il serait approprié d'évaluer cet impact après une plus longue période de suppression virologique (5 ans par exemple). Deuxièmement, notre population comportait des patients naïfs ayant initié un traitement antirétroviral avec une immunodéficience modérée, mais il reste à évaluer si l'initiation de traitement antirétroviral à un taux de CD4+ plus élevé (> 500/mm3), comme c'est désormais recommandé, pourrait diminuer la persistance de l'activation immunitaire post traitement. Troisièmement, l'impact des nouvelles molécules antirétrovirales sur l'activation immunitaire et l'inflammation doit être évalué. Parmi ceux-ci, dolutégravir qui a montré une efficacité supérieure à l'éfavirenz dans l'étude SIMPLE, mérite d'être évalué. Enfin, il serait intéressant d'évaluer l'impact du traitement antirétroviral sur l'activation et l'inflammation chez les patients qui restaurent un niveau de CD4 $> 500/mm^3$.

II. SCIENTIFIC PRODUCTION

A. Published original article

HATTAB S, GUIHOT A, GUIGUET M, FOURATI S, CARCELAIN G, CABY F, MARCELIN A-G, AUTRAN B, COSTAGLIOLA D, KATLAMA C. **Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study.** *BMC Infectious Diseases* 2014 14:122.

B. Submitted article for publication

HATTAB S, GUIGUET M, CARCELAIN G, FOURATI S, GUIHOT A, AUTRAN B, CABY F, MARCELIN A-G, COSTAGLIOLA D, KATLAMA C. S**oluble biomarkers of immune activation and inflammation: impact of two years of effective first-line cART.**

C. Oral communication

HATTAB S, GUIGUET M, CARCELAIN G, FOURATI S, GUIHOT A, AUTRAN B, CABY F, MARCELIN A-G, COSTAGLIOLA D, KATLAMA C. **Impact de différents traitements antirétroviraux (ARV) sur l'évolution des marqueurs d'inflammation et d'activation immunitaire plasmatiques chez les patients infectés par le VIH.** *7eme Conférence Internationale Francophone VIH/HEPATITES*. AFRAVIH 2014, Montpellier, France.

III. ABBREVIATIONS LIST

ABC/3TC: Abacavir/Lamivudine
ACTG: AIDS Clinical Trials Group
AIDS: Acquired Iimmunodeficiency syndrome
ALLRT: AIDS Clinical Trials Group Longitudinal Linked Randomized Trials
APC: Antigen presenting cells.
ARV: Antiretroviral
ATV: Atazanavir
AZT/3TC: Zidovudine/ lamivudine
B_2-microglobulin: Beta2-microglobulin
BMD: Bone mineral density
BMI: Body mass index
cART: Combination antiretroviral therapy
CBA: Cytometric Bead Array
CCR5: C-C chemokine receptor type 5
CMV: Cytomegalovirus
COREVIH : COordination REgionale de lutte contre le VIH
COX-2: Cyclooxygenase type-2
CROI: Conference on Retroviruses and Opportunistic Infections
CRP: C-reactive protein
CTL: Cytotoxic lymphocytes
CXCR4: C-X-C chemokine receptor type 4
CyA: Cyclosporine A
CYP3A4: Cytochrome P450 3A4
DEXA scan: Dual-Energy X-ray Absorptiometry
EACS: European AIDS Clinical Society
EBV: Epstein-Barr virus
EFV: Efavirenz
ELISA: Enzyme-Linked ImmunoSorbent Assay
ESPRIT: Evaluation of Subcutaneous Proleukin in a Randomized International Trial
FDA: Food and Drug Administration
FHDH: French Hospital Database on HIV

FPV:	Fosamprenavir
GALT:	Gut-associated lymphoid tissue
G-CSF:	Granulocyte colony-stimulating factor
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
gp 41:	Glycoprotein 41
gp120:	Glycoprotein 120
HAART:	Highly active antiretroviral therapy
HAND:	HIV-associated neurocognitive disorders
HBV:	Hepatitis B virus
HCV:	Hepatitis C virus
HDAC:	Histone deacetylases
HEAT study:	HIV Study with Epzicom And Truvada
HIV:	Human Immunodeficiency Virus
HLA B*5701:	Human leukocyte antigen B*5701
HPTN study:	HIV Prevention Trials Networks
HR:	Hazard Ratio
Hs-CRP:	Highly sensitive C-reactive protein
IFN-γ:	Interferon-gamma
IL:	Interleukin
INSIGHT:	International Network for Strategic Initiatives in Global HIV Trials
IP-10:	Interferon gamma-induced protein 10
IQR 25-75%:	Inter-quartile 25-75%
LPS:	Lipopolysaccharides
LPV:	Lopinavir
MACS:	Multicenter AIDS Cohort Study
MCP-1:	Chemotactic protein 1
MHC:	Major histocompatibility complex
MI:	Myocardial infarction
MIG:	Monokine induced by interferon-γ
MIP-1 α/β:	Macrophage Inflammatory Proteins-1 alpha/beta
MSM:	Men who have sex with men
MVC:	Maraviroc
NF-κB:	Nuclear factor kappa B
NNRTI:	Non-nucleoside analogue reverse transcriptase inhibitor

NNRTIs:	Non-nucleoside reverse transcriptase inhibitors
NRTI:	Nucleoside analogue reverse transcriptase inhibitor
NtRTI:	Nucleotide reverse transcriptase inhibitor
OR (95% CI):	Odds ratio (95% confidence interval)
PBMCs:	Peripheral blood mononuclear cells
PD1:	Programmed death 1
pDCs:	Plasmacytoid dendritic cells
PI:	Protease inhibitor
PYFU:	Person-years follow-up
RAL:	Rraltegravir
RANTES:	Regulated on activation normal T cell expressed and secreted
RNA:	Ribonucleic acid
SATURN-HIV:	Stopping Atherosclerosis and Treating Unhealthy Bone with Rosuvastatin in HIV
sCD14:	Soluble cluster of differentiation 14
SDF-1:	Stromal cell-derived factor 1
sICAM:	Soluble intercellular adhesion molecule-1
sIL-2R:	Soluble interleukin-2 receptor
SMART study:	Strategies for Management of Antiretroviral Therapy
SMR:	Standardized mortality rate
SPIRAL study:	Switching From PI to RALtegravir in HIV Stable Patients
sTNFR-75:	Soluble tumor necrosis factor receptor-75
sTNF-RI, II:	Soluble tumor necrosis factor receptor-1 and II
sVCAM-1:	Soluble Vascular cell adhesion protein 1
TDF/FTC:	Tenofovir/Eemtricitabine
Th1, Th2:	T-helper cells
Th17:	IL-17-secreting T-helper cells
TNF-α:	Tumor necrosis factor-alpha
US DHHS:	United States Department of Health and Human Services
WHO:	World Health Organization

IV. INTRODUCTION

The use of combination antiretroviral therapy (cART) controls HIV replication in the vast majority of compliant HIV-infected patients. However, it does not fully restore health and patients are still at higher risk of co-morbidities compared to the general population (Shiels MS *et al*, 2009; Lang S *et al*, 2010). In addition, higher levels of immune activation and inflammation markers persist despite the control of HIV viremia when compared to the HIV uninfected population (Reingold J *et al*, 2008; Neuhaus J *et al*, 2010; Alcaide ML *et al*, 2013).

Immune activation and inflammation has received attention in the last years after the observation of the association between inflammation and coagulation markers and all-cause mortality in the SMART study; a finding that modified the concepts about HIV pathogenesis (Kuller LH *et al*, 2008). In this study, patients who had IL-6 and D-dimer levels in the upper quartile at study entry were at 3 to 4 times increased risk of mortality compared to patients who had levels in the lower quartile. Following this observation, several studies have shown an association between elevated levels of inflammatory markers and increased risk of mortality (Tien PC *et al*, 2010; Sandler NG *et al*, 2011) as well as non-AIDS defining morbidities, notably cardiovascular disease and non-AIDS defining cancers (Duprez DA *et al*, 2012; Borges AH *et al*, 2013; Tenorio AR *et al*, 2014). In addition, studies have shown an association between high levels of immune activation and lesser increases in CD4+ T-cell count under cART (Lederman MM *et al*, 2011; Zhang X *et al*, 2013).

Several mechanisms have been proposed as potential causes for the persistence of immune activation and inflammation. Of these, HIV reservoirs and residual viremia have been shown to induce immune activation through HIV-antigenic stimulation of the immune system (Ostrowski SR *et al*, 2008; Mavigner M *et al*, 2009). Secondly, mucosal immune dysfunction characterized by profound depletion of CD4+ T-cells during the early acute phase of HIV infection can lead to a gradual loss of the intestinal barrier function, allowing translocation of the intestinal flora into the systemic circulation leading to immune activation (Brenchley JM *et al*, 2006; Sandler NG *et al*, 2012). In addition, the reactivation of other chronic viral infections such as cytomegalovirus and Epstein-Barr virus induces immune activation through non-HIV antigenic stimulation of the immune system (Petrara MR *et al*, 2012; Wittkop L *et al*, 2013).

The impact of antiretroviral therapy on soluble markers of immune activation and inflammation has not been well documented. Few studies have examined this impact in naïve HIV-infected patients with variable results. While IL-6 levels were found to decrease in the HEAT, MERIT and the ACTG A5224 studies (Smith KY *et al*, 2009; Funderburg N *et al*, 2010; McComsey GA *et al*, 2012), the evolution of other markers such as IP-10 and sCD14 varied between studies. While one study showed that IP-10 levels fell and sCD14 remained elevated (Cassol E *et al*, 2010), another found that both markers fell following therapy (Taiwo B *et al*, 2013). While these discrepancies might reflect differences in baseline HIV disease status, different ART regimens and treatment durations, we think that the major methodological problem in these studies is the inclusion of patients with different levels of virological response.

Given this research problem, my work was devoted to unravel the impact of ART on the dynamics of immune activation and inflammation markers in HIV-infected patients who initiate cART with rapid and persistent viral control over two years, to control the potential impact of viral replication on these markers. First, I evaluated marker levels in HIV-infected patients in comparison with uninfected controls and identified factors associated with high levels of markers before ART initiation. Then, I evaluated changes of markers over two years of cART and identified factors associated with the persistence of elevated levels. Finally, I compared the impact of different ART components on changes of markers among a group of patients who remained on the initial regimen over the 2 years.

V. STATE OF THE ART

A. Human immunodeficiency virus (HIV)

HIV infection, emerged through its devastating face of AIDS in early 1980s has led to an unprecedented epidemic in the modern era. Indeed, in 1981 the first cases of AIDS were identified in New York and California among young previously healthy homosexual men presenting an unusual clustering of rare diseases, notably *pneumocystis carinii* pneumonia, Kaposi's sarcoma as well as cases of persistent lymphadenopathy (Gottlieb MS *et al*, 1981; Rolston KV *et al*, 1986). The observations regarding the immunopathogenesis of this disease and the pattern of occurrence indicated an infectious agent as the likely cause. Further research led to the isolation of the causing virus in the year 1983, and it was named Lymphadenopathy Associated Virus (LAV) in Europe and Human T cell Lymphotrophic Virus III (HTLV III) in the USA (Barre-Sinoussi F *et al*, 1983; Freedman D *et al*, 1989). It is now known as human immunodeficiency virus (HIV) that belongs to the lentivirus retroviruses family. In 2008, the Nobel Prize was attributed to Françoise Barré Sinoussi for the discovery of HIV.

1. Epidemiology of HIV infection

Since the last decade of last century, HIV/AIDS epidemic has become the greatest challenge in global health. Overall the number of HIV infected individuals has regularly increased. In 2012, it is estimated that 35.3 (range 32.2-38.8) million persons are living with HIV worldwide. It is estimated that 2.3 (range 1.9-2.7) million have became newly infected with HIV and 1.6 (range 1.4-1.9) million died in the year 2012 (Unaids, 2013) compared to 5.6 million new infections and 2.6 million deaths in the year 1999 (Cock KM *et al*, 2000). Nowadays, the global prevalence of HIV infection has decreased and stabilized at 0.8 % (range 0.7% - 0.9%) (Dorrucci M, 2010).

The global statistics on HIV infection mask some important local and regional epidemiologic differences. The sub-Saharan Africa remains the most heavily affected region, with two-thirds of the global burden. In addition, regional differences in the trends and mode of transmission exist: epidemics of HIV in men who have sex with men (MSM) continue to expand in most countries notably in the developed countries while heterosexual transmission remains the main mode of transmission in sub-Saharan Africa (Beyrer C *et al*, 2012). Worldwide HIV/AIDS prevalence is shown in **figure 1**.

In France, according to the French National Institute for Public Health Surveillance, it is estimated that 6400 individuals were newly diagnosed in the year 2012, a stable number since 2007. The number of newly diagnosed cases of HIV infection was stable in all groups except among MSM where the number increased and attributed to 42% of new diagnosed cases in 2012. The increasing number of newly diagnosed cases seems to be the result of a greater use of screening in this population including the use of rapid diagnostic tests (http://www.invs.sante.fr). The incidence of HIV infection was estimated at about 17 per 100 000 person-years. Even the incidence of HIV infection has decreased between 2003 and 2008, it remained high and stable in MSM with an incidence of 1006/100 000 person-years in MSM compared to 86/100 000 person-years in intravenous drug users and 9/100 000 person-years among heterosexuals (Le Vu S *et al*, 2010).

In 2010, it was estimated that 149900 (95% CI; 134700-164900) HIV-infected persons were living in France. Of those, 81% were diagnosed while the remaining 19% ignored their seropositivity. While 74% of HIV-infected patients were receiving care, only 56% of them achieved controlled viral load. In addition, the proportions of diagnosed patients, those under care and those with a perfect response to antiretroviral therapy defined as achieving control of viral replication (controlled viral load) varied according the transmission mode. While the highest proportions were found among drug abusers, the lowest proportions were among non-French heterosexuals (Supervie V *et al*, CROI 2013, Abs. 1030).

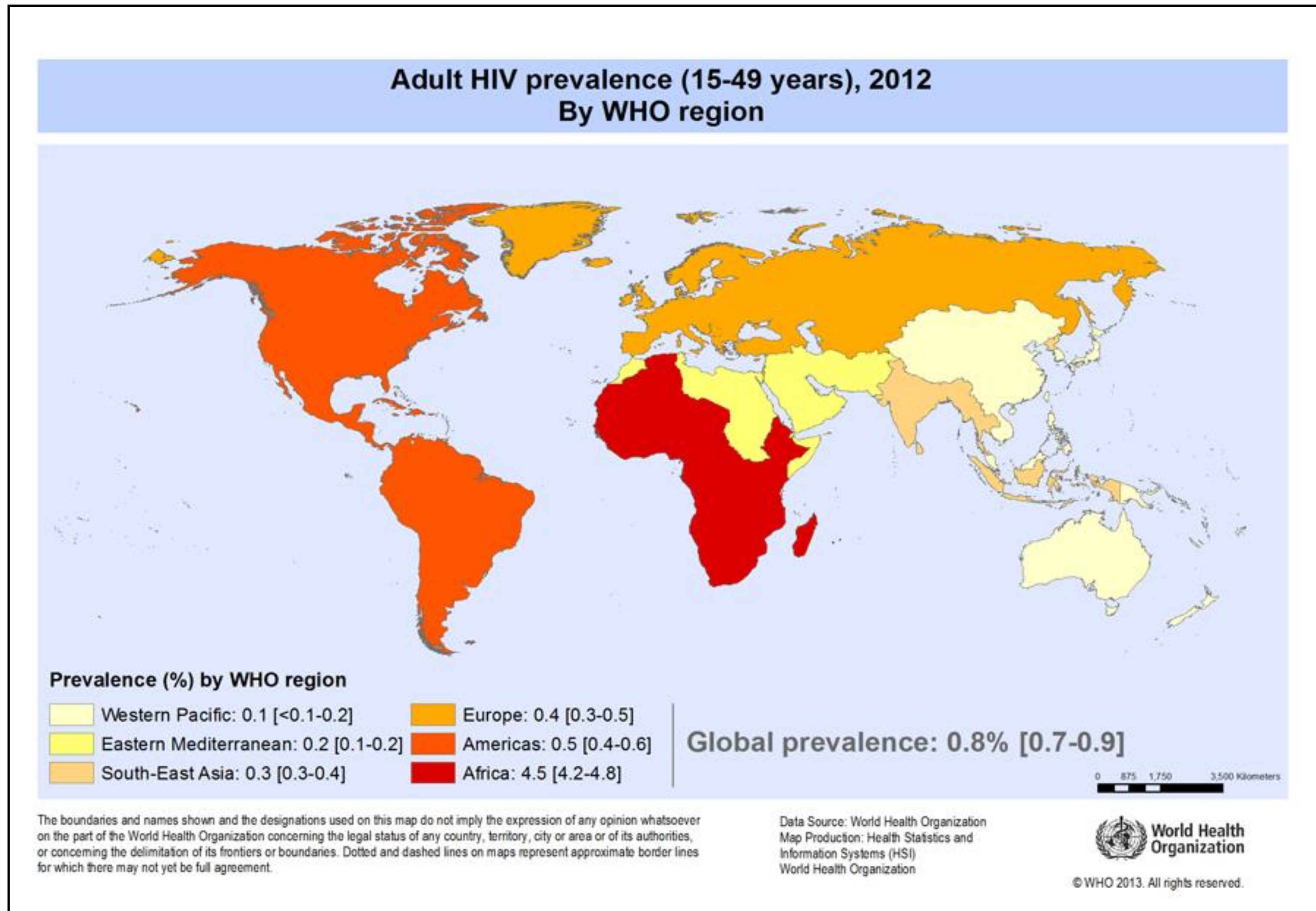


Figure 1: Worldwide HIV prevalence in the year 2012 (available at: http://www.who.int/gho/hiv/hiv_013.jpg?ua=1).

2. HIV replication cycle

Understanding the major steps of HIV replication cycle has been key to investigate molecules that could block its replication and permitted the manufacturing of antivirals directed against important steps of this cycle.

- Step 1 Fusion and entry:

After penetration in the body through mucosa in case of sexual transmission or directly in blood, HIV begins its replication cycle when it binds to different cell types including T-helper lymphocytes, macrophages and dendritic cells. In the mid-nineties, it has been demonstrated that this binding occurs through the reaction of the viral glycoprotein 120 (gp120) to CD4 receptor and one of two co-receptors, CXCR4 or CCR5 (referred to as X4 and R5 respectively) found on the surface of these cells (Deng H *et al*, 1996; Feng Y *et al*, 1996). This binding allows the fusion peptide (gp 41) to penetrate the cell membrane which permits the virus to release the RNA, its genetic material in the cytoplasm of the host cell (Azevedo JM *et al*, 2008).

-Step 2 Reverse transcription:

Once the RNA is released in the cytoplasm of the host cell, an enzyme called reverse transcriptase (RT), specific of retroviruses, copies RNA into a complementary DNA (cDNA) molecule. This major step is error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the immune system (Sarafianos SG *et al*, 2009). The RT also has DNA polymerase activity that creates a sense DNA from the antisense cDNA. Together, the cDNA and its complement form a double-stranded viral DNA that migrate toward the nucleus of the host cell and penetrate it.

-Step 3: HIV integrates the cell genome:

After penetrating the nucleus of the host cell, the integration of viral DNA in the cellular DNA takes place with the aid of viral integrase which catalyzes insertion of the both 3’ viral DNA ends into target DNA (Cherepanov P *et al*, 2011, Krishnan L *et al*, 2012). This integrated viral DNA may then lie dormant, in the latent stage of HIV infection (Taube R, 2013). To actively produce the virus, certain cellular transcription factors need to be present, the most important of these is NF-κB (NF kappa B), which is up regulated when T-cells become activated (Hiscott J *et al*, 2001; Colin L *et al*, 2009). Latent infected cells could

remain inactive for several years. When an infected cell with integrated DNA duplicates, this leads to the production of two cells carrying viral DNA. After being integrated in the host genome, the provirus uses host RNA polymerase to generate messenger RNA (mRNA). This mRNA is then exported from the nucleus to the cytoplasm where it is translated into immature viral proteins.

-Step 4: Protein cleavage and assembly:

The viral protease then cleaves the long chains of HIV proteins into smaller individual proteins (Kohl NE *et al*, 1988) which assemble with HIV RNA genetic material creating a new virus particle. During budding the virus steals part of the cell outer envelope. This envelope, which acts as a covering is studded with HIV glycoproteins (gp120 and gp41) necessary for the virus to bind CD4 receptor and co-receptors to infect other cells.

The final step of the viral life cycle is called budding. With its genetic material tucked away and a new outer coat made from the host CD4 cell's membrane, the newly formed HIV pinches off and enters into circulation, ready to start the whole process again. Different steps of HIV replication are shown in **figure 2**. Using a mathematical model, Perelson AS *et al* estimated that the minimum duration of the HIV-1 life cycle in vivo is 1.2 days on average, and that the average HIV-1 generation time, defined as the time from release of a virion until it infects another cell and causes the release of a new generation of viral particles, is 2.6 days (Perelson AS *et al,* 1996).

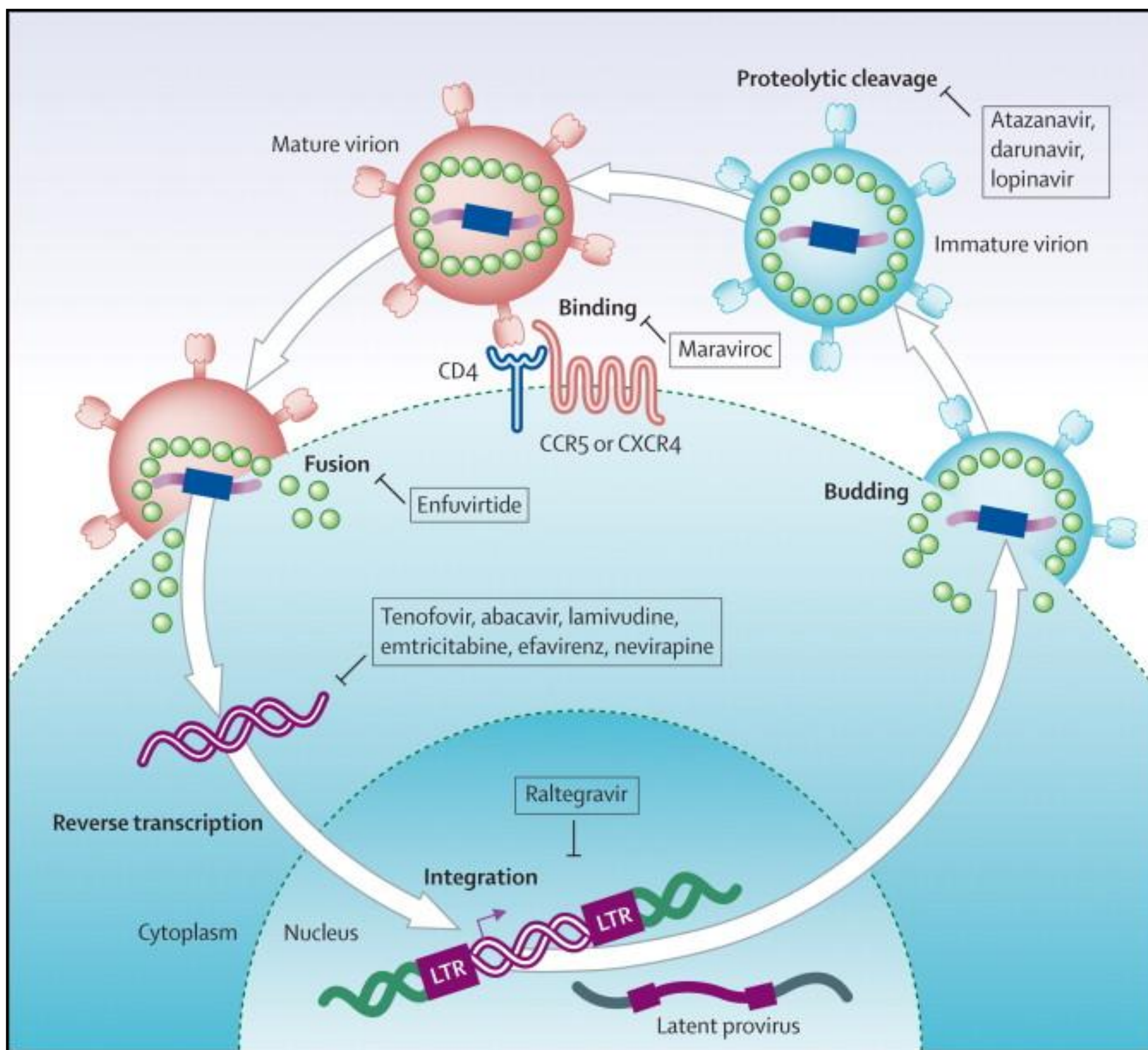


Figure 2: HIV replication cycle (Adapted from Volberding P *et al*, 2010).

3. Immune response against HIV infection

The first response against the HIV infection takes place at the site of infection in order to prevent viral entry. Mucosal epithelium mediates innate defenses through signaling system with Toll-like receptors and provides an array of inhibitory molecules such as SDF-1, MIP-1 α/β and RANTES (SDF-1 blocks CXCR4 while MIP-1 α/β and RANTES block CCR5). The vaginal inoculation of virus leads to the expression of chemokines (MIP-3α) that recruit interferon (IFN)-α/β producing plasmacytoid dendritic cells (pDCs) and to the production of pro-inflammatory cytokines (GM-CSF, IL-1, IL-6 and IL-8) that recruit neutrophils, macrophages and lymphocytes to the endocervix (Haase AT *et al*, 2010). The response of

pDCs results in the induction of inflammatory cytokines, which are involved in directly setting up an antiviral state, and indirectly activating other antiviral cells of the innate immune system (Carrington M *et al*, 2012). Natural killer cells mediate antiviral control, through the recognition of virally infected cells through a network of receptors called the killer immunoglobulin-like receptors (Alter G *et al*, 2011).

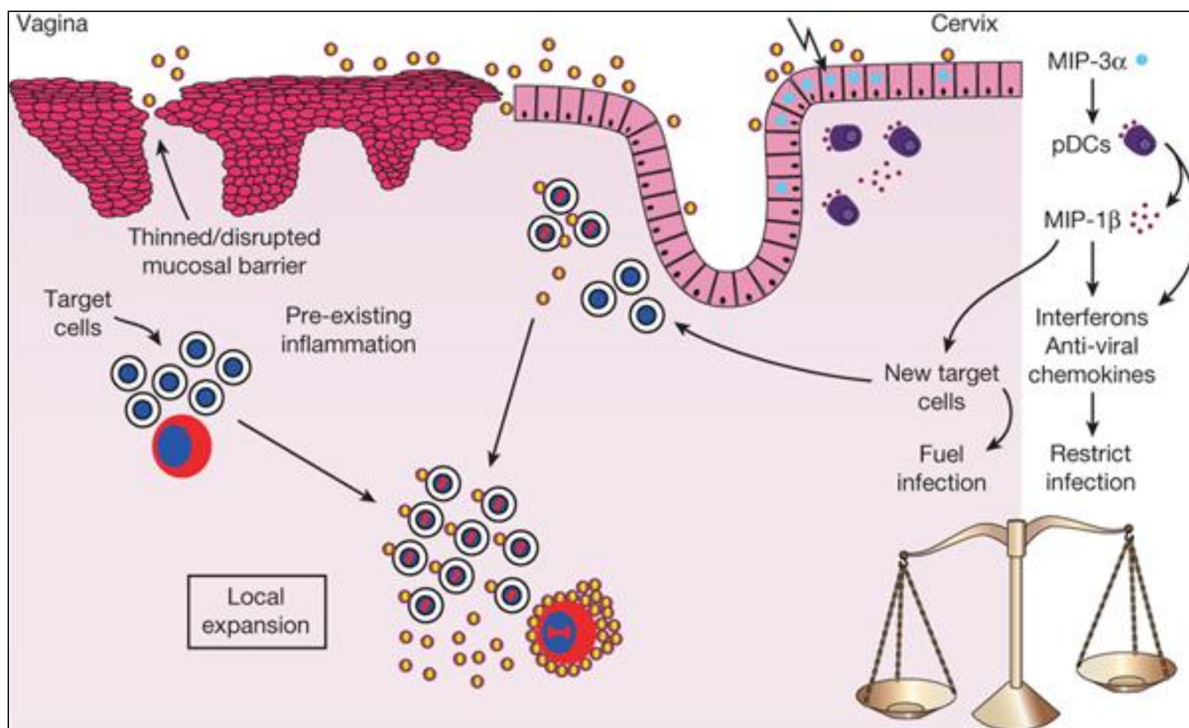


Figure 3: Early immune response upon HIV transmission (Adapted from Haase AT *et al*, 2010).

Paradoxically, these innate antiviral and inflammatory defense mechanisms may facilitate virus transmission, by increasing target cell availability, and by creating conditions for highly efficient cell-to-cell spread of infection. The innate immune response, the inflammatory response and the large increases in IFN-γ expression brings in large numbers of target cells to create a generally favorable environment to fuel expansion of HIV (**Figure 3**). The virus migrates to the gut-associated lymphoid tissue (GALT) and viral replication takes place and reaches its peak that can exceed 10 million copies/mL. This peak is associated with a cascade of elevations in cytokine and chemokine levels (cytokine storm). Stacey AR *et al* quantified levels of 30 cytokines and chemokines using sequential plasma samples collected during the eclipse and exponential viral expansion phases from subjects acquiring HIV-1. The increase in plasma viremia in acute HIV-1 infection was found to be associated with

elevations in plasma levels of multiple cytokines and chemokines (**Figure 4**), including rapid and transient elevations in IFN-α and IL-15 levels; a large increase in IP-10 levels; rapid and more-sustained increases in TNF-α and MCP-1 levels; more slowly initiated elevations in levels of additional pro-inflammatory factors including IL-6, IL-8, IL-18, and IFN-γ; and a late-peaking increase in levels of the immune-regulatory cytokine IL-10 (Stacey AR *et al*, 2009).

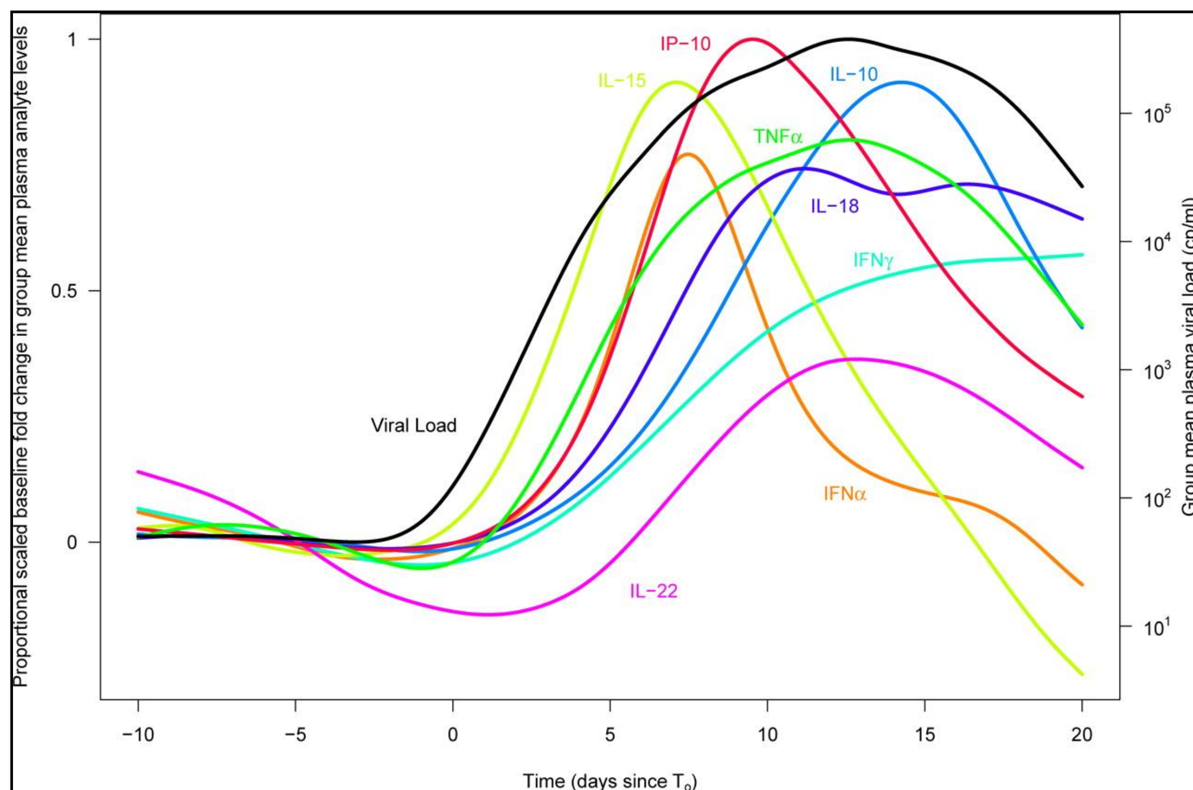


Figure 4: The cytokine storm associated with HIV replication (Adapted from Stacey AR *et al*, 2009).

Although the cytokines/chemokines produced in acute HIV infection contribute to the control of viral replication, their role is not sufficient. The cellular immune responses appear at the end of the second week of infection following antigen expansion and peak replication. These responses are considered late because they cannot clear the virus locally or prevent systemic spread.

Cellular immune responses consist of both helper and cytotoxic T-lymphocytes. Helper T-lymphocytes differentiation into Th1 or Th2 cells is induced by antigen presenting cells (APC). Th1 lymphocytes produce IL-2, IL-12, IFN-γ, and TNF-α which help in the development of cytotoxic lymphocytes (CTL) against HIV. Th2 lymphocytes produce specific

interleukins (IL-4, IL-5 and IL-10) and induce the differentiation of B lymphocytes into plasma cells which produce different antibodies against HIV proteins.

Cytotoxic T lymphocytes play a key role in the immune response against HIV infection particularly before antibodies production. They mediate dual antiviral suppression function by both cytolytic and non-cytolytic mechanisms. The predominant cytolytic mechanism requires direct contact of these lymphocytes with HIV-infected major histocompatibility complex (MHC) class I matched target cells presenting the antigen on their surfaces. This contact leads to the lyses of infected cells mediated by the secretion of granzymes and perforin (Bots M *et al*, 2006; Voskoboinik I *et al*, 2006). The second mechanism is mediated by soluble inhibitory factors produced upon T-cells activation (RANTES, MIP-1 α/β). These three factors contribute to an inflammatory response by recruiting leukocytes to the site of infection and inhibit HIV replication by binding its receptor (CCR5) (Demers KR *et al*, 2013). **Figure 5** illustrates the cellular immune response against HIV infection.

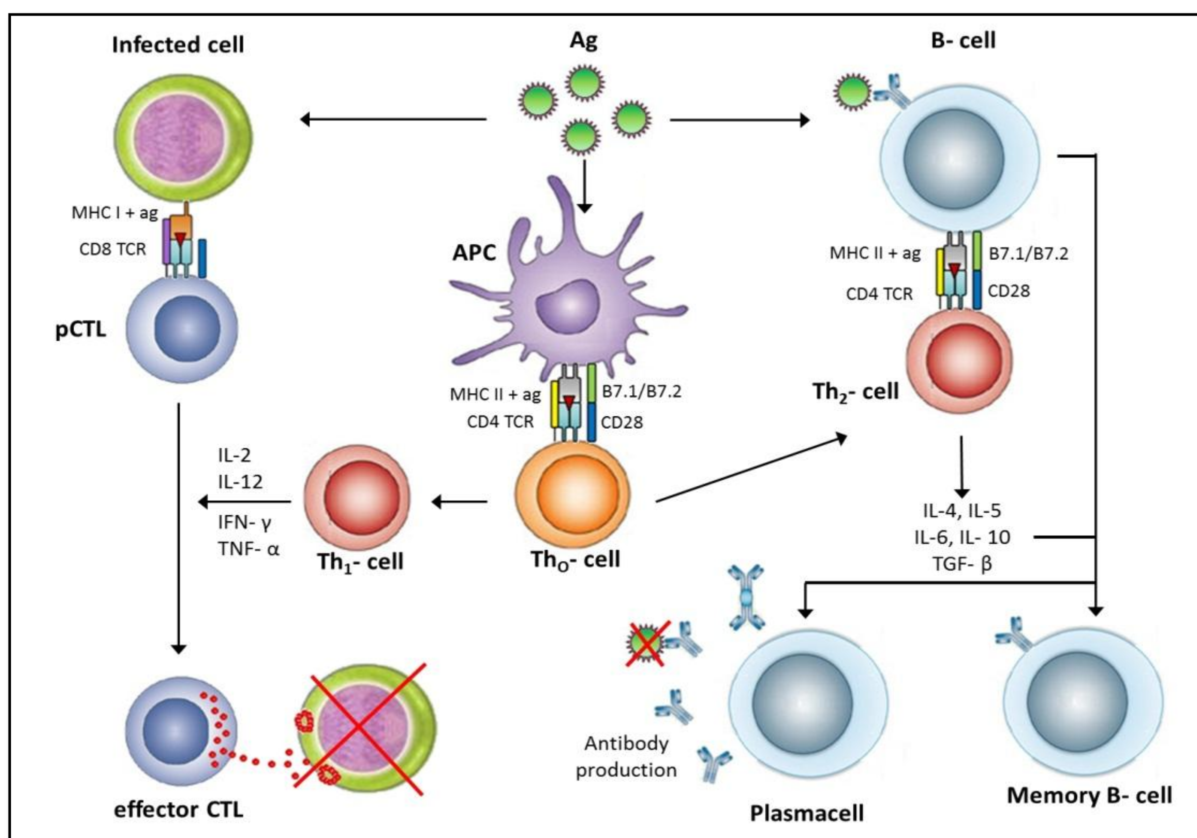


Figure 5: The cellular immune response against HIV infection (Available at: http://www.intechopen.com/books/immunodeficiency).

Humoral immune response against HIV infection consists of antibodies against the virus envelope and capsid (p24, p18, RT and nef) proteins. These antibodies appear 3 to 12 weeks after the contamination with the virus and persist till the progression of the disease where they start to decrease. Only neutralizing antibodies could have protecting role but they appear 2-6 months after the contamination (Alter G *et al*, 2010). The initial group of antibodies defined some of the sites of HIV-1 vulnerability on the envelope spike which consists of three g120/gp41 heterodimers. These sites included the CD4 binding site (CD4bs); the N160 glycan-dependent site associated with the V1/V2 loops; the N332 glycan dependent site at the base of the V3 loop; and the membrane-proximal external region (MPER) on gp41 (Mascola JR *et al*, 2010). Recently, additional sites of vulnerability are being identified as exemplified by 8ANC195 and 3BC176 that recognize yet to be defined epitopes (Klein F *et al*, 2013). In addition to the late appearance of these antibodies, the gene encoding the HIV envelope displays an enormous amount of diversity allowing the virus to escape these antibodies.

4. The natural course of HIV-1 infection

HIV infection initiates a process that leads to progressive destruction of CD4+ lymphocytes, the preferred target cell for HIV-1 infection. The course of HIV-1 infection varies from person to person but a typical pattern is characterized by three phases that occur over a period of years:

- Primary infection

Corresponds to a cascade of biological events that follow the penetration of HIV and lasts 2 to 8 weeks. Following the local innate immune response and the production of inflammatory cytokines, the virus rapidly migrates, probably via draining lymph nodes to the gut-associated lymphoid tissue (GALT) where the establishment of productive infection is dictated by the availability of target CD4+ T cells that express the chemokine receptor CCR5 (Moir S *et al*, 2011). HIV replicates aggressively reaching levels of plasma viremia as high as 10 million copies/mL inducing a cascade of elevations in cytokine and chemokine levels (cytokine storm). This phase is accompanied by a dramatic depletion of CD4+ T cells in the peripheral blood as well as a massive depletion of CCR5+ memory CD4+ T cells in the GALT, a determinant factor of disease progression. Specific losses in mucosal immune function have been associated with the preferential depletion of IL-17-secreting (Th17) CD4+ T cells, which are a subset of T helper cells that are involved in mucosal host defense against

extracellular bacteria (Brenchley JM *et al*, 2008). This depletion leads to the translocation of microbial products into the systemic circulation and the induction of immune activation. Another important event that occurs in this phase is the establishment of the resting CD4+ T cell reservoirs and the dissemination of HIV in other lymphoid organs (discussed later). Early events associated with HIV infection are shown in figure 6.

Clinically, patients usually present signs and symptoms of viral infections including fever, lymphadenopathy, pharyngitis and cough during this phase (Touloumi G *et al*, 2000). However, primary infection remains asymptomatic in some patients. Patients with symptomatic primary infection may progress to have AIDS more rapidly than people with low-grade symptoms or asymptomatic primary infection (Henrard DR *et al*, 1995). Viral replication is massive with over million viruses found in blood and the contagiosity of the newly infected subject is very high. Recognition of this brief syndrome is of major importance to prevent further viral dissemination. Then, with the emergence of immune responses in particular HIV-specific CD8+ T cell responses, HIV viral loads starts to decline precipitously (Touloumi G *et al*, 2000).

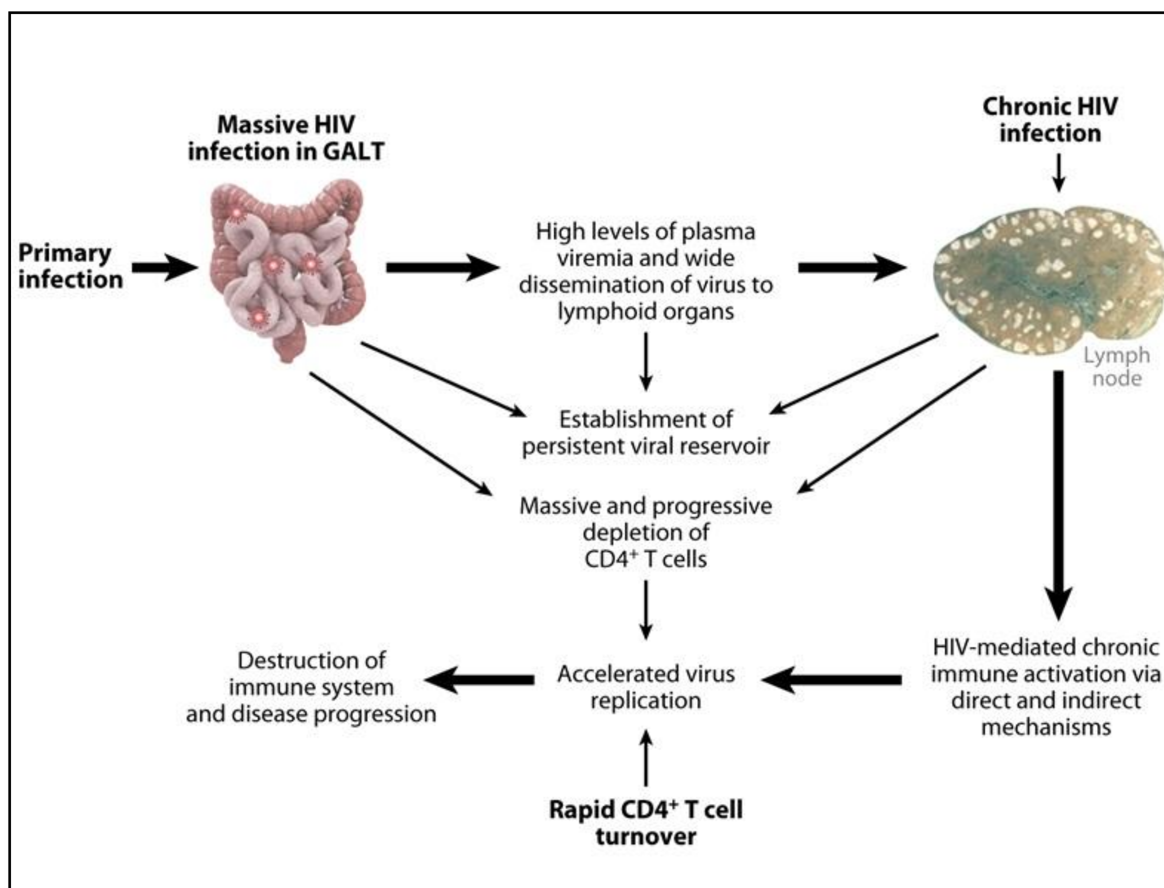


Figure 6: Early events associated with acute HIV infection (Adapted from: Moir S *et al*, 2011)

❖ The asymptomatic phase of HIV infection

Is a dynamic process of virus production and clearance by the immune responses which maintain HIV-RNA stabilized around a set point (Pantaleo G *et al*, 1995). As the immune response is not sufficient to control the virus, the replication continues in the presence of the activated immune system (clinical latency) and leads to slow and gradual depletion of CD4+ T lymphocytes count and this decline varies among individuals. Depending on a rate of CD4 decline between 50-100 cells/mm3 per year, the asymptomatic phase may last 10 years in some individuals. Without any symptoms, HIV-infected individual are however a source of contamination for their sexual partners.

❖ The symptomatic phase:

Prior to the development of AIDS, the homeostasis between the viral replication and the immune responses breaks and leads to rapid depletion of the total T cells and eventually in immune collapse. The reason behind this break may be due to the exhaustion of the proliferative capacity of lymphocytes and progressive deterioration of lymphoid organs as well as the HIV-induced immune activation. It is in this phase where AIDS defining diseases such *pneumocystis jirovecii* pneumonia, Kaposi's sarcoma and tuberculosis appear mainly when CD4 counts drop below 200 cells/mm^3. Figure 7 illustrates the natural course of HIV infection.

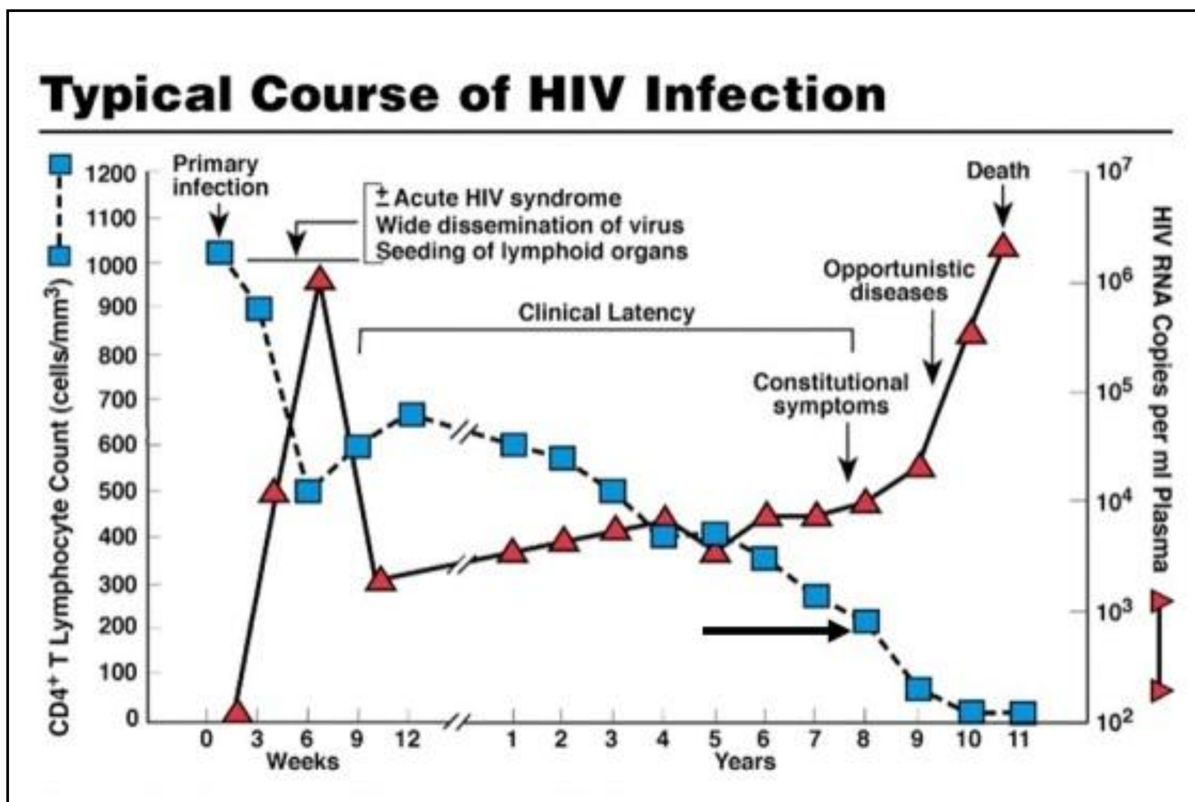


Figure 7: The natural course of HIV infection (Adapted from Fausi AS *et al*, 1996)

5. Predictive biomarkers in the natural course of HIV infection

From the beginning of the epidemics, before being able to directly quantify virus production in blood, different biomarkers have been investigated as surrogate markers to predict HIV disease progression namely occurrence of AIDS, or death in the different attempts to identify drug targets for clinical interventions. These biomarkers could reflect the intensity of viral replication, the degree of immune system activation and the degree of immune deficiency. Beside the predictive value of CD4+ cell count and percentage, neopterin, B_2-microglobulin (immune activation markers) and p24 antigen were identified as predictive biomarkers of HIV disease progression and all-cause mortality before the availability of viral load measurement. Later in 1995, the HIV-1 RNA revealed as strong predictor of HIV progression to AIDS and death independently of CD4+ lymphocyte count (Mellors JW *et al*, 1995; Mellors JW *et al*, 1997). Since then, CD4+ cell count and HIV-1 RNA are the most accurate predictive biomarkers in HIV infection and the standard investigation in HIV infected patients. In addition to markers of immune activation, different markers of inflammation (sICAM-1, E-selectin and IL-6) were predictive of all-cause mortality. In a

cohort of 606 ART-naïve HIV-infected women, CRP was predictive of maternal progression to AIDS, maternal mortality and child mortality. **Table 1** summarizes studies that investigated predictive biomarkers in the natural course of HIV infection in untreated HIV-infected patients.

Table 1: Predictive biomarkers of HIV disease in the natural course of infection in untreated patients

Author	N	Study population	Outcome	Predicvtive markers
Fahey JL *et al*, 1990	395	Homosexual men initially free of AIDS	Progression to AIDS	-neopterin (HR: 6.1, CI:3.4 to 11.0) -B2-microglobulin (HR: 3.2, CI: 1.7 to 6.1)
Hofmann B *et al*, 1990	50	HIV seroconverters	CD4 cell count fall	-B2-microglobulin increased levels correlated inversely with the CD4 cell count ($p<0.001$)
Zangerle R *et al*, 1998	47	Treated or non-treated with AZT monotherpy enrolled in 1991	CD4 cell count fall	-neopterin -B2-microglobulin -sTNFR-75 increased levels correlated inversely with the CD4 cell count ($r=-0.51$, $r=-0.41$, $r=-0.42$ respectively; $p< 0.01$)
Ledergerber B *et al*, 2000	169	Chronically infected patients enrolled between 1993-1994	Progression to AIDS	-p24 antigen: increased p24 levels predicted progression to AIDS ($p=0.043$)
Sipsas NV *et al*, 2003	64	HAART-naive patients enrolled between 1990-1993	AIDS-related death	-sIL-2R -sICAM-1 increased levels associated with time to death ($p=0.008$ and 0.003, respectively)
Feldman JG *et al*, 2003	209	HIV-1-infected women enrolled between 1994-1995	All-cause mortality	-CRP: predicted mortality ($p< .01$) after adjusting for age, BMI, serum albumin, CD4 cell count and HIV-1 RNA.
Mildvan D *et al*, 2005	152	Subsample of ACTG 116B/117, a randomized trial that compared clinical benefit of didanosine and zidovudine monotherapy	Progression to AIDS	- neopterin -endogenous interferon elevated values were associated with disease progression ($p=.0002$, $p=.0053$ respectively) after adjustment for CD4+ cell count and HIV-1 RNA level

Drain PK *et al*, 2007	606	HAART-naïve HIV-infected women	- Maternal progression to AIDS or mortality - Child mortality	-CRP high maternal CRP was associated with progression to stage 4 or death (HR:2.26, CI:1.64 to 3.12) and greater risk of child mortality (HR: 3.03, CI: 1.85-4.96)
Erikstrup C *et al*, 2008	198	ARV-naive HIV-1-infected individuals from the Mupfure Schistosomiasis and HIV Cohort in Zimbabwe	- Progression to AIDS - All-cause mortality	-p24 antigen 10-fold higher was associated with mortality (HR: 2.3, CI: 1.6 to 3.0) and progression to AIDS (HR: 2.0, CI: 1.3 to 3.3)

B. Highly active antiretroviral therapy: the revolution

Since HIV was isolated in 1983, there has been intensive research to identify drugs that could inhibit viral production. Zidovudine, a nucleoside analogue reverse transcriptase inhibitor (NRTI) has been the first antiretroviral drug to demonstrate a benefit in clinical course of HIV disease (Fischl MA *et al*, 1987). However, the benefit was short term mainly due to the insufficient antiviral potency of this drug used as a monotherapy for a sustained clinical benefit. With the continuing development of drugs in the same class, the next step has been the evaluation of dual NRTI combination. Combination therapy with zidovudine and didanosine produced better outcomes in terms of virological control and CD4+ cell counts increases than zidovudine therapy alone (Collier AC *et al*, 1993). Similar results were obtained when combining zidovudine and lamivudine (Eron JJ *et al*, 1995). In 1996, for the first time, the combination of three dugs with the combination of 2 NRTIs and a protease inhibitor (Murphy RL *et al*, 2001) followed soon by the non-nucleoside analogue reverse transcriptase inhibitor (NNRTI) class as third agent, led to a durable clinical benefit and durable control of viral replication (Rey D *et al*, 2001) with a massive decrease in AIDS incidence and mortality. With the concomitant development of virological assays allowing direct quantification of viral replication and therefore the measurement of viral control, antiretroviral strategy has entered a new era. The concept of highly active antiretroviral therapy (HAART) or combined antiretroviral therapy (cART) was born and remains the gold standard. By controlling viral replication, cART allows immune restoration, prevent occurrence of viral resistance and reduces viral transmission (Palella FJ Jr *et al*, 2006; Grinsztejn B *et al*, 2014).

1. Classes of antiretroviral therapy

There has been a constant development of antiretroviral drugs since 20 years. More than twenty antiretroviral drugs have been licensed belonging to six classes and acting through 5 different sites of HIV replication cycle. The first generation of them in the NRTI and the PI class has progressively disappeared and replaced by more potent, more robust or better tolerated drugs. In 2008, a new class of drug; integrase inhibitor (raltegravir) capable to prevent HIV to integrate cell genome was discovered. An important step to improve simplicity and treatment compliance has been the development of once daily regimen combined in one pill once a day.

a) Reverse transcriptase inhibitors

Three types of reverse transcriptase inhibitors exist, nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitor (NtRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). The mode of action of NRTIs and NtRTI is essentially the same. They are analogues of deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. Thus, when NRTI or NtRTI is incorporated, viral DNA synthesis is halted, a process known as chain termination (Cihlar T *et al*, 2010). NTRIs and NtRTI are classified as competitive substrate inhibitors. Different NTRIs and NtRTI approved for use are summarized in **table 2**.

All current guidelines worldwide recommend that ART should be initiated with a combined triple antiretroviral therapy, consisting of a backbone of 2 NRTI agents plus a third agent that could be either a NNRTI , a PI or an integrase inhibitor in some countries. Beside the effectiveness of NRTIs, they have the advantage of being co-formulated in single pills which permits a better adherence. Another advantage is the low potential for interactions with other non-ARV drugs and is not affected by the meals. This class of antiretroviral agents have moderate genetic barrier for resistance.

The combination of zidovudine/lamivudine, widely used in the past is no more recommended as a first line treatment due to toxicity such as digestive intolerance, anemia and mitochondrial toxicity (Brogan KL *et al*, 1990; Van der Valk M *et al*, 2004). However, this association is still recommended as an alternative choice in special cases such as pregnancy (Sturt AS *et al*, 2010). This combination has been replaced by two combinations depending on their efficacy, tolerance and easy use once they are marketed in single tablet: tenofovir/emtricitabine (Truvada®) and abacavir/lamivudine (Kivexa®) (Arribas JR *et al*, 2008; Smith KY *et al*, 2009). TDF/FTC has the advantage that both of the two molecules have anti-hepatitis B effect and recommended for the treatment of coinfected patients (Benhamou Y, 2006). TDF/FTC is today the most widely used NRTI backbone. Renal tubular and long term bone toxicity are the main side effects associated with TDF use (Munoz RM *et al*, 2006).

The main disadvantage of the use of ABC/3TC is the hyper-sensibility reaction to ABC among a group of patients and thus, the use of ABC among patients who are positive for HLA B*5701 should be avoided (Mallal S *et al*, 2008). In addition, this association should not

be initiated among patients with viral load exceeding10^5 copies/mL to avoid virological failure (Sax PE *et al*, 2009).

With regard to the impact of NRTIs on changes of inflammation markers, the impact of TDF/FTC and ABC/3TC has been evaluated in two randomized trials. A decline in IL-6, sVCAM-1, sICAM, sTNF-RI, sTNF-RII, and TNF-a levels was observed in the ACTG A5224s and the HEAT studies which evaluated the impact of cART, consisting of two NRTI combined to a third agent initiation in naïve patients. Hs-CRP levels decreased with both combinations in the HEAT study while remained stable under TDF/FTC and increased with ABC/3TC in the ACTG A5224s study (Smith KY *et al*, 2009; McComsey GA *et al*, 2012). In the MACS cohort, the use of abacavir-containing therapy was associated with a fall in IL-6 and D-dimer levels but not with hs-CRP (Palella FJ Jr *et al*, 2010).

Table 2: NRTIs and NtRTIs approved for use in the treatment of HIV infection

	International Nonproprietary Name	**Commercial name**
NRTIs / NtRTIs	Zidovudine	*Retrovir®*
	Didanosine	*Videx®*
	Stavudine	*Zerit®*
	Abacavir	*Ziagen®*
	Lamivudine	*Epivir®*
	Emtricitabine	*Emtriva®*
	Tenofovir	*Viread®*
2 NRTIs	Abacavir + Lamivudine	*Kivexa®*
	Tenofovir + Emtricitabine	*Truvada®*
	Zidovudine + Lamivudine	*Combivir®*
3 NRTIs	Abacavir + Lamivudine + Zidovudine	*Trizivir®*
2 NRTIs + 1 NNRTI	Tenofovir + Emtricitabine + Efavirenz	*Atripla®*
2 NRTIs +1 II+ booster	Tenofovir + Emtricitabine + Elvitegravir + cobicistat	*Quad®*

Non-nucleoside reverse-transcriptase inhibitors (NNRTIs) have a different mode of action. They block reverse transcriptase by binding on the enzyme itself. They are not incorporated in the viral DNA but instead inhibit the movement of protein domains of reverse transcriptase needed to carry out the process of DNA synthesis. NNRTIs are classified as non-competitive inhibitors of reverse transcriptase (Joly V *et al*, 2000). There are two generations of NNRTIs; the first consists of Efavirenz and Nevirapine while the second consists of Etravirine and Rilpivirine. Despite the virological response associated with their use, the low genetic barrier to resistance is a major disadvantage of this class with greater risk of resistance at the time of failure or treatment interruption (Delaugerre C *et al*, 2001). NNRTIs have a potential for drug-drug interaction since they are extensively metabolized in the liver through cytochrome P450, leading to pharmacokinetic interactions with compounds utilizing the same metabolic pathway, particularly protease inhibitors (Joly V *et al*, 2000). The availability of efavirenz in a fixed-dose combination with TDF/FTC makes this agent the first NNRTI choice for treatment-naive and treatment-experienced patients (Sheran M, 2005).

With regard to inflammation markers, the use of efavirenz combined to two NRTIs was associated with a fall in IL-6, sVCAM-1, sICAM, sTNF-RI, sTNF-RII, TNF-a while hs-CRP levels remained stable at 96 weeks of treatment in the ACTG A5224s study (McComsey GA *et al*, 2012). In a sub-study of MERIT, efavirenz use was associated with a fall in IL-6 and D-dimer while CRP levels increased (Funderburg N *et al*, 2010).

b) Protease inhibitors

Protease inhibitors (PIs) prevent viral replication by inhibiting HIV-1 protease which cleaves long chains of nascent HIV proteins into smaller individual proteins which assemble with HIV RNA genetic material creating a new virus particle. The PIs are potent antiretroviral agents with a high genetic barrier to resistance. However, the accumulative use of PIs is associated with a risk for cardiovascular diseases and metabolic complications. In addition, PIs have the potential for interactions with other non-ARV drugs. Among the first generation PIs (saquinavir, ritonavir, indinavir, lopinavir, fosamprenavir), lopinavir has been largely used but no more recommended by the international guidelines. Ritonavir, the first licensed PI is no more recommended as an antiretroviral agent but as a pharmaco-enhancer to boost another PIs as it inhibits particular liver enzymes that metabolize protease inhibitors (cytochrome P-450 CYP3A4) (Zeldin RK *et al*, 2004). Among the second generation PIs, atazanavir and

darunavir are now recommended as a first-line option for treatment-naïve and treatment-experienced patients while tipranavir is rarely used due to side effects.

In addition to the efficacy of atazanavir and darunavir, these agents showed favorable impact with regard to inflammation. In the ACTG A5224s study, the use of atazanavir was associated with a fall in IL-6, sVCAM-1, sICAM, sTNF-RI, sTNF-RII, TNF-a while hs-CRP levels remained stable at 96 weeks of treatment (McComsey GA *et al*, 2012). In another study, the use of darunavir combined to raltegravir was associated with a fall in IP-10, IL-6, D-dimer and sCD14 levels (Taiwo B *et al*, 2013).

c) Integrase inhibitors

This class of drugs has been designed to block the action of integrase, a viral enzyme that inserts the viral genome in the DNA of the host cell (Croxtall JD *et al*, 2009). Rraltegravir (RAL) was the first integrase inhibitor approved by the FDA. Clinical trials indicate that RAL is safe and highly effective in the treatment of both antiretroviral-naïve and –experienced patients (Chirch LM *et al*, 2009). In the STARTMRK, the non-inferiority of RAL was shown in comparison with efavirenz (Lennox JL *et al*, 2009). Furthermore, RAL showed better tolerability and more rapid decay of the viral load (Markowitz M *et al*, 2007). The last EACS and US DHSS guidelines introduced RAL as a first line third agent to be combined to NRTI backbone. French guidelines did not recommend it yet because of the high cost and the necessity for twice daily administration (Morlat P *et al*, 2014).

Beside the favorable impact of RAL on inflammation markers in ART-naïve patients, mentioned above, a switch from protease-based tri-therapy to a raltegravir-containing tri-therapy in patients with suppressed viremia led to a decrease in biomarkers associated with inflammation, insulin resistance and hyper-coagulability in the SPIRAL study (Martínez E *et al*, 2012).

Elvitegravir, approved in 2013, is an integrase inhibitor which is licensed in combination with a parmaco-enhancer, cobicistat, and the dual NRTI combination tenofovir/emtricitabine in a single tablet regimen once daily. Elvitegravir share RAL a good safety profile but has a low genetic barrier to resistance (Sax PE *et al*, 2012).

Dolutegravir, a second-generation integrase inhibitor, has the advantages of long half-life that does not need to be pharmacologically enhanced and is effective as a once daily drug

(Katlama C *et al*, 2012). Dolutegravir has shown non-inferior efficacy to raltegravir in the SPRING-2 study (Raffi F *et al*, 2013) and superior efficacy to efavirenz (Walmsley SL *et al*, 2013) in the SINGLE study in terms of virological control and tolerability. Dolutegravir has been commercialized in 2014 and the last US DHSS guidelines recommended its use as a first line third agent.

d) Fusion inhibitor

Enfuvirtide (T20) approved initially in 2003 (Lalezari JP *et al*, 2003) is today the only fusion inhibitor available. It is an injectable peptide that requires two daily subcutaneous injections. T20 mimics HIV fusion machinery preventing gp41 creating an entry pole for the capsid of the virus keeping it out of the cell (Hardy H *et al*, 2004). Given its parenteral administration, inadequate for long term therapy, the use of T20 has massively decreased over time. It is currently exceptionally used in antiretroviral strategy since other oral drugs such as second generation PIs or integrase inhibitors allow now to control viral replication in case of treatment failure with viral resistance.

e) Entry inhibitor

Maraviroc (MVC) is a selective antagonist of the chemokine receptor (CCR5), thereby blocking the HIV protein gp120 from associating with the receptor. The efficacy of MVC was established in the MERIT study in comparison with efavirenz, both combined with zidovudine/lamivudine, in treatment-naïve patients with CCR5-tropic HIV-1. In terms of virological control at 96 weks, MVC showed similar effect as efavirenz, and MVC recipients had greater CD4 increases and fewer adverse event discontinuations (Sierra-Madero J *et al*, 2010). MVC is currently the only CCR5 co-receptor inhibitor approved for clinical use in naïve patients or patients experienced therapeutic failure following traditional antiretroviral therapies (Perry CM *et al*, 2010; Cooper DA *et al*, 2010). HIV tropism for CCR5 should be tested before MVC is administered to ensure the efficacy of this drug because HIV can use other co-receptors such as CXCR4. The use of MVC is limited by the need to access viral tropism test before use, a twice daily administration and a high cost.

MVC targets the cellular chemokine CCR5 receptor, which is involved in important inflammatory pathways, the trafficking of immune cells and transitioning an innate immune response into an acquired response (Khan IA *et al*, 2006). This suggested that MVC could have an anti-inflammatory effect reducing immune activation and permits to have an

immunological benefit. In a study of chronically HIV-infected patients receiving stable antiretroviral therapy whose regimen was intensified with 48 weeks of MVC, no changes were detected in CD4+ or CD8+ counts, although a significant decrease was found in the proportion of activated CD4+ and CD8+ T-cells. LPS and sCD14 levels increased (Gutiérrez C *et al*, 2011). Contrastingly, in a trial of 45 HIV-infected subjects with suppressed viremia randomized to MVC intensification or placebo, MVC-treated subjects experienced a greater increase activated CD8+ T cells at week 24, and lesser decline in activated CD4+ T cells compared with placebo-treated subjects (Hunt PW *et al*, 2013).

2. Recommendations for first-line cART initiation

a) When to start?

The question of "when to start" antiretroviral therapy has been debated for many years and still differs in the different guidelines even across countries and particularly since the advent of combined ART. The dramatic effects of combination antiretroviral therapy which was introduced in 1996 on mortality and morbidity, coupled with emerging insights regarding viral dynamics and HIV evolution, supported the interest of early initiation of therapy. The "hit HIV, early and hard" paradigm was adopted in the US Department of Health and Human Services (DHHS) guidelines between 1998 and 2000, which recommended that most patients be offered therapy, including those with asymptomatic disease and a CD4+ cell count above 500 cells/mm^3 (US DHHS guidelines, 2000).

However, after the early enthusiasm of late nineties, the recognition of metabolic toxicity, renal disorders, the fear about the development of resistance, the potential difficulties of implementing ART in all patients from all countries who need it, a switch happened and guidelines went back in their recommendation questioning whether potential benefits of therapy outweighed the potential risks at higher CD4+ cell counts. This led to the sense that medications should be delayed. Several cohort studies during this era indicated that a higher pre-treatment CD4+ cell count was a strong predictor of good outcomes during therapy, with consistent and clear benefits occurring if therapy was initiated before the CD4 declined to below 200 cells/ mm^3, but additional benefit was also apparent when therapy was initiated at a CD4 of 200 to 350 cells/ mm^3 (Hogg RS *et al*, 2001; Egger M *et al*, 2002; Palella FJ *et al*, 2003). The 2001 version of the DHHS guidelines was modified accordingly such that therapy was strongly recommended for those with a CD4 <200 cells/ mm^3, generally recommended

for those with a CD4 count of 200 to 350 cells/ mm^3 and not recommended in patients with higher CD4 cell counts (US DHHS guidelines, 2001).

In the recent years, the availability of well tolerated, less toxic and co-formulated antiretroviral drugs made the rationale for delaying antiretroviral therapy less evident. The SMART study (El-Sadr WM *et al*, 2006) has been a key study that has deeply modified the concepts about HIV clinical pathogenesis. This study randomized HIV-infected patients who had a CD4+ cell count of more than 350/mm^3 to the continuous use or episodic use of antiretroviral therapy. Episodic use involved the deferral of therapy until the CD4+ count decreased to less than 250/mm^3 and then the use of therapy until the CD4+ count increase to more than 350/mm^3. Opportunistic diseases or death from any cause occurred more in the interruption use group compared to the continuous use group. The risk of all-cause mortality was associated with high levels of inflammation and coagulation biomarkers suggesting that interrupting therapy may increase the risk of death (Kuller LH *et al*, 2008). Recent recommendations delivered in 2012 recommend treatment for all HIV-infected individuals with different strength of recommendations (US DHHS guidelines, 2012) and the concept "Treatment as prevention' was adopted in the last guidelines delivered in 2013 which recommend that antiretroviral therapy should be started if a patient is requesting treatment and ready to start, and in sero-different partners to decrease HIV transmission. **Table 3** shows different recommendations on when to start antiretroviral therapy according to different guidelines.

Table 3: Different guidelines on when to start antiretroviral therapy?

Guideline	AIDS or HIV-related symptoms	CD4+ Cell Count		
		< 350	350-500	> 500
US DHSS 2014	Yes	Yes	Yes	Yes
FRANCE 2013	Yes	Yes	Yes	Yes
EACS 2013	Yes	Yes	Yes	Questionable
WHO 2013	Yes	Yes	Yes	NA

b) What to start?

Most treatment guidelines recommend the initiation of cART, based mainly on a backbone of two nucleoside reverse transcriptase inhibitors (NRTI) combined with non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI). The last US guidelines delivered in May 2014 added dolutegravir while the European guidelines added raltegravir to their guidelines. The choice of first line cART should be individualized taking in consideration other variables such as possible interactions with other concomitant medications, the resistance genotyping, the expected tolerance, the price and the availability of therapy.

Two backbones of NRTIs are nowadays widely used depending on their efficacy, tolerance and easy use once they are marketed in single tablet: TDF/FTC and ABC/3TC. The combination of AZT/3TC is no more recommended as a first line treatment due to side effects except in the WHO guidelines. **Table 4** shows different antiretroviral combinations recommended by different guidelines.

Table 4: Different guidelines on what to start in first line antiretroviral therapy?

Guideline	NRTI	NNRTI	PI	INI
US DHSS 2014	TDF/FTC ABC/3TC	EFV	ATV/r DRV/r	RAL DTG
FRANCE 2013	TDF/FTC ABC/3TC	EFV RPV	ATV/r DRV/r	No INI
EACS 2013	TDF/FTC ABC/3TC	EFV RPV	ATV/r DRV/r	RAL
WHO 2013	TDF/ 3TC (or FTC) ABC/3TC AZT/3TC	EFV NVP	No PI	No INI

3. Benefits of antiretroviral therapy (ART)

a) HIV replication control and immune system restoration

Nowadays, we are in the era where most of HIV-infected patients who have access to ART have achieved controlled plasmatic viral loads. Data from the FHDH shows that 89% of patients under cART in the year 2012 achieved controlled viremia (<50 copies/mL) compared to 79% in the year 2007. The control of HIV viremia has a substantial role in the management of HIV infection and its consequences.

The control of viremia has been translated in a series of immunological and clinical benefits. The restoration of CD4+ cell count occurs following the initiation of cART and a rise of 50 to 100 cells/mm3 is usually observed in the first year of therapy. In a long term observational study that prospectively followed patients initiating cART, the majority of increase in CD4 cell counts was in the first two years with little increase afterwards (Lok JJ *et al*, 2010). In some patients, the viral suppression under ART is not associated with CD4 count restoration, a phenomenon that is predictive of worse outcomes. Controlled viral load and restored immunity on combination antiretroviral therapy (cART) resulted in significant differences in the incidence of AIDS-defining illnesses (esophageal candidiasis, Kaposi's sarcoma, pulmonary and extrapulmonary tuberculosis, non-Hodgkins lymphoma, bacterial pneumonia, *Pneumocystis jirovecii* pneumonia and recurrent herpes simplex) before and after cART as well as over the different periods of cART (Mocroft A *et al*, 2013; Hleyhel M *et al*, 2013).

In addition to the immunological and clinical benefits, the control of viremia (<50copies/mL) has been shown to prevent the selection of resistance mutations by the HIV. In patients with persistent low-level viremia under cART, new resistance mutations were detected and the magnitude of low-level viremia was the primary driver of evolution rate at emergent drug resistance mutation (Taiwo B *et al*, 2011; Vardhanabhuti S *et al*, 2014).

b) Decreased mortality and morbidity with increased survival probabilities

Since the introduction of cART, dramatic decrease in the overall mortality has been observed (Mocroft A *et al*, 2003; Palella FJ Jr, 2003). A recent study compared mortality rates in the SMART and ESPRIT trials with the mortality rates in the general population. Patients who were not users of injection drugs, aged 20-70 years, from the continuous ART control arms of ESPRIT and SMART, were included if the HIV plasma viral load was ≤400

copies/ml in the SMART study and ≤500 copies/ml in the ESPRIT study. Mortality rate was increased compared with the general population with a CD4+ cell count between 350 and 499 cells/mm^3 [SMR 1.77, 95% CI 1.17-2.55] while no evidence for increased mortality was seen with CD4+ cell counts greater than 500 cells/mm^3 (SMR 1.00, 95% CI 0.69-1.40) (Rodger AJ *et al*, 2013).

Along with the decrease in mortality, survival probabilities among HIV-infected patients initiating effective cART increased. In the UK Collaborative HIV Cohort Study where patients aged more than 20 years who started ART during 2000-2010 (excluding injection drugs users), were followed for mortality until 2012, the expected age at death of 35-year-old men with CD4 cell count of at least 350cells/mm^3 was 77 (72-81) years, compared with 78 years for men in the general UK population (May MT *et al*, 2014). These results suggest that newly diagnosed, successfully treated individuals can have a normal life expectancy.

c) Decreased heterosexual transmission of HIV

Beside the individual benefits, cART has led to a decrease of heterosexual transmission of HIV to non-infected partners through the control of viral replication. Data from prospective cohort analysis of serodiscordant couples showed a 92% reduction of HIV transmission between patients started treatment and those not receiving it. Transmission occurred more among couples in whom non-treated HIV-infected partner with low CD4 cell counts and higher plasma HIV-1 concentrations (Donnell D *et al*, 2010). In the HPTN 052 Study, 1763 sero-discordant couples in which HIV-1–infected participants had a CD4 count of 350 to 550 cells/mm^3 were randomly assigned to receive antiretroviral therapy either immediately or after a decline in the CD4 count or the onset of HIV-1-related symptoms. Of the 28 linked transmissions, only 1 occurred in the early-therapy group suggesting a relative reduction of 96% in the number of linked HIV-1 transmissions resulting from the early initiation of antiretroviral therapy, as compared with delayed therapy (Cohen MS *et al*, 2011). A meta-analysis of studies evaluated sexual HIV transmission rates between heterosexual serodiscordant couples, suggest a minimal risk of transmission when the HIV-positive partner is tolerant to cART, has full virologic suppression and do not have sexually transmitted infections (Loutfy MR *et al*, 2013). The PARTNER study has recruited 1110 couples where the partners have differing HIV status, and nearly 40% of them are gay couples. Couples have to be having sex without condoms at least some of the time. The HIV negative partner cannot be using post-exposure or pre-exposure prophylaxis and the HIV positive partner has to be on

ART, with the most recent viral load below 200 copies/mL. In this study, there have been no transmissions within couples from a partner with an undetectable viral load (Rodger A *et al*, CROI 2014, Abs. 153LB).

4. Complications of cART

The use of cART has been associated with increasing life expectancy in HIV-infected patients. While the median age of HIV-infected patients is increasing, concerns arise concerning the long term toxicities and morbidities associated with the use of cART. Many patients under antiretroviral therapy displayed lipodystrophy, metabolic alterations, increased risk of cardiovascular diseases and reduced bone mineral density. Now that ART is recommended for lifelong, the challenge is to maximize antiviral efficacy and to minimize toxicity (Guaraldi G *et al*, 2014).

a) Lipodystrophy

Lipodystrophy is defined as abnormal fatty tissue distribution. It could present as lipoatrophy (loss of fat tissue in the face and the extremities), lipohypertrophy (accumulation of fat tissue in the abdomen and posterior neck) or a mixture of lipoatrophy and lipohypertrophy. Several overlapping risk factors attribute for the development of lipodystrophy (Martinez E *et al*, 2001). There is an evidence that HIV-1 infection on its own contributes to the development of the lipodystrophic phenotype by interfering with some key genes of adipocyte differentiation and mitochondrial function in patients which have not received antiretroviral treatment (Giralt M *et al*, 2006). Beside the role of HIV, the use of thymidine analogues NRTIs (zidovudine and stavudine) are mainly responsible for peripheral lipoatrophy. NRTIs induce mitochondrial dysfunction and modify adipocyte phenotype and adipose tissue pattern of secretion of cytokines and adipokines through the production of reactive oxygen species (Capeau J *et al*, 2006). This deleterious effect was one of the causes behind the replacement of thymidine analogues with other nontoxic NRTIs such as abacavir or tenofovir which leaded to improvement in lipoatrophy. The use of PIs is associated with the development of fat hypertrophy (Caron-Debarle M *et al*, 2010). PIs increase cytokine and decrease adiponectin secretion and expression (Lagathu C *et al*, 2004). In a recent study, Guaraldi G *et al* have shown that CD8+ T-cell activation was associated with lipodystrophy and the relative amount of visceral abdominal adipose tissue in antiretroviral therapy-

controlled, virologically suppressed, HIV-infected patients suggesting that CD8+ activation may be involved in the accumulation of central fat (Guaraldi G *et al*, 2013).

b) Metabolic alterations

Beside the morphological changes (lipodystrophy) associated with PIs use, the APROCO study, which has investigated the prevalence of lipid and glucose alteration found that 23% of HIV-infected patients have glucose metabolism alterations, 28% have hypertriglyceridemia and 57% have hypercholesterolemia. Age was significantly associated with different phenotypes of lipodystrophy and metabolic alterations while among antiretrovirals, only ritonavir was associated with hypertriglyceridemia (Saves M *et al*, 2002). These abnormalities were associated with increased plasma levels of apolipoproteins (Bard JM *et al*, 2006). Compared to HIV-uninfected population, the incidence of type-2 diabetes mellitus in HIV-infected men with cART exposure was greater than 4 times in the multicenter AIDS cohort study (Brown TT *et al*, 2005). Several cohorts reported an increased incidence of diabetes mellitus among patients on cART and ranged from 4.4 person-years follow-up (PYFU) in the Swiss HIV cohort study to 14.1 PYFU in the APROCO-COPILOTE Cohort Study (Ledergerber B *et al*, 2007; De wit S *et al*, 2008; Capeau J *et al*, 2012). In addition to traditional risk factors, the use of NRTIs (stavudine, zidovudine, lamivudine and didanosine) and the protease inhibitor (indinavir) containing regimens was associated with the risk of developing diabetes mellitus.

c) Myocardial infarction

The risk of myocardial infarction (MI) among HIV-infected patients is higher than general population (Lang S *et al*, 2010). Endothelial dysfunction is possibly the most plausible link between HIV infection and atherosclerosis (Mu H *et al*, 2007). The mechanisms underlying the regulation of endothelial function in HIV-infected persons appear to be multifactorial, including direct effects of HIV on the endothelium, indirect effects of HIV on lipids and inflammatory cytokines and ART-related effects (Mondy KE *et al*, 2008). After the reported association between the use of ART and the risk of MI in the DAD study (Worm SW *et al*, 2010), several studies have reported an association between abacavir use and risk of MI. However, in a case-control study (matched for age, sex, and clinical center) nested within the French Hospital Database on HIV found that the risk of MI was increased by cumulative exposure to all the studied PIs except saquinavir, whereas the association with abacavir cannot be considered causal (Lang S *et al*, 2010).

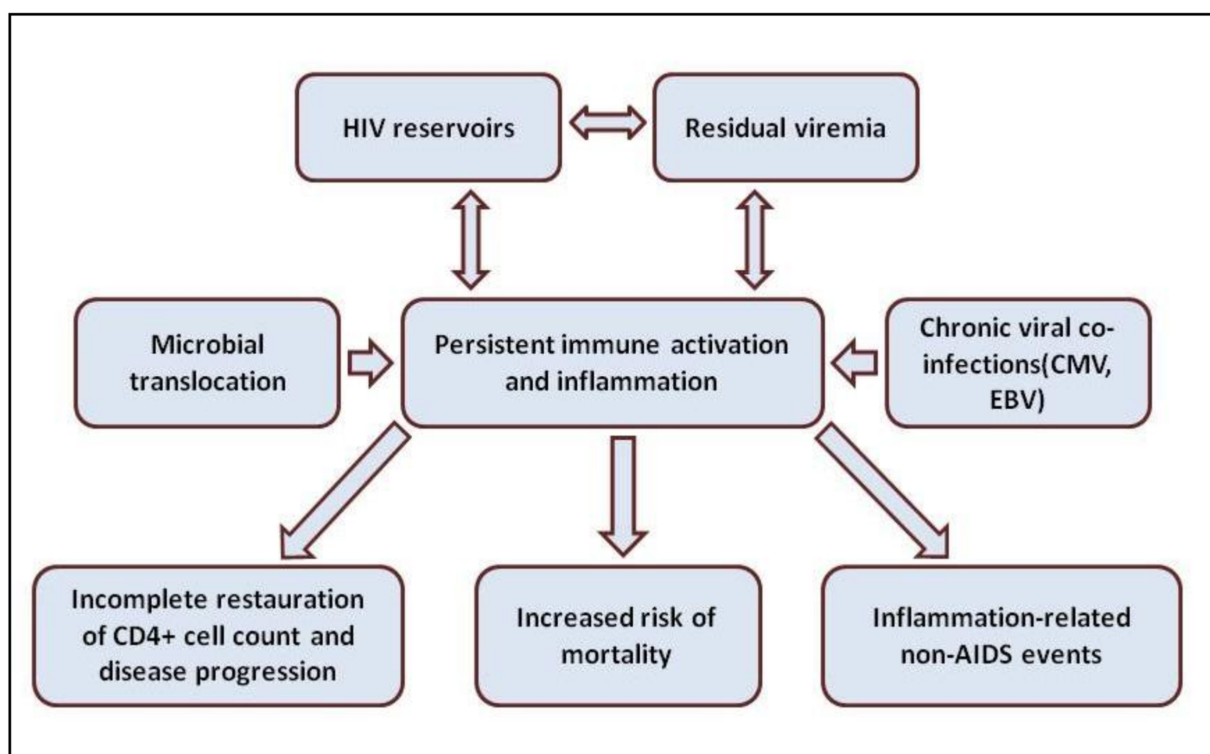
d) Reduced bone mineral density and increased risk of fractures

Reduced bone mineral density (osteoporosis and osteopenia) is another complication of both HIV and current antiretroviral drugs. In a meta-analysis of 20 studies, the prevalence of osteoporosis was more than three times greater compared to HIV-uninfected controls. In addition, ART-exposed and PI-exposed patients had a higher prevalence of osteoporosis (Brown TT *et al*, 2006). The change in bone mineral density (BMD) was compared in a randomized trial that assigned the patients into three treatment arms: an NNRTI and a PI/r, a PI/r and NRTIs or an NNRTI and NRTIs using DEXA scan. After 1 year, the decrease in lumbar spine BMD was more in patients receiving PI/r-containing regimen compared with NNRTI and NRTIs suggesting that BMD should be monitored during lifelong antiretroviral therapy (Duvivier C *et al*, 2009). Another study evaluated osteopenia in HIV-1-infected men receiving cART for a median of 7.5 years, showed that tenofovir exposure was independently associated with a larger decline in BMD at lumbar and hip spine (Assoumou L *et al*, 2013).

Beside the evaluation of BMD, some studies evaluated the risk of fractures. In Danish population-based cohort study evaluating the development of low and high energy fractures in HIV-infected patients, an increased risk of fracture compared with population controls was found. Among HIV-monoinfected patients, the increased risk was observed for low-energy but not for high-energy fractures, and the increased risk of low-energy fracture was only observed in HAART-exposed patients (Hansen AB *et al*, 2012). Further study found that the cumulative exposure to TDF and lopinavir/ritonavir was independently predictive of increased risk of osteoporotic fracture in the HAART era (Bedimo R *et al*, 2012).

C. Beyond cART: failure of eradication, residual viremia and persistent immune activation and inflammation[1]

The expanded use of cART has changed the course of HIV infection. Nowadays, about 90% of patients on ART have plasma HIV-RNA levels below the levels of detection for long periods of time. Despite this success, failure of HIV eradication due to established reservoirs, residual viremia and persistent immune activation and inflammation remain a challenge in HIV research (Katlama C, 2013). This chapter demonstrates these unresolved issues with more focus on the causes and the consequences as well as the therapeutic interventions to decrease persistent immune activation and inflammation. The following diagram summarizes these issues.



[1] Main source :

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1. HIV reservoirs and failure of eradication

In the mid-nineties, the persistence of small detectable pool of latently infected, CD4+ T-cells carrying replication-competent virus was documented in several independent studies among patients who received clinically effective ART (Wong JK *et al*, 1997; Finzi D *et al*, 1997; Chun TW *et al*, 1997). In addition, several studies demonstrated rapid viral rebound after discontinuation of therapy in patients in whom sustained suppression of plasma viremia had been achieved for prolonged periods (Harrigan PR *et al*, 1999; Davey RT *et al*, 1999; Garcia F *et al*, 1999). These latently infected, CD4+ T-cells serve as HIV reservoir and present an impediment to viral eradication.

The establishment of HIV reservoir is initiated early in the course of infection at time of virus penetration. This conclusion is based on the observation that the initiation of cART in infected patients as early as 10 days after the onset of symptoms of primary HIV-1 infection did not prevent generation of latently infected, resting CD4+ T cells carrying integrated HIV-1 DNA as well as infectious HIV-1 despite the successful control of plasma viremia shortly after institution of cART (Chun TW *et al*, 1998).

To study the establishment of HIV reservoirs, researchers in Thailand enrolled 68 patients presented with acute HIV infection, diagnosed within 3 days and enumeration of total and integrated HIV DNA in PBMCs was performed 2 days before the initiation of raltegravir-containing antiretroviral therapy. There were 24 patients characterized as Fiebig 1 (positive HIV RNA, negative p24 antigen and HIV antibodies on 3rd generation ELISA), seven patients were characterized as Fiebig 2 (positive HIV RNA and p24 antigen, negative for HIV antibodies on ELISA) while 36 patients were characterized as Fiebig 3 (positive HIV RNA, p24 antigen and HIV antibodies on ELISA, negative on a Western blot). At the time of diagnosis, 92% of the Fiebig 1 group had undetectable integrated HIV DNA in their PBMCs, compared to 29% of Fiebig 2 and 53% of Fiebig 3. Patients in Feibig 2 and Feibig 3 stages showed substantial reductions in HIV DNA within 12 weeks of starting ART, and reached undetectable levels of integrated HIV DNA in PBMCs by week 24. Seven out of ten patients who underwent sigmoid biopsy, and who had detectable integrated HIV DNA at baseline, had undetectable HIV DNA by week 24 of treatment (Ananworanich J *et al*, CROI 2013, Abs. 47).

Considerable progress has been achieved regarding our knowledge in the pathogenesis of HIV reservoirs and the mechanisms of viral latency even though a lot remains to be understood. When the viral DNA is integrated, infected cells with replication-competent pro-virus are transcriptionally silenced by corepressor complexes that contain histone deacetylases (HDAC), histone methyltransferases, and heterochromatin proteins; active methylation of the long terminal repeat might also play a part. Additional restrictions limiting cellular levels of the essential Tat cofactor P-TEFb and nuclear factor kappa B ensure that the provirus remains silenced unless the host cell is activated (Colin L *et al*, 2009; Karn J *et al*, 2011). Two hypotheses are suggested to explain the persistence of HIV reservoir despite the effective cART use for long time. First, the memory phenotype with the long half-life of the latently infected resting CD4+ T cells which enables these cells keep the virus without expressing viral antigens on their surface, thus enabling them to escape host immune responses. Second, ongoing low-level of HIV replication with de novo viral infection, due to partial suppression and/or to inadequate drug penetration (Chun TW *et al*, 2012; Passaes CP *et al*, 2014).

The most widely discussed approach to eradicate HIV reservoirs is reactivating HIV genomes in resting CD4+ T cells. To date, HDAC inhibitors have been tested as potential latency-reversing agents. Of these, valproic acid has been tested in different studies with no measurable effect (Siliciano JD *et al*, 2007; Sagot-Lerollr N *et al*, 2008; Archin MM *et al*, 2008). Other HDAC inhibitors (vorinostat, romidepsin, panobinostat, givinostat and belinostat) have been tested ex vivo and showed promising results in the reactivation of virus in latently infected cells. Disulfiram, which has been used in the treatment of alcoholism, is being evaluated (Hochreiter J *et al*, 2012). The use of cytokines has been envisioned as an additional therapeutic strategy. Interleukin-2 use was not successful (Kulkosky J *et al*, 2002). Nowadays, Interleukin-7 is being tested in combination with ART intensification with raltegravir and maraviroc to prevent proliferation or reseeding of the latent reservoir.

Clinical trials of latency-reversing agents have raised the issue of how eradication efforts will be monitored. To date, the gold standard assay is a limited dilution virus culture assay (Siliciano JD *et al*, 2005). However, it is time-consuming and so, viral DNA detection using PCR is being used to monitor these efforts. Studies have shown a big difference in the level of DNA detected by PCR compared to cultures. This was due to the detection of the DNA of defective non-replicative viruses (Kieffer TL *et al*, 2005). Thus, improved assays to follow eradication efforts are needed.

2. Residual viremia

Using sensitive single-copy assays for HIV-1 RNA, residual amounts of free virus are still detected in the plasma of patients on suppressive cART (Dornadula G *et al*, 1999) and the source of residual viremia is controversial. Ongoing viral replication as a source of residual viremia is debated. Several lines of evidence support the hypothesis that the source of such viremia is the release of HIV-1 from cells infected before the initiation of HAART rather than ongoing replication. First, clinical experience has shown that adherent patients who maintain plasma viral loads less than 50 copies/ml do not develop resistance to therapy. Secondly, intensification studies that added potent antiretroviral drug to suppressive cART did not find any further decrease in the amount of residual viremia. Third, phylogenetic studies of residual plasma viremia show no sequence evolution in patients on effective HAART (Hermankova M *et al*, 2001; Dinoso JB *et al*, 2009; Gandhi RT *et al*, 2010).

In contrast, some experts believe that the decrease in CD8+ T-cell activation and the transient increase of episomal HIV-1 DNA observed after the intensification of HAART with raltegravir, provide evidence that active HIV-1 replication continues despite HAART (Buzon MJ *et al*, 2010; Llibre JM *et al*, 2012).

3. Persistent immune activation and inflammation among HIV-infected patients

Immune activation reflects usually the mounting of antiviral immunity and may be seen as normal and positive observation in case of infection with any pathogen including HIV. Immune activation usually subsides after the acute phase which results in the clearance of the pathogen or the transition of the pathogen in latent state. In the case of HIV infection, immune system is highly activated in concert with peak viremia but in chronic phase, where the level of viremia decrease considerably, immune activation does not subside which is unique for HIV infection.

HIV-associated chronic immune activation engages a range of molecular and cellular processes including both innate and adaptive immunity. This includes higher frequencies of T and B cells with activated phenotype, increased lymphocyte turnover with abnormalities in cell cycle regulation. On the innate side, monocytes, macrophages and dendritic cells are activated. Beside, certain circulating cytokines and chemokines are increased.

a) Causes of persistent Immune activation and inflammation

The specific mechanisms underlying the persistence of chronic immune activation in chronic HIV infection are still unclear. It seems that HIV induces persistent immune activation by multiple and complex mechanisms with many of these acting synergistically.

(1)HIV reservoirs and residual viremia

In a pilot study including 32 cART-treated patients with undetectable HIV-1 RNA followed prospectively over 24 months; residual viremia was associated with increased blood levels of soluble immune activation markers (Ostrowski SR *et al*, 2008). In addition, intensification of HAART with raltegravir was associated with a significant decrease in CD8+ T-cell activation and transient increase of episomal HIV-1 DNA suggesting that residual viremia results from low-level viral replication and drive immune activation (Llibre JM *et al*, 2012; Massanella M *et al*, 2012). Another study has characterized the residual viruses that are still produced in HIV-infected patients under effective cART for a median duration of seven years, found evidence that the residual viremia could be due to the release of archival virus from reservoir cells and/or ongoing virus replication in some patients. This residual viremia correlated to persistent immune activation suggesting that low-level virus production might contribute to persistent immune dysfunction in patients under cART (Mavigner M *et al*, 2009).

The relationship between residual viremia, HIV reservoirs and immune activation and inflammation seems to be bidirectional. Pro-inflammatory cytokines and immune activation might result in increased frequency of activated CD4+ T cells, which could contribute to persistent low-level viremia and subsequent replenishment of reservoirs (Decrion AZ *et al*, 2005; Jones LE *et al*, 2007; Klatt NR *et al*, 2013).

(2)Microbial translocation

Several studies have indicated that microbial translocation is a driver of chronic immune activation among HIV-infected patients (Lackner AA *et al*, 2009). During the early phase of HIV infection, profound depletion of CD4+ T cells in the intestinal mucosa occurs. This depletion involves preferentially CD4+ T helper cells (Th17) that produce interleukin-17 which is thought to be crucial for the maintenance of mucosal immunity. The depletion of these cells leads to a breakdown of physical and biological mucosal barrier and increased permeability of the mucosa (Brenchley JM *et al*, 2008; ElHed A *et al*, 2010). This results in

the translocation of bioactive microbial products such as lipopolysaccarides (LPS); a major component of the outer membrane of gram-negative bacteria and flagellin from the intestinal lumen to the systemic circulation. These products may cause a broad activation of the immune system by stimulating various immune cell types.

The presence of causal link between microbial translocation and chronic immune activation is supported by the presence of higher LPS levels among HIV- infected patients and the positive correlation between its levels and markers of immune activation. The use of suppressive cART decreases microbial translocation (LPS) levels partially and does not restore completely the intestinal mucosa function (Brenchley JM *et al*, 2006; Douek D *et al*, 2007). **Figure 8.b** demonstrates this phenomenon.

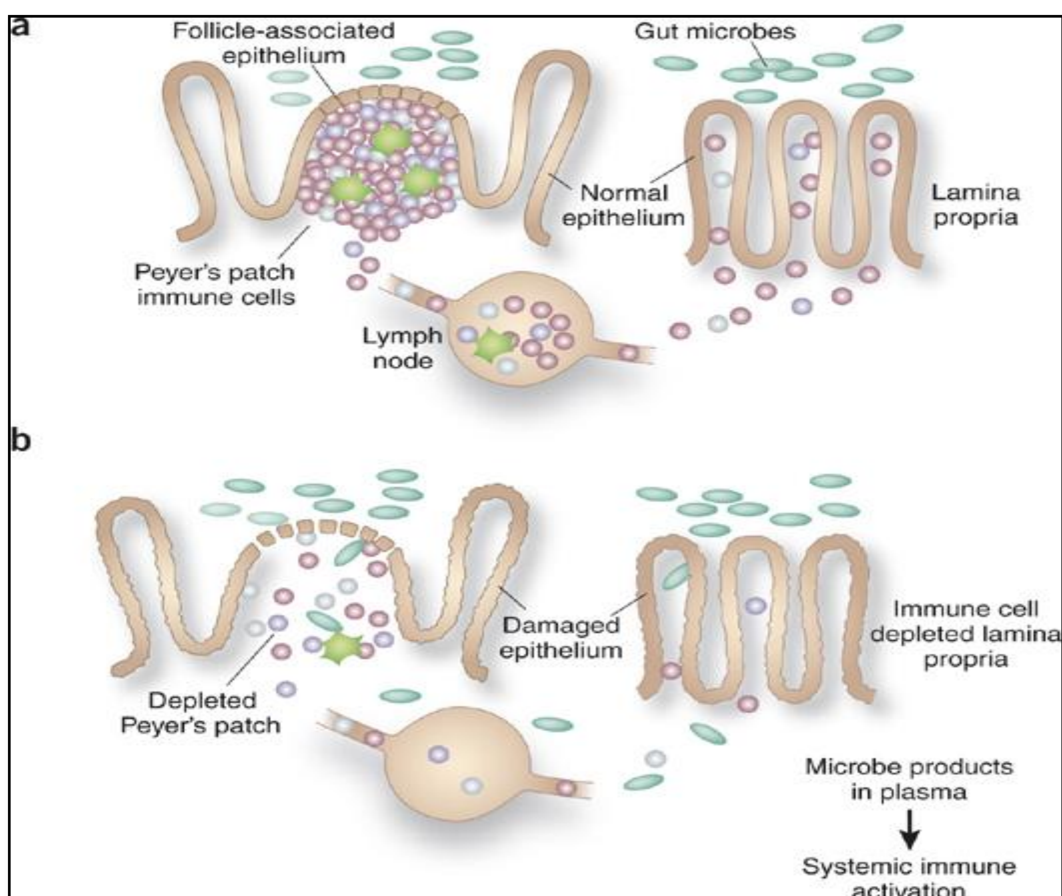

Figure 8: Microbial products translocation via the gut mucosa (Adapted from brenchley JM *et al*, 2006).

(3)Reactivation of other latent viruses

HIV also induces immune activation through indirect pathway. During HIV-1 infection, the depletion of CD4+ T cells may result in suboptimal immune control of other persistent viruses such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV). The suboptimal control permits their reactivation and replication. Recent studies have shown significant activation of EBV- and CMV-specific CD8+ T cells in HIV-1 infected patients receiving cART (Petrara MR *et al*, 2012; Wittkop L *et al*, 2013). Hence, sustained antigen-mediated immune activation occurs in HIV-1 infected patients due to HIV-1, but also to other viruses (EBV and CMV).

b) Consequences of immune activation and inflammation

(1)Immune system damage and insufficient recovery of CD4+ cell count

It is well established that chronic immune activation plays a role in the pathogenesis of HIV infection. Despite suppressive cART, some HIV-infected patients fail to normalize their CD4+ T-cell count. Ledermann *et al* have found that higher levels of cellular (CD38+ and HLA-DR expression) and soluble markers (sCD14) of immune activation markers in patients who did not normalize CD4+ cell count compared to patients who normalized CD4+ T-cell count independently of nadir CD4+ T-cell count, age at ART initiation and other clinical indices (Lederman MM *et al*, 2011). In addition, among HIV-infected patients who maintain suppressed viremia in the absence of cART (elite controllers), Hunt et al found that higher CD4+ and CD8+ T-cell activation (CD38+ and HLA-DR+) was associated with lower CD4+ T-cell count. These studies demonstrate the role of persistent immune activation in the damage of immune system in the era of cART (Hunt PW *et al*, 2008).

The mechanism by which immune activation causes the loss of the CD4+ T cells seems to be complex. One mechanism derives from the ability of HIV to preferentially infect and kill activated CD4+ T helper cells which are a key component of the host immune system. The presence of activated CD4+ T cells and a virus infecting activated CD4+ T cells may result in a viscous cycle of new infection, virus production, and further death of CD4+ T cells (Appay V *et al*, 2008).

A second mechanism by which immune activation impacts immune system is the dysregulation of the architecture of tissues that are crucial for T cell regeneration and function such as bone marrow, thymus and lymph nodes. This damage translates into ineffective T cell regeneration despite ART (Estes JD *et al*, 2009). A third mechanism is the differentiation of CD4+ and CD8+ T cells toward a state of replicative senescence driven by excessive levels of immune activation (Appay V *et al*, 2007).

(2)Increased mortality compared to general population

HIV-infected patients still have higher rates of mortality compared to non-infected population (Antiretroviral Therapy Cohort Collaboration, 2008) and the proportion of deaths attributable for non-AIDS related conditions has increased and included hepatic, cardiovascular as well as non-AIDS malignancies (Mocroft A *et al*, 2002; Krentz HB *et al*, 2005; Palella FJ *et al*, 2006). The SMART study was the first to demonstrate an association between elevated levels of inflammation markers (IL-6, D-dimer and sCD14) and all cause mortality (Kuller LH *et al*, 2008; Sandler NG *et al*, 2011). Following this observation, several markers of inflammation, immune activation and coagulation have been extensively studied in association with mortality in HIV-infected patients receiving cART (**Table 5**). Of these, IL-6 and CRP were widely studied. In a recent study, Hunt PW *et al* assessed immunologic predictors of mortality in a case-control study. Cases matched by duration of ART-mediated viral suppression, nadir CD4+ T cell count, age, gender, and prior cytomegalovirus retinitis; plasma gut epithelial barrier integrity markers (intestinal fatty acid binding protein and zonulin-1), sCD14, kynurenine/tryptophan ratio, sTNF-R1, hsCRP, and D-dimer levels all strongly predicted mortality, even after adjustment for CD4+ cell count. Higher proportions of (CD38+/HLA-DR+) CD8+ T cells, frequencies of senescent (%CD28-CD57+), exhausted (PD1+), naïve, and CMV-specific T cells did not predict mortality (Hunt PW *et al*, 2014). These results suggest that gut epithelial barrier dysfunction, innate immune activation, inflammation, and coagulation (but not T cell activation, senescence, and exhaustion) independently predict mortality in treated HIV-infected individuals.

(3)Inflammation-related non-AIDS events

Despite massive reduction of clinical HIV-related events, combination antiretroviral therapy does not restore full health and HIV-infected adults have excess risk of cardiovascular, liver, kidney, bone, and neurologic diseases. Many markers of inflammation

are elevated in HIV disease and strongly predictive of the risk of morbidity (Deeks SG *et al*, 2013).

Several studies have shown an association between plasma markers of inflammation and the risk of non AIDS-defining events. With regard to cardiovascular diseases, the SMART study was the first to show an association between high levels of inflammation and cardiovascular disease (Duprez DA *et al*, 2012) followed by several studies which found that higher levels of CD8+ T-cell activation (CD38+ and HLA-DR+) and soluble markers of immune activation and inflammation (CRP, sCD14, sTNFR-1, IL-6, P-selectin and sVCAM-1) are associated with atherosclerosis expressed as increased carotid intima-media thickness in HIV-infected patients receiving cART (Hsue PY *et al*, 2009; Merlini E *et al*, 2012; Longenecker CT *et al*, 2013; Barbour JD *et al*, 2014). Venous thromboembolism is also found to be associated with increased plasma levels of P-selectin, D-dimer and hyaluronic acid in a case-control study (Musselwhite LW *et al*, 2011).

While several studies have evaluated the association between markers and cardiovascular disease, little is known concerning the potential association with other non-AIDS defining events such as diabetes and cancer risk. Brown et al reported that sTNFR-1 is associated with the risk of diabetes (Brown TT *et al*, 2010) while the SILCAAT study group (INSIGHT SMART) found an association between inflammation markers (CRP and IL-6) and the development of both AIDS- related and unrelated cancers (Borges AH *et al*, 2013).

HIV-associated neurocognitive disorders (HAND) is another non-AIDS defining event that remains prevalent among HIV-infected patients despite cART. Higher levels of sCD14 in plasma (Lyons JL *et al*, 2011) and higher levels of IL-8, MCP-1, IP-10, sCD14 and G-CSF and IFN-γ in the cerebrospinal fluid correlated with impaired neurocognitive function (Kamat A *et al*, 2012; Yuan L *et al*, 2013; Correia S *et al*,2013).

Table 5: Association between markers of inflammation, immune activation and coagulation with mortality in HIV-infected patients receiving cART

Author	**Study design**	**Study population**	**Outcome**	**Predicvtive markers**
Kalayjian RC *et al* (2010)	Case-control	HAART-naive participants randomized in ACTG protocols 384 (stavudine and didanosine or lamivudine and zidovudine, in combination with either nelfinavir, efavirenz, or both) and 5015 (stavudine and emtricitabine with lopinavir/r). Median age and CD4 cell count were 44 years, 42/mm^3 in cases (41) compared to 37years, 62/mm^3 in controls (111).	-AIDS defining illness -Mortality	OR (95% CI) for upper vs. lower quartiles: -sCD40L: 2.46 (1.20-5.04) -sTNFR-1:3.10 (1.44-6.67)
Tien PC *et al* (2010)	Cohort	922 participants from the Study of Fat Redistribution and Metabolic Change in HIV infection. Median age and CD4 cell count were 45 years, 189/mm^3 in cases compared to 43years, 389/mm^3 in controls.	All-cause mortality	OR (95% CI) for highest tertile vs. lowest tertile: -Fibrinogen: 2.57 (1.46-4.52) -CRP: 2.68 (1.46-4.93)
Mangili A *et al* (2011)	Cohort	327 HIV-positive men and women from the Cardiovascular AIDS Risk Evaluation (CARE) who were on ART for 10 years. Median age 44 years and CD4 cell count; 458/mm^3	All-cause mortality	HR (95% CI) for hsCRP > 3mg/L: 2.38 (1.15-4.9)
Koethe JR *et al* (2011)	Cohort	142 Naïve patients with malnutrition and advanced HIV in Lusaka and Zambia. Median age 32 years and CD4 cell count; 34/mm^3	All-cause mortality	HR (95% CI) for hsCRP >15 mg/L vs. < 5 mg/L : 1.96 (1.12, 3.44)
Ledwaba L *et al* (2012)		Case-control study of naïve patients with advanced HIV in South Africa. Median age and CD4 cell count were 35 years, 39/mm^3 in cases (187) and 35 years, 42/mm^3 in controls (374).	All-cause mortality	OR (95% CI) for the highest vs. lowest quartile: -hsCRP : 3.5 (1.9-6.7) -IL-6 : 3.8 (1.8-7.8) -D-dimer : 2.6 (1.4-4.9)

McDonald B *et al* (2013)	Case-control	Naïve patients from the Tshepo study (Gaborone, Botswana) initiating cART consisting of NRTI+1NNRTI. Median age and CD4 cell count were 36 years, 170/mm^3 in cases (32) and 35 years, 164/mm^3 in controls (64).	All-cause mortality	OR (95% CI) for high level IL-6 : 1.25 (1.05-1.48)
Fuster D *et al* (2014)	Cohort	400 naïve patients with alcohol problems who were recruited in 2001-2003, and were followed until 2010 for mortality. Median age 43 years and CD4 cell count; 75/mm^3	All-cause mortality	HR (95% CI) for high IL-6 levels: 2.57 (1.58-4.82)

c) Therapeutic interventions to decrease immune activation and inflammation

Different therapeutic approaches are currently being developed with the goal of decreasing residual immune activation and inflammation. These approaches include strategies that target residual viremia, microbial translation, co-infections and therapies that target immune activation directly.

(1)Interventions to decrease residual viremia

Antiretroviral therapy intensification has been proposed to target residual viremia and reduce residual immune activation. Intensification with efavirenz, lopinavir or atazanavir carried for 10 weeks showed that median levels of viremia were not significantly different between pre- and post-intensification indicating that low-level viremia is related to the size of stable reservoirs established prior to cART initiation (Dinoso JB *et al*, 2009). However, other studies showed that intensification with raltegravir, decreased CD8+ T cells activation (Buzon MJ *et al*, 2010; Llibre JM *et al*, 2012). In this way, a French study (ANRS 147 OPTIPRIM) has recently compared the impact of maximized (penta-therapy) vs. conventional (tri-therapy) HAART- on HIV reservoirs, as assessed by cell-associated HIV-DNA levels at 24-month, in patients with primary HIV-1 infection. At month 24, a similar efficacy of the two regimens on levels of viral reservoirs (Cheret A *et al*, abstract 549LB, CROI, 2014). In 2014, there are probably less arguments with our current tools and the current available drugs to envisage a major role of treatment intensification in the decrease of residual viremia.

(2) Interventions to decrease microbial translocation

Interventions targeted at blocking microbial translocation are currently being investigated. Alteration in gut micobiota is present early in HIV infection and thus, prebiotics and probiotics can be used to modify this alteration. In a pilot study where untreated HIV-infected individuals received a prebiotic oligosaccharide mixture, microbiota composition improved substantially and there was a significant reduction in levels of sCD14, a measure of microbial translocation (Gori A *et al*, 2011). Studies in ART-suppressed HIV-infected patients are necessary to confirm whether supplementing ART with prebiotics/probiotics could be helpful. Other ongoing studies include the administration of oral antibiotic with a broad spectrum that is concentrated in the gastrointestinal tract as well as oral anti-inflammatory drug used to treat inflammatory bowel disease that acts locally on the gut tissue.

(3) Interventions to treat coinfections

Treatment of co-infections is hypothesized to reduce residual immune activation. In a recent randomized, placebo controlled study, ART-treated HIV-infected and CMV-seropositive individuals with CD4+ cell count less than 350 cells/mm^3 were randomized to receive valganciclovir versus placebo for 8 weeks. The valganciclovir group had a significant decrease in CD8+ T-cells activation markers (CD38+ and HLA-DR+) (Hunt PW *et al*, 2011). Additional strategies that allow the treatment of common co-infections in HIV-infected individuals with more tolerated drugs should be developed.

(4) Interventions that directly target immune activation

(a) Cyclosporine A (CyA)

CyA is a powerful immune-suppressive agent that has been used in recipients of organ transplants and in the treatment of autoimmune disorders. In a randomized study on 54 treatment-naïve patients that received ART with or without CyA was conducted; there was no difference between the 2 groups in terms of the level of proviral DNA or CD4+ T cell counts (Markowitz M *et al*, 2010). Based on the disappointing results of this study, it was concluded that cyclosporine does not provide any advantage.

(b) Glucocorticoids

Based on their immune-suppressive activities and following the hypothesis that immune activation was a major component of the deleterious effects of HIV, prednisolone was administered in combination with antiretroviral therapy to HIV-infected individuals and resulted in decreased immune activation (Mccomsey GA *et al*, 2001; Andrieu JM *et al*, 2004). While of potential interest, the long-term use of glucocorticoids is limited by the serious side effects and thus, more selective glucocorticoid receptor modulators with safer toxicity profiles should be considered for future therapeutic studies in HIV-infected individuals.

(c) Hydroxyurea

Studies that investigated the role of hydroxyurea, an anticancer drug, in clinical practice with anti-HIV drugs have produced conflicting results. The combination of stavudine and didanosine with and without hydroxyurea was studied in 144 HIV-positive patients and after therapy, CD4$^+$ T cell counts increased less in the hydroxyurea-containing arm, reflecting the lymphopenic effect of hydroxyurea (Rutschmann OT *et al*, 1998). In another study, the

use of hyroxyurea, showed cytostatic effect inhibiting T-cell proliferation, induced antiviral suppression without affecting immune activation (Lori F *et al*, 2005).

(d) Cyclooxygenase type 2 (COX-2) inhibitors

Cyclic AMP may be induced by prostaglandin E_2 following LPS-induced up-regulation of COX-2 in monocytes due to the elevated LPS levels in patients with chronic HIV infection. Celecoxib, a COX-2 inhibitor, was tested for 12 weeks in HIV-infected patients without antiretroviral treatment in a randomized trial. Celecoxib reduced CD8+ T cell activation marker (CD38+), IgA levels and the inhibitory surface receptor programmed death 1 (PD-1) on CD8+ T cells (Pettersen FO *et al*, 2011).

(e) Statins

Based on the hypothesis that HMGco-A reductase inhibitors (statins) may modulate the immune response to HIV infection, a randomized, placebo-controlled trial investigated the effect of atorvastatin in patients not receiving ART on HIV-1 RNA and cellular markers of immune activation. Although HIV-1 RNA level was unaffected by the intervention, atorvastatin use resulted in reductions in circulating proportions of activated CD4+ and CD8+ T cells (Ganesan A *et al*, 2011). The second study evaluated the effect of atorvastatin in patients with undetectable viral load, and at week 48, cellular immune activation was significantly lower while with no difference in hs-CRP was observed between those received atorvastatin and those did not receive it (De Wit S *et al*, 2011). A third study evaluated the effect of lovastatin in ART-naïve patients, and found that at 12-month follow-up, there was no effect of lovastatin on the activation level of T cells (Montoya CJ *et al*, 2012). The last study on statin effect was the SATURN-HIV trial which evaluated the effects of rosuvastatin on markers of cardiovascular disease risk in ART-treated HIV-infected subjects. After 24 weeks, significant decreases in plasma levels of sCD14 and in the proportions of tissue factor-positive patrolling (CD14(Dim)CD16+) monocytes while no change in levels of T-cell activation, hs-CRP, IL-6 or D-dimer was observed (Funderburg NT *et al*, 2014; Eckard AR *et al*, 2014). In this context, a French study (CESAR trial) is now evaluating the effect of rosuvastatin in patients receiving suppressive cART with no indication for a statin treatment on markers of immune activation.

(f) TNF inhibitors

Etanercept is an artificially engineered receptor that binds to tumor necrosis factor (TNF) and reduces its effect. Data indicates that HIV infection is associated with excessive production of TNF-α, which may contribute to immune dysfunction (De SK *et al*, 2002). This data provides a rationale to test the therapeutic potential of etanercept in the management of HIV-infected patients.

VI. MATERIALS AND METHODS

A. Hypothesis and objectives of the study

1. Hypothesis

Considering the fact that antigenic stimulation with the HIV is the main source of immune activation and inflammation (Cohen Stuart JW *et al*, 2000), we hypothesized that rapid and persistent viral control among treatment-naïve HIV-infected patients initiating cART for two years, will decrease markers of immune activation and inflammation. In addition, we hypothesized that different regimens of ART could have different impact on the evolution of these markers.

2. Objectives

1. To identify relationships between patients characteristics and levels of immune activation and inflammation markers at cART initiation.
2. To evaluate the evolution in marker levels after 2 years of effective cART, comparing marker levels at cART initiation and two years post-cART with levels observed in HIV-uninfected controls.
3. To identify factors associated with abnormal levels of these markers two years post-cART.
4. To evaluate the impact of different regimens/drugs of ART on the evolution of these markers.

B. Study design

When evaluating the evolution of immune activation and inflammation markers, different confounding factors that might influence the evolution of these markers should be controlled by the study design. It is important to control for viral replication, which is a major cause of immunological activation and inflammation. In addition, it will not be appropriate to enroll patients who were already exposed to ARV. In order to achieve this design, different inclusion and non-inclusion criteria were chosen.

1. Inclusion criteria of patients

- ART-naive HIV-1 infected patients initiating a first cART between January 2006 and December 2009 and followed up for two years.

- Patients receiving one of the recommended ART combinations consisting of:
    - (TDF/FTC) + LPV/r, ATV/r, FPV/r or EFV
    - (ABC/3TC) + LPV/r, ATV/r, FPV/r or EFV
- Patients achieving rapid and persistent virological response defined as:
    - Plasma HIV-1 viral load below 400 copies/mL at 6 months.
    - Plasma HIV-1 viral load below 50 copies/mL at 24 months.
    - No viral rebound (viral load >1000 copies/ml) between month 6 and month 24.
- Available data on biological exams at cART initiation and at 24 months.

In the analysis that compared the impact of different antiretroviral drugs on the evolution of markers, only patients who maintained the same regimen through the two years study period were analysed to control the impact of treatment switching on the interpretation of results.

2. Non-inclusion criteria

- HIV-2 infected patients.
- The non-availability of sufficient quantity of plasma either at cART initiation or at month 24.

3. Controls

The control population consisted of 20 HIV-seronegative healthy blood donors. In France, donors can donate blood between 18 and 70 years of age. Marker levels among controls were evaluated in the context of ACTIVIR study, which was performed at the immunology laboratory (INSERM, UMR_S 1135, CIMI, F-75013, Paris, France) in 2010 to characterize T-cell and monocyte activation among HIV-infected patients with controlled viremia on antiretroviral therapy.

4. Ethical aspects

The study was approved by the Pitié-Salpétrière institutional review board and eligible patients were asked to sign a consent form before the use of their medical information and their plasma samples according to the French law after having enough time to read the information note about the study (information note and consent are found in the annexes).

C. Database resources

1. Department of infectious diseases/ Pitié-Salpétrière hospital

The department of infectious and tropical diseases at the Pitié-Salpêtrière is one of the referral centers that provide care to HIV-infected patients in Ile-de-France. HIV-infected patients, newly diagnosed patients and people who are accidentally exposed to blood particles receive care in one of the two divisions of the “HIV and immune depression unit”: the day-case unit where patients present for acute health problem or for programmed visit or in the consultation unit where patients are followed-up. In addition to the consultations and the day-case units, there is also an HIV clinical research unit where patients are proposed to participate in national and international trials investigating new therapeutic strategies, new antiretroviral drugs or new vaccines or in international cohorts. Beside the patients followed-up in the infectious diseases department, some patients are followed-up in the internal medicine department. Up to December 2013, a total of 4073 HIV-infected individuals were followed-up in the Pitié-Salpêtrière hospital. Their median age was 49 (IQR 25-75%; 41-55) years and their median follow up was 8 years (IQR 25-75%; 4-12). Of those, 3933 (96%) were receiving antiretroviral therapy and 90% of them had controlled viral load (<50 copies/mL). In 2013, 323 individuals were recruited including 132 newly diagnosed individuals.

The HIV and immune depression unit is a part of the COREVIH (COordination REgionale de lutte contre le VIH), Ile-de-France Centre. COREVIH began in the 2008 in the different regions of FRANCE. The objectives of COREVIH are to promote coordination of health professionals on clinical and therapeutic expertise, screening, prevention, health education, clinical and epidemiological research, training, as well as patients associations and users of the health system. In addition, COREVIH participates in improving the quality and safety of care for patients. Furthermore, COREVIH analyzes medical and epidemiological data concerning HIV infection in France.

2. NADIS®

Nadis is a secured electronic medical record, designed by health professionals to help improve the quality of HIV, HCV and HBV infected patients management and allows the participation in national assessments and epidemiological surveillance. In addition, it facilitates clinical research, the evaluation and the development of scientific projects since the

structure is identical in all hospitals using this software. Each patient file contains several variables including:

- Social characteristics of the patient
- Clinical data on HIV and hepatitis infection
- Medical history
- Treatment history
- Clinical examination
- Different biological results related or unrelated to HIV or hepatitis infection
- Genotyping
- Prescribed drugs (ARVs including doses and concomitant drugs)

In NADIS®, clinical data are collected during medical encounters while laboratories data are imported directly through computerized system to minimize collection errors. In 2013, biological tests were performed at the Pitié-Salpêtrière laboratories for 89% of patients followed-up in the hospital. Data quality is ensured by automated checks during data capture, by regular controls, by annual assessments, and by ad hoc processes before any analysis is performed.

D. Patients selection, clinical data collection and exportation

Using the criteria described above, NADIS® was used to screen patients for eligibility and a list of different variables for each eligible patient was obtained. Paper medical forms were then used to complete missing information as well as to ensure the information extracted from NADIS. The collected variables included characteristics of patient as well as clinical, biological and therapeutic status of patients. These variables were:

- Sex.
- Age.
- Weight and height.
- Smoking status.
- Hepatitis B (AgHBs positive) and C (HCV antibody positive) co-infections.
- HIV exposure group.
- Prior AIDS event.

- Pre-therapeutic and post therapeutic CD4+ cell count, CD8+ cell count and HIV-1 load.
- Initial cART regimen and changes of ART through the two years.

Collected clinical data described above were entered in a paper form (found in the annexes) and recorded using EpiInfo software (version 3.5.3). Another file was created with results of biomarker measurements. Files were merged and exported to be analysed using STATA.

E. Plasma collection and preparation

For the majority of patients, blood samples are drawn at the time of follow-up visit. In France, the medical insurance system offers the patients the choice to have their biological exams performed either in the hospital or in the private laboratories. For this reason, plasma was not available for some of the patients. Once samples are used to perform biological tests (CD4, CD8 cell counts, HIV-1 RNA, etc…), residual blood sample is centrifuged and plasma is frozen at the virology laboratory bank.

After the selection of patients, plasma tubes were retrieved from the plasma bank with the help of Dr. Slim Fourati from the virology laboratory. At this step, patients for whom sufficient quantity of plasma was not available either at cART initiation or at month 24 were excluded. Frozen plasma samples were allowed to defreeze for 50 minutes at room temperature. Samples were then centrifuged for 5 minutes at 1000 rpm. Aliquots were prepared by distributing different quantities of plasma into eppendorf tubes according to quantities indicated by each marker kit. These tubes were stored immediately at -20 C° to be used for cytokines measurement.

F. Biomarkers selection and measurement

In collaboration with our colleges in the immunology laboratory (Pr. Brigitte Autran, Dr. Guislaine Carcelain, and Dr. Amelie Guihot), we selected markers of immune activation and inflammation that can be reliably measured on frozen plasma. In addition, the association between these markers and different outcomes such as death and non-AIDS events has been reported in the literature. We evaluated IL-6 and hs-CRP as markers of inflammation, soluble CD14 (sCD14) as a marker of monocyte activation, and interferon-γ-inducible protein 10 (IP-10/CXCL-10) and monokine induced by interferon-γ (MIG/CXCL-9) as markers of T-

lymphocyte, monocyte and macrophage activation. The fact that the measurement of D-dimer on frozen plasma is not reliable precluded the evaluation of this marker of coagulation.

Using Enzyme-Linked ImmunoSorbent Assay (ELISA), we quantified IL-6, hs-CRP and sCD14 while IP-10 and MIG levels were determined with Cytometric Bead Array kits (BD™ CBA) on a BD FACS Canto I. These techniques were chosen taking into account their sensitivity (the ability to detect the marker at lower threshold) for measuring biomarker levels. Dr. Amelie Guihot and the technicians in the immunology laboratory teached me how to measure cytokines using ELISA and CBA techniques. I performed all the experiments running 80 plasma samples per kit per day for the ELISA and then, 50 plasma samples per day for the CBA. I used to dilute plasma as recommended by the kit manufacturers and when values were higher than the highest standard value, they were further diluted and retested. Standards provided with the kits were measured in duplicate and the mean value was used as reference. All D0 and M24 samples from each patient were tested in the same run. All the results of biomarker measurements were then validated by Dr. Guislaine Carcelain, and Dr. Amelie Guihot. Biomarker levels among controls were evaluated in the ACTIVIR study using the same techniques used in this study (results are shown in table 1). Hs-CRP was not tested in ACTIVIR and so, control levels for hs-CRP were not available.

Table 1: Biomarker levels among the control group (pg/mL)

	IL-6	**IP-10**	**MIG**	**sCD14**
Sample 1	2,01	344,56	1192,17	1230000
Sample 2	0,04	251,41	1009,74	125
Sample 3	0,04	255,89	564,64	1020000
Sample 4	0,04	249,6	548,68	7269,2
Sample 5	0,04	258,54	534,82	2440000
Sample 6	3,37	148,9	516,05	1450000
Sample 7	1,35	246,86	506,53	2485000
Sample 8	0,04	258,54	454,77	1540000
Sample 9	6,54	217,63	449,66	125
Sample 10	2,01	201,5	449,66	241000
Sample 11	3,23	352,54	444,51	1316,3
Sample 12	5,31	217,86	439,33	125

Sample 13	0,27	353,2	393,53	5397
Sample 14	0,04	190	382,23	1685000
Sample 15	5,43	308,53	379,37	2210000
Sample 16	0,64	246,86	328,04	1870000
Sample 17	0,04	180,3	308,44	5733,6
Sample 18	0,27	163,3	246,48	2320000
Sample 19	0,64	94,35	166,6	1470000
Sample 20	0,04	92,11	155,92	1870000

1. Enzyme-Linked ImmunoSorbent Assay (ELISA)

ELISA is an assay, which was developed independently and simultaneously by the research group of Peter Perlmann and Eva Engvall at Stockholm University in Sweden and by the research group of Anton Schuurs and Bauke van Weemen in the Netherlands. Different types of ELISA exist but they share the same principle using at least one capture antibody with specificity for a particular antigen (biomarker in our case), a second antibody coupled with an enzyme and color change to identify an antigen. In our study we used sandwich ELISA kit described below (figure 1).

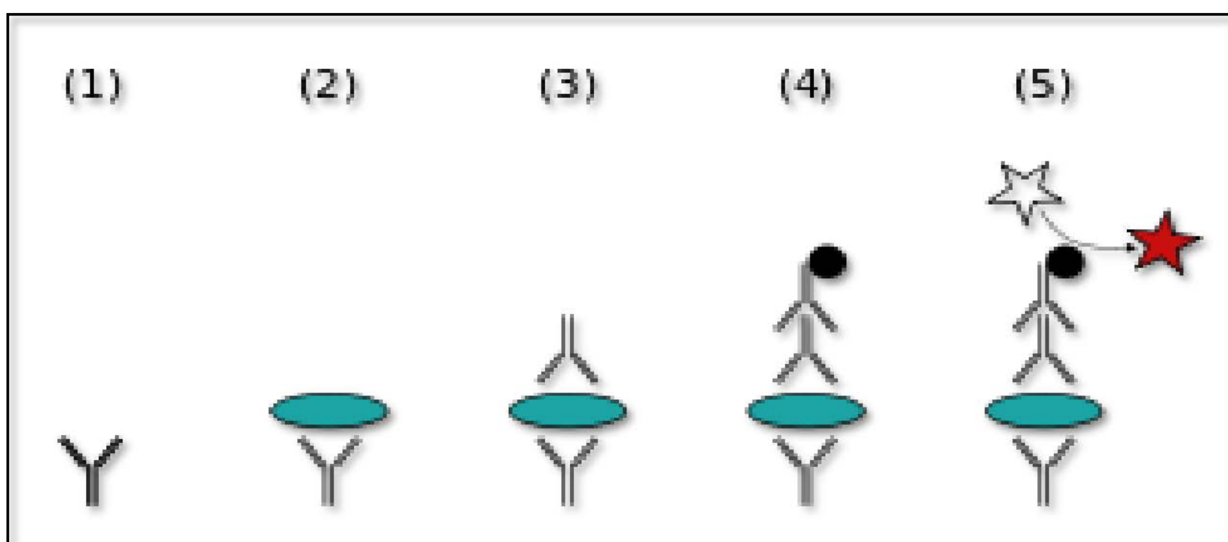

Figure 1: Different steps of marker identification using ELISA[2]

1. A surface is prepared to which a known quantity of capture antibody is bound. Any nonspecific binding sites on the surface are blocked.
2. The antigen-containing sample is applied to the plate and then the plate is washed to remove unbound antigen.
3. A specific antibody is added, and binds to antigen (hence the 'sandwich': the Antigen is stuck between two antibodies)
4. Enzyme-linked secondary antibodies are applied as detection antibodies that also bind specifically to the antibody. The plate is then washed to remove the unbound antibody-enzyme conjugates.
5. A chemical is added to be converted by the enzyme into a color or fluorescent or electrochemical signal.
6. The absorbency or fluorescence or electrochemical signal of the plate wells is measured to determine the presence and quantity of antigen.

2. Cytometric Bead Array (CBA)

The introduction of flow cytometric bead-based technology has added a new approach for investigators to simultaneously measure multiple analytes in biological samples. The cytometric bead array (CBA) allows the rapid evaluation of multiple analytes in a single sample using minimal sample volumes with reproducibility and results comparative with traditional immunoassays. Principles of Cytometric Bead Array are described below (figure 2):

1. Each capture bead in the array has unique fluorescence intensity and is coated with a capture antibody specific for a single analyte.
2. A combination of different beads is mixed with a sample or standard and a mixture of detection antibodies that are conjugated to a reporter molecule (phycoerythrin (PE)).
3. Following the incubation and subsequent washing, the samples are acquired on a flow cytometer. The FCAP Array analysis software gates on each individual bead population and determines the median fluorescence intensity (MFI) for each analyte in the array. It generates a standard curve and performs interpolation of sample

[2]From http://en.wikipedia.org/wiki/File:ELISA-sandwich.svg

concentrations compared to the standard curve and generates an analysis report in tabular format.

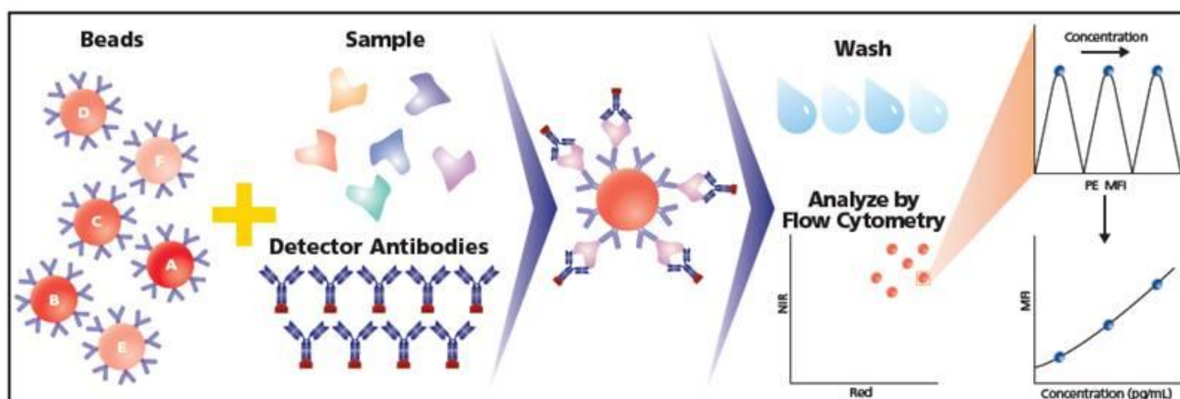

Figure 2: Principles of Cytometric Bead Array used in this study [3]

Information regarding the ELISA and CBA kits used in our study, manufacturer and the variation coefficients of these kits are found in table 2.

Table 2: ELISA and CBA kits used in our study

	Marker	Manufacturer	Reference	Coefficient of variation	Limit of detection (pg/ml)
ELISA	**IL-6**	R/D Quantikine®	HS600B	6.9-7.8%	0.039 pg/ml
	hs-CRP	Calbiotech®	CR120C	7.9–8.5%	0.004 mg/L
	sCD14	R/D Quantikine®	DC140	4.8–7.4%	125 pg/ml
CBA	**IP-10**	BD™ CBA	558280	4%	0.50 pg/ml
	MIG	BD™ CBA	558286	9–13%	1.10 pg/ml

G. Statistical analyses

Under the supervision of Marguerite Guiguet, I started my first experience in the field of statistics carrying out the descriptive analyses of the study population. After that, I learned to perform statistical analyses such as the comparison of medians, the comparison of means, the comparison of proportions, linear regression, interaction tests and logistic regression. The two summer school sessions I assisted were helpful in understanding these analyses. I used STATA 12 software for all statistical analyses in my study.

[3] (developed from http://www.bdbiosciences.com/research/cytometricbeadarray/formats/index.jsp)

1. Relationships between biomarker levels and patients characteristics at cART initiation

To investigate relationships between biomarker levels and patient characteristics at cART initiation, biomarker levels were compared among the HIV-infected patients according to their sex, age, body mass index (BMI), smoking status, hepatitis B serology, prior AIDS-defining events, CD4 cell count, CD4/CD8 ratio and viral load, using Wilcoxon tests. When more than one factor was associated with higher levels of a given biomarker ($p<0.15$) in univariable models, the relevant factors were entered in a backward logistic stepwise regression model, and variables with p values >0.10 were removed from the model.

2. Changes in biomarker levels after two years of effective cART

Changes in marker levels after 2 years of cART were determined using the median difference between marker values at D0 and M24 and tested using one-sample sign rank test.

3. Factors associated with persistent elevated marker levels after 2 years of cART

Biomarker levels were considered “elevated” if they exceeded the mean value plus two standard deviations in the control group. Logistic regression models were used to identify factors associated with elevated biomarker values after two years of cART. These factors were: sex, age, body mass index, smoking status, hepatitis B serology, prior AIDS events, CD4 cell count and HIV-1 load at cART initiation, viral blips between M6 and M24, the change of CD4 cell count, and the CD4/CD8 ratio at M24. Factors associated with elevated biomarker values in univariable analysis ($p<0.15$) were included in multivariable analysis.

4. Comparative impact of different ART components on the evolution of biomarkers

As mentioned above, these analyses were performed in a subgroup of patients who remained under the initial cART regimen for two years. Marker values at D0 and M24 were $\log_e$-transformed and the geometric mean of their ratio was determined (mean fold change). Linear regression models were used to investigate the impact of the two NRTI backbones (TDF/FTC and ABC/3TC) and the different third agents (EFV, LPV/r and ATV/r) on the biomarker changes. The results are expressed as the estimated percentage difference between the mean fold changes observed with a given drug, using TDF/FTC and EFV as the reference

groups for the comparison. Relationships between baseline covariables and changes in each biomarker were examined in univariable linear regression models. These covariables were sex, age, body mass index, smoking status, hepatitis B or C virus coinfection, prior AIDS-defining events, and the pre-ART CD4 cell count and viral load. Baseline covariables associated with changes in at least one biomarker ($p<0.10$) and viral blips above 50 copies/ml between M6 and M24 were retained in all multivariable linear regression models in order to control for factors that might have influenced the choice of cART regimen or affected biomarker levels. Age and smoking status were included in the multivariable model since these variables are known to influence marker levels (Deeks SG, 2013, Pine SR, 2011). Interaction terms between the NRTI backbone and the third agent were tested for each marker.

5. Sensitivity analyses

In the analyses that evaluated changes in marker levels after 2 years of cART in the overall population, sensitivity analyses excluding patients who experienced viral “blips” between month 6 and month 24 were conducted, while hepatitis C coinfection was considered as a non-inclusion criterion. When investigating the impact of different ART components on the evolution of biomarkers, hepatitis C coinfection was not considered as a non-inclusion criterion and sensitivity analyses were conducted, excluding patients with hepatitis coinfection.

VII. RESULTS

A. Impact of two years of effective first-line cART on soluble biomarkers of immune activation and inflammation

1. Summary of the study

We evaluated changes in IL-6, IP-10, MIG and sCD14 among patients who initiated first-line cART between January 2006 and December 2009 with rapid and persistent virological control. One hundred thirty-nine patients with a median age of 40 years, a median HIV-1 RNA level of 4.8 log_{10} copies/mL and a median CD4+ cell count of 294 filled the inclusion criteria and were enrolled in the study. Biomarker levels before and after cART initiation were compared with levels found among HIV-seronegative controls.

This work has been submitted for publication:

HATTAB S, GUIGUET M, CARCELAIN G, FOURATI S, GUIHOT A, AUTRAN B, CABY F, MARCELIN A-G, COSTAGLIOLA D, KATLAMA C. ***Soluble biomarkers of immune activation and inflammation in HIV infection: impact of two years of effective first-line cART.***

At cART initiation, we found that all biomarker levels were higher in HIV-infected patients than in controls. Higher levels of IL-6 and sCD14 were associated with prior AIDS events. Higher levels of IP-10 were associated with a low CD4/CD8 ratio and with high viral load, while higher levels of MIG were associated with high viral load.

After 2 years of effective cART, IL-6, IP-10 and MIG levels fell significantly to levels not significantly different from controls, while sCD14 levels did not change significantly and remained higher than levels found in controls. Age was the only associated factor with persistent elevated IP-10 (OR, 1.60/10-year; p=0.047) and MIG levels (OR, 1.92/10-year; p=0.007). In the supplementary analysis, elevated sCD14 level at 2 years was associated with none of the studied variables.

These results suggest that effective cART is useful but might not be sufficient to attenuate immune activation over 2 years, in particular, among older patients. Earlier initiation and/or longer successful cART might be necessary to drive immune activation to

normal levels in a context where the age of persons at HIV diagnosis is increasing (Costagliola D, 2014).

2. Submitted article 1

Soluble biomarkers of immune activation and inflammation in HIV infection: impact of two years of effective first-line cART

Suhaib Hattab*[1,2], Marguerite Guiguet[1,2], Guislaine Carcelain[3,4,5], Slim Fourati[1,2,6], Amelie Guihot[3,4,5], Brigitte Autran[3,4,5], Fabienne Caby[1,7], Anne-Geneviève Marcelin[1,2,6], Dominique Costagliola[1,2], Christine Katlama[1,2,7].

1: INSERM, UMR_S 1136, Pierre Louis Institute of Epidemiology and Public Health, F-75013, Paris, France.

2: Sorbonne Universities, UPMC Univ Paris 06, UMR_S 1136, Pierre Louis Institute of Epidemiology and Public Health, F-75013, Paris, France.

3: INSERM, UMR_S 1135, CIMI, F-75013, Paris, France.

4: Sorbonne Universities, UPMC Univ Paris 06, UMR_S 1135, CIMI, F-75013, Paris, France.

5: AP-HP, Hôpital Pitié-Salpêtrière, Département d'immunologie, F-75013, Paris, France.

6: AP-HP, Hôpital Pitié-Salpêtrière, Service de virologie, F-75013, Paris, France.

7: AP-HP, Hôpital Pitié-Salpêtrière, Service des maladies infectieuses, F-75013, Paris, France.

Corresponding author name: Suhaib Hattab

Corresponding author e-mail: suhaibhattab@yahoo.com

Address: 56, Boulevard Vincent Auriol, CS 81393, 75646 Paris Cedex 13, FRANCE

Telephone: +33 1.42.16.01.75

Fax: +33 1.42.16.01.26

Running title: Immune activation and inflammation under effective cART

Key words: HIV, cART, immune activation, inflammation, biomarkers, age

Short title: 49/50 character

Abstract: 250/250 words

Text: 3411/3500

References: 41

Tables: 4/6

Abstract

Objective: To assess the impact of rapid and persistent viral control by combination antiretroviral therapy (cART) on HIV-associated immune activation and inflammation.

Methods: In this longitudinal observational study, we examined changes in interleukin-6 (IL-6), interferon-γ-inducible protein-10 (IP-10), monokine induced by interferon-γ (MIG) and soluble CD14 (sCD14) levels during two years of effective first-line cART. Biomarker levels before and after cART were compared to those observed in HIV-uninfected controls, using the Wilcoxon signed rank test. Elevated biomarker levels were defined with respect to healthy control values (mean + 2SD). Factors associated with persistently elevated biomarker levels after 2 years of cART were identified by logistic regression.

Results: We studied 139 patients with a median HIV-1 RNA level of 4.8 log_{10} copies/mL and a median CD4 cell count of 294/mm^3 at cART initiation. At cART initiation, all biomarker levels were higher than in controls ($p<0.05$). After 2 years of cART, IL-6, IP-10 and MIG levels fell significantly, by a median of 0.54, 420 and 1107 pg/mL, respectively (all $p<0.001$), and were no longer elevated in >75% of patients. In contrast, sCD14 levels did not change significantly ($0.18*10^6$ pg/mL; p=0.102) and remained elevated. Older age was associated with elevated levels of IP-10 (OR, 1.60/10-year; p=0.047) and MIG (OR, 1.92/10-year; p=0.007) after 2 years of cART.

Conclusions: Rapid and persistent viral suppression by first-line cART reduces IL-6, IP-10 and MIG to normal levels while sCD14, a marker of monocyte activation remain elevated. High levels of IP-10 and MIG tend to persist in older patients.

Introduction

Combination antiretroviral therapy (cART) controls HIV plasma viral load in most patients, leading to a reduction in morbidity and mortality [1,2]. However, persistent low-level viral replication and immune activation despite treatment remain an important therapeutic challenge [3].

Immune activation is associated with more rapid disease progression and with less efficient CD4+ cell recovery during cART [4–6], while plasma biomarkers of inflammation have been linked to a risk of mortality and non AIDS-defining events such as cardiovascular disease and non AIDS-defining cancers [7–11]. An association between these biomarkers and mortality was first noted in the SMART study [12]. Persistent immune activation in this setting may be related to residual viremia driven by HIV cellular reservoirs [13] and by other chronic viral infections (cytomegalovirus, Epstein-Barr virus, etc.) [14,15]. In addition, mucosal immune dysfunction characterized by profound depletion of CD4+ T-cells during the early acute phase of HIV infection can lead to a gradual loss of the intestinal barrier function, allowing translocation of the intestinal flora into the systemic circulation [16,17].

Soluble biomarkers are a convenient way of assessing immune activation and inflammation in HIV-infected patients receiving cART. Interleukin-6 (IL-6) is a biomarker of inflammation and was found to be associated with all-cause mortality and cardiovascular diseases in the SMART study [12]. IP-10 (interferon-γ-inducible protein-10) and MIG (monokine induced by interferon-γ) are two chemokines produced by different cells and target lymphocytes, particularly, activated T cells. Elevated plasma IP-10 levels during the primary phase of HIV-1 infection were predictive of earlier decline in the CD4+ cell count in a recent study [18]. Soluble CD14 (sCD14) is a marker of monocyte activation that binds to lipopolysaccharides in plasma. Plasma levels of sCD14 are an independent predictor of mortality among HIV-infected patients [19].

The impact of first-line ART on soluble biomarkers of inflammation and immune activation has been examined in few studies, with variable results. For example, IL-6 levels were consistently found to fall in the HEAT, ACTG A5224, MERIT and MACS studies [20–23]. However, while one study showed that IP-10 levels fell and that sCD14 levels remained elevated on cART [24], another study showed that both IP-10 and sCD14 levels fell, after the initiation of an NRTI-sparing regimen [25]. These discrepancies might be due differences in baseline HIV disease status, the virological response, and/or the treatment regimen.

In this longitudinal observational study, we examined the impact of rapid and persistent virological control during two years of first-line cART on levels of soluble biomarkers of immune activation and inflammation, and sought to identify factors associated with persistently elevated biomarker levels. The patients were representative of chronically HIV-infected patients who started cART with moderate immunodeficiency in the recent cART era.

Methods

Study population

With their informed consent, all HIV-infected patients receiving care in the Infectious Diseases Department of Pitié-Salpêtrière Hospital (Paris, France) have their clinical, biological and therapeutic data prospectively recorded in standardized electronic medical records (NADIS database). Biological data obtained in Pitié-Salpêtrière hospital laboratories, including HIV RNA levels and CD4 and CD8 cell counts, are directly imported into the database from the laboratory computer system, thus minimizing collection errors. The quality of the database is ensured by automated checks during data capture, and by regular controls and annual assessments. Routine blood tests are performed at each hospital visit, and residual plasma is stored frozen, identified by a serial number.

All HIV-1-infected patients who started a first line of cART between January 2006 and December 2009 were screened for eligibility for this study, using the NADIS database. Patients were eligible if they received a first-line combination recommended by contemporary French guidelines (2006 and 2008) (tenofovir-emtricitabine (TDF/FTC) or abacavir-lamivudine (ABC/3TC), combined with either efavirenz or a ritonavir-boosted protease inhibitor (atazanavir (ATV/r), fosamprenavir (FPV/r) or lopinavir (LPV/r)) and had a rapid and persistent virological response, defined by plasma HIV-1 viral load (VL) below 400 copies/mL at 6 months and below 50 copies/mL at 24 months, with no values above 1000 copies/mL between month 6 and month 24, to control for the possible effect of residual viral replication on immune activation and inflammation. Hepatitis C virus (HCV) coinfection (positive HCV antibody) was a non inclusion criterion, as HCV infection can also influence biomarker levels. Eligible patients were enrolled if frozen plasma samples obtained at the time of cART initiation (D0) and at month 24 (M24) were available. The control population consisted of 20 HIV-seronegative healthy blood donors. In France, donors can donate blood between 18 and 70 years of age. The study was approved by the Pitié-Salpêtrière institutional review board, and eligible patients were

asked to give their written consent to the use of their medical information and plasma samples, as required by French law.

Sample collection and plasma soluble biomarker assays

We selected biomarkers of inflammation and immune activation that can be reliably measured in frozen plasma. We evaluated IL-6 as a biomarker of inflammation, IP-10 and MIG as biomarkers of immune activation including T lymphocytes and monocytes, and sCD14 as a biomarker of monocyte activation.

Enzyme-linked immunosorbent assays (ELISA) were used to quantify IL-6 and sCD14, **according to their manufacturer's instructions** (R&D Quantikine®, HS600B and DC140 respectively). IP-10 and MIG levels were determined on thawed diluted plasma with Cytometric Bead Array kits (BD™ CBA) on a BD FACS Canto I device, according to **manufacturer's instructions.** Coefficients of variation were 6.9-7.8 % for IL-6, 4.8–7.4% for sCD14, 4% for IP-10 and 9–13% for MIG while the limits of detection were 0.039, 125, 0.50, and 1.10 pg/ml respectively*.* Plasma samples were allowed to thaw for 50 minutes at room temperature before centrifugation for 5 minutes at 1000 rpm, followed by distribution into Eppendorf tubes in the amounts required for each assay kit. Plasma was diluted as recommended by the kit manufacturers. Standards provided with the kits were measured in duplicate and the mean value was used as reference. Samples with values higher than the highest standard value were further diluted and retested. D0 and M24 samples were tested in the same run. The same biomarkers were measured with the same kits in the control population.

Statistical analysis

To investigate relationships between biomarker levels and patient characteristics at cART initiation, biomarker levels were compared among the HIV-infected patients according to their sex, age, body mass index (BMI), smoking status, hepatitis B (HbsAg) serology, prior AIDS-defining events, CD4 cell count, CD4/CD8 ratio and viral load, using Wilcoxon tests. When more than one factor was associated with higher levels of a given biomarker

(p<0.15) in univariable models, the relevant factors were entered in a backward stepwise regression model, and variables with p values >0.10 were removed from the model. The two-sample Wilcoxon rank-sum test was used to compare biomarker levels between the HIV-infected patients and controls.

Among the HIV-infected patients, changes in the level of each biomarker during the first two years of cART were expressed as the difference between M24 and D0, and median differences were tested with a one-sample sign rank test.

Biomarker levels **were considered “elevated” if they exceeded the** mean value plus two standard deviations in the control group. Changes in the proportion of patients with elevated biomarker values at between D0 and M24 **were assessed using McNemar’s test.** Logistic regression was used to identify factors associated with elevated biomarker values after two years of cART. Factors associated with elevated biomarker values in univariable analysis (p<0.15) were included in multivariable analysis. A sensitivity analysis excluded patients who experienced viral **“blips” (VL transiently above 50 copies/mL) b**etween month 6 and month 24. STATA 12 software was used for all statistical analyses, and p values <0.05 were considered to denote statistical significance.

Results

Patient characteristics at cART initiation (Table 1)

Between January 2006 and December 2009, a total of 539 patients began first antiretroviral therapy and remained under care over two years in our department. Of those, 370 patients had a rapid and persistent virological response over the two years. Of them, 280 patients who began therapy with abacavir/lamivudine or tenofovir/emtricitabine plus efavirenz, atazanavir/r, lopinavir/r or fosamprenavir/r were eligible for the study. The remaining 90 patients were not considered since they either received no longer recommended antiretroviral therapy such as combivir and invirase (n=64) or were included in clinical trials evaluating new drugs such as darunavir and rilpivirine. Frozen plasma samples at baseline and after two years were available for 147 patients. Characteristics of patients who had stored plasma were not different from those did not have it for age, CD4 cell count, HIV-RNA level, AIDS defining events and for prescribed antiretroviral therapy. Six patients coinfected with HCV were not eligible and 2 patients who withheld their consent were excluded.

The study population thus consisted of 139 patients. Most of the patients were male, and median age was 40 years (range; 22 to 74). At cART initiation the median CD4 cell count was 294/mm^3 and the median plasma HIV RNA level was 59 340 copies/mL. Fifty-seven patients (41%) had plasma HIV RNA levels above 100 000 copies/mL.

Nineteen patients (14%) had experienced a total of 21 AIDS-defining events, consisting of ***Pneumocystis jirovecii*** pneumonia (n=8), Kaposi**'s** sarcoma (n=5), tuberculosis (n=4), cerebral toxoplasmosis (n=3) and esophageal candidiasis (n=1). Seven patients had HBV coinfection (HBsAg positivity); all of them received tenofovir/emtricitabine.

The prescribed ART regimens are shown in table 1. Seventy-five patients (54%) remained on the same cART regimen during the first two years, while 64 patients (46%) had ART modifications, all for reasons other than virological failure. These changes were between drugs of the same class (n=23), between a protease inhibitor and efavirenz

(n=25), or towards a regimen including raltegravir (n=12), maraviroc (n=2), or bitherapy (n=2).

Biomarker levels at cART initiation

At cART initiation, median IL-6, IP-10, MIG and sCD14 levels were 1.64, 717, 1660 and 2.60×10^6 pg/mL respectively. Factors associated with high biomarker levels in univariable analyses are shown in table 2. In stepwise regression analyses, higher levels of IL-6 were associated with prior AIDS events (p<0.001). Higher levels of IP-10 were associated with a low CD4/CD8 ratio (p=0.007) and with high viral load (p=0.001), while higher levels of MIG were associated with high viral load (0.024). High sCD14 levels were associated with prior AIDS events (p=0.01). Median levels of all the biomarkers were significantly higher in the patients than in the controls (table 3). However, the majority of patients had IL-6 levels below the upper limit of normal (<5.7 pg/mL), while a majority had abnormal IP-10 (>378 pg/mL) or MIG (>955 pg/mL) levels. One-third of patients had sCD14 level above the upper limit of normal (<2.98 $\times 10^6$ pg/mL). Coefficients of variation among the control group were 1.34 for IL-6, 0.32 for IP-10, 0.52 for MIG and 0.88 for sCD14.

Evolution of immunovirological status after two years of effective cART

As required by the eligibility criteria, plasma HIV RNA levels were below 400 copies/mL in all the patients at M6 including 123 patients (88%) who had levels below 50 copies/mL. The median time to viral suppression (<400 copies/mL) was 2 months. Between M6 and M24, 29 patients experienced a single viral blip above 50 copies/mL (median 77 copies/mL; range 51 to 804); none was associated with treatment modification. HIV RNA levels were below 50 copies/mL in all the patients at M24.

The CD4 cell count rose by a median of 224/mm^3 between D0 and M24, and the median count at M24 was 523/mm^3 (IQR 357 to 676). The median CD4/CD8 ratio rose from 0.29 at cART initiation to 0.76 (IQR 0.48 to 1.05) at M24. Seventy-one patients (51%) had CD4 cell counts above 500/mm^3 at M24, and 52 patients (37%) had a CD4/CD8 ratio

above 0.9. All the patients were clinically stable and none of them experienced a clinical event (AIDS, myocardial infarction or cancer) during the study period.

Changes in biomarkers of immune activation and inflammation after two years of effective cART

As shown in Table 3, after two years of effective cART, IL-6, IP-10 and MIG levels fell by a median of 0.54 pg/mL (-33%), 420 pg/mL (-59%) and 1107 pg/mL (-67%), respectively (all p<0.001), while sCD14 levels did not change significantly ($-0.18*10^6$ pg/mL (-7%)). No difference in IL-6, IP-10 and MIG levels was found between the patients and controls at M24, while sCD14 levels remained higher in the patients, even after excluding those with viral blips.

Factors associated with persistently elevated biomarker levels after 2 years of cART

Only 4 patients (3%) had elevated IL-6 levels at M24 compared to 11% at cART initiation (p=0.008), so we were unable to investigate factors associated with persistence of this biomarker. Twenty-nine patients (21%) had elevated IP-10 levels at M24 compared to 86% at cART initiation (p<0.001). As shown in table 4, older age was associated with persistently elevated IP-10 levels (OR, 1.60 per 10-year increment). Twenty-two patients (16%) had elevated MIG levels at M24 compared to 81% at cART initiation (p<0.001), and age was again the only factor associated with persistently elevated levels of this biomarker (OR, 1.92 per 10-year increment). Soluble CD14 levels remained stable during the 2 years of cART: 24% of patients had elevated levels at M24, compared to 32% of patients at cART initiation (p=0.109). Therefore, we did not investigate factors associated with persistence of this biomarker. Eighty-One patients (58%) had normal values of all 4 biomarkers and only one patient had elevated values of the 4 biomarkers. In a sensitivity analysis excluding patients with viral blips, the proportions of patients who had elevated biomarker levels at M24 were similar: 4% for IL-6, 18% for IP-10, 13% for MIG, and 25% for sCD14.

Discussion

This longitudinal observational study showed that IL-6, IP-10 and MIG levels fell significantly during the first two years of virologically effective cART, to values that were not significantly different from those of healthy controls. In contrast, sCD14 levels remained relatively stable, at a significantly higher median value than in the healthy controls. In addition, IP-10 and MIG levels remained elevated in some patients, who tended to be older.

Studies of the impact of first-line antiretroviral therapy on biomarkers of immune activation and inflammation must control for viral replication, as it is a major cause of persistent immunological activation and inflammation. Thus, the intent-to-treat approach used in randomized trials, which includes all patients independently of their virological response, may not be appropriate and could partly explain discrepancies between published results. Our study was restricted to patients in whom viral load was controlled throughout the two-year study period. In addition, all the patients under care in our department were screened for eligibility, thus avoiding any major selection bias. We included patients who experienced isolated viral blips, in order to investigate the possible impact of blips on residual immune activation and inflammation. These patients were excluded from sensitivity analyses. Due to the fact that the sample was selected retrospectively, we could not account for other coinfections (cytomegalovirus, sexually transmitted diseases) or other inflammatory conditions that might influence biomarker levels.

IL-6, IP-10, MIG and sCD14 levels before cART initiation were significantly higher than in the healthy controls. IL-6 and sCD14 levels were particularly high in patients who had already experienced AIDS-defining events, in keeping with previous reports [26,27]. Patients with high baseline viremia also had higher levels of IP-10 and MIG, in keeping with the fact that antigenic stimulation by HIV is the main source of immune activation [28–31].

Along with the control of HIV viremia, IL-6, IP-10 and MIG levels all fell significantly during two years of effective cART, even though most of the patients had IL-6 levels within the normal range at treatment initiation. In the majority of patients, levels of these three biomarkers reached values not significantly different from those of the healthy controls, while sCD14 levels did not decline and thus remained higher than in the controls. These results are consistent with those obtained in a study conducted in the ALLRT cohort, in which HIV viremia was constantly controlled during the one-year study period [10]. There are several possible explanations for these stably high sCD14 levels despite two years of virologically effective cART. First, factors other than HIV viremia may be responsible for persistent sCD14 elevation. In particular, two years of suppressive cART may not be sufficient to restore the intestinal barrier function, allowing bacterial translocation to persist [24]. Second, the cART regimens used in our study may not be as effective as raltegravir-containing regimens which have been shown to reduce sCD14 levels [25,32]. However the very small number of patients who changed their initial regimen to raltegravir precluded any specific investigation in our study. This finding is of importance given the association between sCD14 levels and subclinical vascular disease independently of traditional risk factors found in a recent study [33]. Even if we observed that the level of IL-6 returned to normal level in most patients, it does not contradict the fact that high levels of IL-6 are associated with increased risk of cardiovascular disease in HIV-infected patients as well as in the general population [11,34]. The contrast in the proportion of patients who had elevated IL-6 level after treatment and the proportion of patients who had elevated IP-10 or MIG level could be explained in two ways. First, the majority of the patients had IL-6 levels within the normal range at treatment initiation, even if values were in the upper range of normal values. Second, the variability in IL-6 levels was more important than the variability of IP-10 and MIG levels, as illustrated by the higher coefficient of variation.

IP-10 and MIG levels remained elevated in some patients despite two years of effective cART. Interestingly, while levels of these biomarkers were not related to age at time of

cART initiation, once HIV viremia was suppressed, older patients were more likely to retain elevated levels after treatment. Each 10-year age increment was associated with a 60% increase in the risk of retaining elevated levels of biomarkers of immune activation at M24. These results are compatible with the proposed link between immune activation and premature aging [35,36], and may partly explain the slower CD4 cell recovery in older patients [37]. Older patients might thus need personalized therapy to attenuate this immune activation. It is possible that different cART components could have different impact on residual immune activation and inflammation. The impact of different cART regimens on the biomarker dynamics have been evaluated among the 54% of patients who remained on the initial regimen over the study period. The only difference was a smaller fall in IP-10 and MIG with atazanavir/r than with efavirenz [38].

Transient low-level viremia (blips) may predict subsequent virological failure [39]. No association was found between such blips and the persistence of immune activation in this study, but we may have had insufficient power to show an association since only 21% of patients presented viral blips. A recent study showed an association between T cell activation and the risk of subsequent viral blips, but no causal relationship was shown [40].

The persistence of immune activation and inflammation markers particularly in older patients despite the virological suppression remains a challenge given that the age of individuals at HIV diagnosis has increased over time [41]. Whether the initiation of cART at higher CD4+ cell count, as now recommended by many recent guidelines, could normalize immune activation levels remains to be assessed. The impact of new antiretroviral drugs on immune activation and inflammation markers is a clinically relevant question that merits investigation.

Acknowledgments

SH, MG, GC, AG, A-GM, DC and CK contributed to conception and design. Plasma samples were collected by SH, SF and A-GM. Biomarkers measurements were made by SH, AG, GC and BA while collection and assembly of data were made by SH, MG, SF, AG and FC. Statistical analyses were performed by SH, MG and DC. All authors contributed to critical revision of the article for scientific accuracy and final approval. Authors thank Damien Egault and Rachid Agher for the technical assistance. Biomarker measurements were made in collaboration with the INSERM UMR_S 1135 immunology laboratory.

Conflicts of interest and source of funding

No members of the study team have any financial or personal relationships with people or organizations that could inappropriately influence this work. Marguerite Guiguet, Guislaine Carcelain, Anne-Geneviève Marcelin, Dominique Costagliola and Christine Katlama have received at some stage in the past travel grants, consultancy fees, honoraria or study grants from various pharmaceutical companies including Bristol-Myers-Squibb, Gilead Sciences, Janssen-Cilag, Merck-Sharp & Dohme-Chibret and ViiV Healthcare. For the remaining authors none was declared. This study was funded by INSERM recurrent grants (INSERM UMR_S 1136).

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Table 1. Patient characteristics at cART initiation

N=139	N (%) or median (interquartile range)
Sex, Male	112 (81%)
Age, years	40 (34-47)
Body mass index (kg/m^2)	23 (21-25)
Current smokers	50 (36%)
Hepatitis B coinfection (HBsAg positivity)	7 (5%)
Exposure group	
Men who have sex with men	55 (42%)
Heterosexual men or women	59 (40%)
Others	25 (18%)
Time since HIV-1 diagnosis (months)	6 (1- 43)
Prior AIDS events	19 (14%)
CD4 /mm^3	294 (190-384)
CD4 %	16% (13 to 21)
CD8 /mm^3	900 (571-1240)
CD8 %	57% (47 to 62)
CD4/CD8 ratio	0.29 (0.19-0.45)
HIV-1 viral load (log_{10} copies/mL)	4.8 (4.3-5.3)
Initial cART regimen	
TDF/FTC/LPV/r	26 (19%)
TDF/FTC/ATV/r	25 (18%)
TDF/FTC/FPV/r	10 (7%)
TDF/FTC/EFV	52 (37%)
ABC/3TC/LPV/r	8 (6%)
ABC/3TC/ATV/r	12 (9%)
ABC/3TC/FPV/r	2 (1%)
ABC/3TC/EFV	5 (3%)

HBsAg, hepatitis B surface antigen; cART, combination antiretroviral therapy; TDF, tenofovir; FTC, emtricitabine; ABC, abacavir; 3TC, lamivudine; LPV/r, lopinavir/ritonavir; ATV/r, atazanavir/ritonavir; FPV/r, fosamprenavir/ritonavir; EFV, efavirenz.

Table 2. Relationships between patient characteristics and biomarker levels at cART initiation (N=139)

		IL-6 (pg/mL)		IP-10 (pg/mL)		MIG (pg/mL)		sCD14 (10^6 pg/mL)	
	N	Med (IQR)	P- value	Med (IQR)	P- value	Med (IQR)	P- value	Med (IQR)	P- value
Sex									
Male	112	1.7 (1.1-2.8)	0.347	721 (466-1035)	0.713	1715 (1075-2759)	0.908	2.6 (2.1-3.1)	0.991
Female	27	1.6 (0.9-2.7)		666 (488-1102)		1494 (1093-4038)		2.6 (2.2-3.1)	
Age (years)									
≤35	44	1.6 (1.1-2.8)	0.355	741 (519-1128)	0.970	1519 (1113-2675)	0.923	2.6 (2.1-3.1)	0.913
35-44	47	1.4 (1.0-3.1)		713 (468-1055)		1553 (980-3719)		2.6 (2.2-3.1)	
≥44	48	1.9 (1.3-2.7)		709 (450-1102)		2174 (1093-2713)		2.6 (2.0-3.3)	
BMI (kg/m^2)									
<27	113	1.6 (1.0-2.6)	0.401	675 (468-1005)	0.042	1622 (1059-2804)	0.314	2.6 (2.1-3.1)	0.519
≥27	26	1.9 (1.3-2.9)		1035 (666-1173)		2217 (1113-4393)		2.8 (2.2-3.1)	
Smoking									
Current smoker	50	1.9 (1.3-2.9)	0.055	660 (526-1126)	0.833	1963 (1193-2675)	0.251	2.7 (2.1-3.3)	0.171
Non-smoker	89	1.6 (0.99-2.5)		721 (466-1055)		1453 (1040-2873)		2.5 (2.1-3.1)	
Hepatitis B coinfection									
Positive	7	1.0 (0.6-7.4)	0.461	660 (314-855)	0.270	1445 (924-2249)	0.360	2.0 (1.7-3.9)	0.281
Negative	123	1.6 (1.1-2.8)		711 (468-1102)		1666 (1091-2873)		2.6 (2.1-3.1)	
AIDS events									
Yes	19	4.55 (1.9-7.4)	<0.001	1306 (812-2017)	<0.001	1827 (1004-2831)	0.767	3.17 (2.5-4.2)	0.006
No	120	1.54 (1.0-2.4)		665 (461-988)		1612 (1091-3719)		2.54 (2.1-3.0)	
CD4 at D0 (mm^3)									
≤200	35	1.9 (1.2-4.3)	0.267	850 (537-1306)	0.082	1481 (1113-3474)	0.684	2.5 (2.1-3.4)	0.799
200-350	52	1.6 (1.1-2.3)		700 (460-1026)		2123 (1204-2804)		2.6 (2.0-3.1)	
≥ 350	35	1.6 (1.0-2.9)		653 (439-907)		1641 (992-2852)		2.7 (2.1-3.1)	
CD4/CD8 ratio D0									
≤0.22	38	2.0 (1.3-4.4)	0.060	1065 (761-1563)	<0.001	1937 (1170-3474)	0.592	2.9 (2.5-3.6)	0.004
0.22-0.37	41	1.4 (1.1-2.5)		640 (460-781)		1622 (1113-2633)		2.6 (2.2-3.1)	
≥0.37	41	1.6 (1.0-2.5)		647 (447-881)		1544 (1017-2712)		2.4 (1.8-2.9)	
HIV-1 viral load D0									
<5log	82	1.56 (1.0-2.5)	0.087	639 (437-855)	<0.001	1452 (924-2336)	<0.001	2.57 (2.0-3.1)	0.201
≥5log	57	1.78 (1.2-4.0)		892 (641-1376)		2262 (1392-4153)		2.66 (2.1-3.4)	

- IL-6, Interleukin-6; IP-10, Interferon-γ-inducible protein 10; MIG, Monokine induced by interferon-γ; sCD14, soluble CD14; BMI, body mass index; D0, time of cART initiation. Results are presented as median (IQR 25-75%).

Table 3. Biomarker levels among controls (HIV-) and HIV-infected (HIV+) patients at D0 and at M24; comparisons with controls and changes during cART

	HIV- N= 20	HIV+ D0 N= 139	HIV+ D0 vs. HIV-	HIV+ M24 N= 139	HIV+ M24 vs. HIV-	Change from D0 N= 139	Change from D0*** N= 110
IL-6 (pg/mL)	0.45 (0.04-2.62)	1.64 (1.06-2.80)	p=0.005	1.14 (0.68-1.82)	p=0.074	-0.54 (-1.63 to 0.14)	-0.46 (-1.27 to 0.09)
Elevated value* ≥ 5.7 pg/mL		11%		3%			
P-value**						< 0.001	< 0.001
IP-10 (pg/mL)	246 (185-258)	717 (471-1065)	p<.0001	263 (187-346)	p=0.152	-420 (-723 to -212)	-407 (-673 to -212)
Elevated value ≥378 pg/mL		86%		21%			
P-value						< 0.001	< 0.001
MIG (pg/mL)	447 (353-525)	1660 (1091-2831)	p<.0001	473 (332-664)	p=0.385	-1107 (-2167 to -594)	-1025 (-1873 to -579)
Elevated value ≥955 pg/mL		81%		16%			
P-value						< 0.001	< 0.001
sCD14 (10^6pg/mL)	1.34 (0.56-1.87)	2.60 (2.09-3.10)	p<.0001	2.35 (1.97-2.95)	p<.0001	-0.18 (-0.75 to 0.55)	-0.16 (-0.63 to 0.58)
Elevated value ≥2.98*10^6 pg/mL		32%		24%			
P-value						0.102	0.327

D0, time of cART initiation; M24, month 24; IL-6, Interleukin-6; IP-10, Interferon-γ-inducible protein 10; MIG, Monokine induced by interferon-γ; sCD14, soluble CD14.
*, Calculated among HIV-uninfected controls (mean+2SD). **, Signed rank test. ***, Analyses excluding patients with viral blips.
Results are presented as median (IQR 25-75%).

Table 4: Factors associated with persistently elevated biomarker levels at M24

	IP-10 > 378 pg/mL n=29/139 (21%)				MIG > 955 pg/mL n=22/139 (16%)	
	Univariable		**Multivariable**		**Univariable**	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Sex (Male)	0.70 (0.26-1.85)	0.472			1.10 (0.34-3.57)	0.872
Age (per 10 years)	1.49 (0.98-2.27)	0.063	1.60 (1.01-2.56)	0.047	1.92 (1.19-3.09)	0.007
Body mass index > 27	2.46 (0.96-6.3)	0.061	2.37 (0.76-6.70)	0.384	0.65 (0.18-2.37)	0.509
Current smoking	1.11 (0.47-2.59)	0.805			0.62 (0.23-1.71)	0.357
Hepatitis B coinfection	1.42 (0.26-7.74)	0.684			0.81 (0.09-7.08)	0.849
Prior AIDS event	1.01 (0.31-3.32)	0.983			0.99 (0.26-3.75)	0.996
CD4 at D0 (log2)	0.79 (0.59-1.05)	0.112	0.77 (0.56-1.07)	0.126	0.92 (0.65-1.31)	0.653
HIV-1 viral load at D0 > 5 log	1.01 (0.44-2.34)	0.963			1.24 (0.49-3.11)	0.644
Viral blip between M6-M24	2.03 (0.80-5.10)	0.134	2.11 (0.70-6.30)	0.186	1.13 (0.38-3.40)	0.815
Change in CD4 from D0 (per 50 cells/mm^3)	0.88 (0.76-1.02)	0.085	0.88 (0.74-1.04)	0.123	1.03 (0.88-1.21)	0.650
CD4/CD8 ratio at M24 > 0.9	0.73 (0.31-1.72)	0.475			1.03 (0.41-2.60)	0.948

- IP-10, Interferon-γ-inducible protein 10; MIG, Monokine induced by interferon-γ; OR, odd ratio; D0, day 0; M6, month 6; M24, month 24.

3. Supplementary data: Factors associated with persistently elevated sCD14 levels at M24

sCD14 > $2.98*10^6$ pg/mL n=34/139 (24%)				
	Univariable		Multivariable	
	OR (95% CI)	p	OR (95% CI)	p
Sex (Male)	2.96 (0.83-10.5)	0.094	2.68 (0.53-13.5)	0.230
Age (per 10 years)	1.42 (0.95-2.13)	0.086	1.43 (0.93-2.23)	0.106
Body mass index > 27	0.35 (0.10-1.27)	0.113	0.28 (0.06-1.38)	0.121
Current smoking	0.88 (0.38-2.01)	0.765		
Hepatitis B coinfection	1 (omitted)			
Prior AIDS event	1.57 (0.54-4.53)	0.402		
CD4 at D0 (log2)	0.77 (0.58-1.02)	0.072	0.77 (0.56-1.07)	0.127
HIV-1 viral load at D0 > 5 log	0.76 (0.33-1.70)	0.509		
Viral blip between M6-M24	0.63 (0.22-1.83)	0.403		
Change in CD4 from D0 (per 50 cells/mm^3)	1.00 (0.88-1.14)	0.942		
CD4/CD8 ratio at M24 > 0.9	0.46 (0.19-1.09)	0.078	0.59 (0.21-1.65)	0.318

B. Comparative impact of different ART components on the evolution of immune activation and inflammation markers

1. Summary of the study

Out of the overall population (139 patients), 78 remained on the same ART regimen over the 2 years allowing to assess the impact of different ART components. The impact of the NRTI backbone and the third agent on changes of biomarkers were analyzed using a factorial design. Results are expressed as the estimated percentage difference between the mean fold changes observed with a given drug, using TDF/FTC and EFV as the reference groups for the comparison. Characteristics of patients were similar to those of the overall population.

This work was published in the BMC Infectious Diseases 2014:

HATTAB S, GUIHOT A, GUIGUET M, FOURATI S, CARCELAIN G, CABY F, MARCELIN A-G, AUTRAN B, COSTAGLIOLA D, KATLAMA C. ***Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study.*** BMC Infectious Diseases 2014 14:122.

During the 2-years study period, changes of marker levels were similar to those observed in the overall population: significant decline of IL-6, IP-10 and MIG levels was observed while sCD14 levels remained stable. Changes of all biomarkers were not significantly different across the NRTI backbones while the choice of the third agent influenced the degree of decline in markers of T-lymphocyte and monocyte activation IP-10 and MIG. A smaller decline of IP-10 and MIG levels was observed with ATV/r than with EFV (IP-10Δ -57%, $p = 0.011$; MIGΔ -136%, $p = 0.007$), while no difference was noted between LPV/r and EFV.

The differential impact of the third agent on changes of immune activation markers suggests that the use of IP-10 and MIG could be useful along with classical criteria (HIV-1 RNA and CD4 cell count) when evaluating new antiretroviral drugs.

2. Published article 2 (next page)

Hattab et al. BMC Infectious Diseases 2014, 14:122
http://www.biomedcentral.com/1471-2334/14/122

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study

Suhaib Hattab[1,2*], Amelie Guihot[3,4,5], Marguerite Guiguet[1,2], Slim Fourati[1,2,6], Guislaine Carcelain[3,4,5], Fabienne Caby[1,7], Anne-Geneviève Marcelin[1,2,6], Brigitte Autran[3,4,5], Dominique Costagliola[1,2] and Christine Katlama[1,2,7]

Abstract

Background: Few studies have compared the impact of different antiretroviral regimens on residual immune activation and inflammation with discordant results. Aim of the study was to investigate the impact of various antiretroviral regimens on markers of immune activation and inflammation during the first two years of effective therapy.

Methods: We studied HIV-infected antiretroviral-naïve patients who began cART with either abacavir/lamivudine or tenofovir/emtricitabine, combined with ritonavir-boosted lopinavir (LPV/r), atazanavir (ATV/r) or efavirenz (EFV). All the patients had a virological response within 6 months, which was maintained for 2 years with no change in their ART regimen. C-reactive protein (hs-CRP), interleukin-6 (IL-6), soluble CD14 (sCD14), monokine induced by interferon-γ (MIG) and interferon-γ-inducible protein-10 (IP-10) were measured in stored plasma obtained at cART initiation and 24 months later. Mean changes from baseline were analyzed on $\log_e$-transformed values and multivariable linear regression models were used to study the effect of the treatment components, after adjusting for factors that might have influenced the choice of ART regimen or biomarker levels. Differences were expressed as the mean fold change percentage difference (Δ).

Results: Seventy-eight patients (91% males) with a median age of 43 years met the inclusion criteria. Their median baseline CD4 cell count was $315/mm^3$ and HIV-1 RNA level 4.6 $\log_{10}$ copies/ml. During the 2-years study period, IL-6, IP-10 and MIG levels fell significantly, while hs-CRP and sCD14 levels remained stable. IP-10 and MIG levels declined significantly less strongly with ATV/r than with EFV (IP-10Δ -57%, $p = 0.011$; MIGΔ -136%, $p = 0.007$), while no difference was noted between LPV/r and EFV. The decline in IL-6 did not differ significantly across the different treatment components.

Conclusions: After the first 2 years of successful cART, IL-6, IP-10 and MIG fell markedly while hs-CRP and sCD14 levels remained stable. The only impact of ART regimen was a smaller fall in markers of immune activation with ATV/r than with EFV. Our results suggest that these markers could be worthwhile when evaluating new antiretroviral drugs.

Keywords: HIV, cART, Immune activation, Inflammation, Markers

* Correspondence: suhaibhattab@yahoo.com
[1]INSERM, UMR_S 1136, Pierre Louis Institute of Epidemiology and Public Health, Paris F-75013, France
[2]Sorbonne Universities, UPMC Univ Paris 06, UMR_S 1136, Pierre Louis Institute of Epidemiology and Public Health, Paris F-75013, France
Full list of author information is available at the end of the article

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Background

Antiretroviral therapy (ART) leads to a dramatic reduction in HIV-related morbidity and mortality [1] and currently suppresses viral replication in the vast majority of compliant patients [2]. Most treatment guidelines recommend early initiation of ART, based mainly on nucleoside reverse transcriptase inhibitors (NRTI) combined with non-nucleoside reverse transcriptase inhibitors (NNRTI) or protease inhibitors (PI) [3,4]. However, despite viral suppression and quantitative immune restoration in most patients, signs of immune activation and inflammation persist [5,6]. This may be due in part to ongoing low-level viral replication [7], the coinfection with other chronic viruses such as cytomegalovirus and Epstein-Barr virus [8,9] and to the consequences of mucosal immune dysfunction that is characterized by a profound depletion of CD4 + T-cells during the early acute infection, and a progressive loss of the ability to maintain the intestinal barrier function, allowing translocation of the intestinal microbial flora into the systemic circulation which induces immune activation and inflammation cascades [10]. An additional factor that may contribute to this persistence is the perturbation of immune regulatory mechanisms, such as regulatory T-cells or the immune regulatory cytokines such as interleukin-10 [11].

By comparison with HIV-uninfected individuals, several studies have shown an excess of comorbidities in HIV-infected patients on virologically effective treatment, including metabolic disorders [12], decreased bone mineral density with an increased risk of fractures [13], an elevated cardiovascular risk [14], and cancers [15]. These comorbidities have been attributed to a higher prevalence of standard risk factors such as smoking in HIV-infected patients, as well as to past or present HIV-induced immune depression reflected by the CD4 cell nadir or the CD4/CD8 cell ratio [16,17], and to the effect of some antiretroviral drugs [12,18,19]. In addition, elevated levels of markers of inflammation, such as interleukin-6 (IL-6) and C-reactive protein (CRP), have been linked to increased morbidity and mortality in both the general and HIV-infected populations [20-22]. The SMART study was the first to demonstrate that mortality in this setting is linked to HIV replication, inflammatory markers such as IL-6 and soluble CD14 (sCD14), and coagulation markers such as D-dimers [23]. Since then, several studies have shown an association between plasma markers of inflammation and the risk of non AIDS-defining events [24,25]. In the ALLRT cohort, elevated levels of IL-6, sCD14, D-dimers and soluble tumor necrosis factor receptors (sTNFR1 and sTNFR2), both prior to and during ART, were associated with the occurrence of non AIDS-related morbidities and death [26].

Now that ART is recommended for all HIV-infected patients, and life expectancy is greatly prolonged by suppressive ART despite the lack of viral eradication, it is important to compare the potential impact of different antiretroviral strategies on residual immune activation and inflammation. There are some arguments to suggest that different drugs from different classes may have different impact on immune activation and inflammation. In the Spiral study, a switch from PI-based therapy to a raltegravir-containing regimen in patients with suppressed viremia led to a decrease in biomarkers associated with inflammation, insulin resistance and hypercoagulability [27].

Few studies have examined the impact of first-line ART on biomarkers of inflammation and immune activation, and they have given discordant results [28-31]. These discordant results might reflect differences in baseline HIV disease status, and/or differences in the virological response, and/or treatment switches.

We therefore explored the impact of different first-line ARV regimens on soluble markers of inflammation and immune activation during the first two years of effective therapy, while controlling for potential confounders such as HIV replication, and changes in the initial regimen.

Methods

Study design

We compared the impact of commonly used first-line antiretroviral drugs on soluble markers of inflammation and immune activation, while controlling for potential confounders. In order to avoid the influence of previous ARV exposure, active viral replication and treatment switches, we restricted our analysis to a homogenous group of treatment-naive HIV-infected patients who had experienced a rapid and persistent virological response and remained on their initial regimen for 2 years. Because the biomarkers of interest were not part of the patients' routine biological monitoring, we restricted our analysis to patients for whom plasma samples stored at ART initiation and 2 years later were available. The analyses were also adjusted for baseline characteristics that might have influenced the choice of cART regimen or affected biomarker levels, such as the age, smoking status, CD4 cell count, prior AIDS-defining events, plasma HIV-1 viral load, and hepatitis virus coinfection.

Study population

All HIV-infected patients receiving care in the Infectious Diseases department of Pitié-Salpêtrière Hospital (Paris, France) have their clinical, biological and therapeutic findings recorded prospectively in standardized electronic medical records (NADIS). Biological data obtained in the hospital, such as HIV RNA levels and immunological parameters, are directly imported from the laboratory computer system, thus minimizing collection bias. The quality

of the database is ensured by automated checks during data capture, and by regular controls and annual assessments. Routine blood tests are performed at each hospital visit, and residual plasma is stored frozen, being identified by a serial number.

All HIV-1-infected patients who started first-line cART between January 2006 and December 2009 were screened for eligibility, using the hospital database. Patients were included in this study if they received either tenofovir-emtricitabine (TDF/FTC) or abacavir-lamivudine (ABC/3TC), combined with efavirenz or with a ritonavir-boosted protease inhibitor (atazanavir (ATV/r) or lopinavir (LPV/r)). In order to control for potential causes of inflammation/activation due to persistent plasma viral replication, we only studied patients who had a rapid and persistent virological response, defined by a plasma HIV-1 viral load (VL) below 400 copies/mL at 6 months and below 50 copies/mL at 24 months, with no values above 1000 copies/ml between month 6 and month 24. Other eligibility criteria included no change in antiretroviral therapy throughout the 24 months of the study, and the availability of frozen plasma samples obtained at baseline (D0) and month 24 (M24). The study was approved by the Pitié-Salpêtrière institutional review board, and the patients were asked to give their written consent to the use of their medical information and plasma samples, as required by French law.

Sample collection and plasma soluble markers measurements

We selected markers of inflammation and immune activation that can be reliably measured in frozen plasma. We evaluated IL-6 and hs-CRP as markers of inflammation, soluble CD14 (sCD14) as a marker of monocyte activation, and interferon-γ-inducible protein 10 (IP-10) and monokine induced by interferon-γ (MIG) as markers of T-lymphocyte and macrophage activation.

Enzyme-linked immunosorbent assays (ELISA) were used according to manufacturer's instructions to quantify IL-6, sCD14 (R&D Quantikine®, HS600B and DC140 respectively) and hs-CRP (Calbiotech®, CR120C). IP-10 and MIG levels were determined on thawed diluted plasma with Cytometric Bead Array kits (BD™ CBA) on a BD FACS Canto I according to manufacturer's instructions. Coefficients of variation were 6.9-7.8% for IL-6, 7.9–8.5% for hs-CRP, 4.8–7.4% for sCD14, 4% for IP-10 and 9–13% for MIG. Plasma samples were allowed to thaw for 50 minutes at room temperature before centrifugation for 5 minutes at 1000 rpm, followed by distribution into Eppendorf tubes in the amounts required for each assay kit. Plasma was diluted as recommended by the kit manufacturers. Standards provided with the kits were measured in duplicate and the mean value was used as reference. Samples with values higher than the highest standard value were further diluted and retested. D0 and M24 samples from each patient were tested in the same run.

Statistical analysis

Two NRTI backbone combinations (TDF/FTC vs ABC/3TC) and three third agents (LPV/r, ATV/r vs EFV) were compared in a factorial design. Baseline characteristics were compared between treatment groups by using the Wilcoxon and chi-squared tests in order to identify variables associated with the choice of treatment. Because the marker values were not normally distributed, they were $\log_e$-transformed for analysis. For each marker, changes between D0 and M24 (mean fold change) were expressed as the geometric mean of their ratio after $\log_e$ transformation. A paired one-sample t test was used to identify significant differences in the overall changes in each marker.

Linear regression models were used to investigate the impact of the different NRTI backbones and the different third agents on the biomarker changes. The results are expressed as the estimated percentage difference between the mean fold changes observed with a given drug, using TDF/FTC and EFV as the reference groups for the comparison. Relationships between baseline covariables and changes in each biomarker were examined in univariable linear regression models. These covariables were sex, age, body mass index, smoking status, hepatitis B or C virus (HBV or HCV) coinfection, prior AIDS-defining events, and the pre-ART CD4 cell count and viral load. Baseline covariables associated with changes in at least one biomarker ($p < 0.10$) and viral blips above 50 copies/ml between M6 and M24 were retained in all multivariable linear regression models in order to control for factors that might have influenced the choice of cART regimen or affected biomarker levels. Age and smoking status were included in the multivariable model since these variables are known to influence marker levels [32,33]. Interaction terms between the NRTI backbone and the third agent were tested for each marker. Sensitivity analyses were conducted, excluding patients with hepatitis virus coinfection. Statistical analyses were run on STATA 12 software, and p values <0.05 were considered statistically significant.

Results

Baseline characteristics

Between January 2006 and December 2009, a total of 539 patients began first antiretroviral therapy and remained under care over two years in our department. Of those, 370 patients had a rapid and persistent virological response over the two years. Of them, 280 patients who began therapy with abacavir/lamivudine or tenofovir/emtricitabine plus efavirenz, atazanavir/r, lopinavir/r or

fosamprenavir/r were eligible for the study. The remaining 90 patients were not considered since they either received no longer recommended antiretroviral therapy such as combivir and invirase (n = 64) or were included in protocols evaluating new drugs such as darunavir and rilpivirine. Frozen plasma samples at baseline and after two years were available for 149 patients. Characteristics of patients who had stored plasma were not different from those did not have it for age, CD4 cell count, HIV-RNA level, AIDS defining events and for prescribed antiretroviral therapy. Two patients who withheld their consent were excluded. Among the remaining 147 patients, 78 remained on the same antiretroviral regimen throughout the 2-year study period and comprised the study population. Modifications in first ART regimen were due to side effects, drug toxicity, or switch to newly available drugs.

The NRTI backbone consisted of TDF/FTC in 61 patients (78%) and ABC/3TC in 17 patients. The third agent was EFV in 36 patients (46%), ATV/r in 27 patients (35%) and LPV/r in 15 patients. At baseline, the median viral load was 4.6 $\log_{10}$ copies/mL, the median CD4 cell count was 315/mm^3 and the median CD4/CD8 cell ratio was 0.30. Seven patients (9%) had an AIDS-defining event. Four patients were HCV-PCR-positive and two were HBsAg-positive.

Baseline characteristics were well balanced across the treatment groups, with the following exceptions. As shown in Table 1, patients prescribed LPV/r had the lowest median CD4 cell count (177 cells/mm^3), a value significantly different from the median count in patients prescribed ATV/r (330 cells/mm^3) or EFV (342 cells/mm^3) (p = 0.020). Patients prescribed TDF/FTC had a lower median CD4 cell count than patients prescribed ABC/3TC (304 versus 431 cells/mm^3; p = 0.008). Four patients prescribed LPV/r had an AIDS-defining event, compared to only one patient prescribed ATV/r and two patients prescribed EFV (p = 0.030). All the patients with hepatitis virus coinfection were prescribed LPV/r (p < 0.001).

Changes in immunovirological status during the first two years of effective cART

As requested, all the patients had plasma HIV viral loads below 400 copies/ml at M6 and below 50 copies/ml at M24. At M6, 72 patients (92%) had viral loads below 50 copies/ml. Between M6 and M24, twelve patients (15%) had isolated viral loads above 50 copies/ml (median 63 copies/ml; range 54 to 210). Among patients receiving TDF/FTC, 10 patients (16%) had isolated plasma viral loads above 50 copies/ml, compared to 2 patients (12%) receiving ABC/3TC (p = 0.64). Among patients receiving EFV, 3 patients (9%) had isolated viral loads above 50 copies/ml, compared to 4 patients (17%) receiving ATV/r and 5 patients (33%) receiving LPV/r (p = 0.08). During the 2-year study period, the CD4 cell count increased by 216/mm^3 and the CD4/CD8 ratio by 0.42. At M24, the median CD4 cell count was 530/mm^3 (IQR 393 to 683) and the median CD4/CD8 ratio was 0.76 (IQR 0.47 to 1.1). No patient developed AIDS defining event during the two years of treatment, while 11 patients experienced a non-AIDS defining event, consisting of pneumonia (n = 5), nephropathy (n = 4), osteoporosis (n = 1) and idiopathic gout (n = 1). None of the 28 smoking individuals quitted smoking while on treatment.

Changes in markers of inflammation and immune activation during the first two years of effective cART

At baseline, the only difference in plasma biomarkers levels across the treatment groups was a higher IL-6 level in patients prescribed LPV/r (median 2.17 pg/ml) than in patients prescribed ATV/r or EFV (median 1.2 and 1.6 pg/mL, respectively; p = 0.040).

Table 1 Baseline characteristics of the patients

	N (%) or median (interquartile range)					
	Total n = 78	TDF/FTC n = 61	ABC/3TC n = 17	EFV n = 36	ATV/r n = 27	LPV/r n = 15
Sex: Male	71 (91%)	56 (91%)	15 (88%)	34 (94%)	22 (81%)	15 (100%)
Age, years	43 (34-47)	44 (35-49)	38 (32-43)	42 (34-46)	44 (35-48)	42 (33-55)
Body mass index	23 (22-24)	23 (22-25)	22 (21-24)	23 (22-25)	22 (21-25)	22 (21-24)
Current smokers	28 (36%)	25 (41%)	3 (18%)	14 (39%)	6 (22%)	8 (53%)
Viral hepatitis coinfection	6 (8%)	5 (8%)	1 (6%)	0	0	6 (40%)
Prior AIDS	7 (9%)	5 (8%)	2 (12%)	2 (6%)	1 (4%)	4 (27%)
CD4/mm^3	315 (217-409)	304 (190-384)	431 (330-512)	342 (262-402)	330 (263-446)	177 (72-299)
CD8/mm^3	959 (660-1345)	949 (656-1335)	1053 (762-1468)	981 (774-1240)	1111 (646-1506)	709 (454-1109)
CD4/CD8 ratio	0.30 (0.19-0.46)	0.28 (0.17-0.41)	0.49 (0.22-0.59)	0.33 (0.19-0.46)	0.29 (0.24-0.49)	0.16 (0.13-0.38)
Viral load ($\log_{10}$ copies/mL)	4.6 (4.1-5.2)	4.7 (4.3-5.2)	4.4 (3.6-4.9)	4.8 (4.3-5.2)	4.4 (4-4.9)	5.1 (4.6-5.3)
Viral load > 5 $\log_{10}$	36%					

TDF, tenofovir; FTC, emtricitabine; ABC, abacavir; 3TC, lamivudine; EFV, efavirenz; ATV/r, atazanavir/ritonavir; LPV/r, lopinavir/ritonavir.

At M24, IL-6, IP-10 and MIG levels were significantly lower than at baseline (-40%, -59% and -74%, respectively), while sCD14 levels were unchanged (Table 2). The hs-CRP level fell by 23% but the change was not statistically significant.

Impact of individual antiretroviral drugs on markers of inflammation and immune activation

Based on univariable linear regression models, between-group comparisons and the literature, the following variables were selected for multivariable analyses: age, smoking status, prior AIDS-defining events, baseline CD4 cell count, baseline viral load, hepatitis B or C virus coinfection, and viral blips above 50 copies/ml between M6 and M24. After adjustment for these variables and for the ART components (the NRTI backbone and the third agent), no significant difference in the decline in the IL-6 level was found between the TDF/FTC and ABC/3TC groups (mean fold change percentage difference Δ 4%; p = 0.90) (Table 3). Compared to EFV, no significant difference in the change of IL-6 was associated with ATV/r or LPV/r use. In contrast, the choice of treatment regimen influenced the decline in IP-10 and MIG levels: the decline in both IP-10 (Δ = -57%, p = 0.011) and MIG (Δ = -136%; p = 0.007) was significantly smaller with ATV/r than with EFV, while no significant difference was found between LPV/r and EFV (IP-10 Δ = -4%; p = 0.87; MIG Δ = -48%; p = 0.44) or between ABC/3TC and TDF/FTC (IP-10 Δ = 30%; p = 0.09; MIG Δ = 24%; p = 0.47).

No interaction was found between the NRTI backbone and the third agent for any of the marker, p-values of the interaction terms were 0.19, 0.26, 0.72, 0.34 and 0.91 for IL-6, hs-CRP, sCD14, IP-10 and MIG respectively. Similar results were obtained in sensitivity analyses that excluded patients with hepatitis virus coinfection.

Discussion and conclusions

This observational study of changes in markers of inflammation and immune activation during the first two years of unchanged virologically effective first-line cART regimens (tenofovir- or abacavir-based NRTI backbone plus efavirenz, atazanavir/r or lopinavir/r) shows that the choice of the third antiretroviral agent influenced the degree of decline in markers of T-lymphocyte and macrophage activation. Plasma levels of IL-6, IP-10 and MIG fell by at least 40% in all the treatment groups, while the decline in hs-CRP levels failed to reach statistical significance and sCD14 levels were unchanged. The only observed difference between the treatment groups was that the T-lymphocyte and macrophage activation markers IP-10 and MIG fell less markedly in patients receiving ATV/r than in patients receiving EFV; no difference was found between LPV/r and EFV. Our patient population represents the naïve patients who initiated cART between January 2006 and December 2009 with a moderate immune deficit and a median viral load slightly below 100000 copies/ml and who experienced a rapid virological response in usual care in France.

Several aspects of study design are important in studying the influence of different antiretroviral drugs on biomarker variations in patients starting first-line therapy. First, it is important to control for viral replication, which is a major cause of persistent immunological activation

Table 2 Changes from M0 to M24 in plasma markers of inflammation and immune activation ($\log_e$-transformed values)

		Total n = 78	TDF/ FTC n = 61	ABC/ 3TC n = 17	EFV n = 36	ATV/r n = 27	LPV/r n = 15
IL-6	Baseline (pg/ml)	1.6 (1.1-2.5)	1.6 (1.1-2.6)	1.2 (0.99-1.7)	1.6 (1.1-2.5)	1.2 (0.88-2.4)	2.17 (1.1-7.4)
	Mean fold change (95% CI)	0.60 (0.49, 0.74)	0.60 (0.48, 0.77)	0.60 (0.36, 0.99)	0.59 (0.42, 0.83)	0.82 (0.64, 1.05)	0.36 (0.20, 0.67)
	P value*	<0.001					
hs-CRP	Baseline (mg/L)	4 (2.2-15)	4.2 (2.3-15)	2.7 (1.8-9.4)	3.9 (2.2-17.8)	3 (2-5.9)	7.5 (2.9-20)
	Mean fold change (95% CI)	0.77 (0.57, 1.1)	0.81 (0.56, 1.20)	0.66 (0.33, 1.30)	0.76 (0.46, 1.26)	0.88 (0.54, 1.44)	0.63 (0.27, 1.47)
	P value	0.11					
sCD14	Baseline (10^6 pg/ml)	2.6 (2.1-3.1)	2.6 (2.2-3.1)	2.5 (2-3.1)	2.5 (2.2-3)	2.6 (1.7-3.1)	3.1 (2-3.4)
	Mean fold change (95% CI)	1.00 (0.93, 1.1)	1.00 (0.93, 1.10)	0.96 (0.79, 1.15)	0.95 (0.85, 1.06)	1.10 (0.93, 1.26)	1.00 (0.85, 1.20)
	P value	0.82					
IP-10	Baseline (pg/ml)	664 (431-960)	630 (435-974)	721 (431-895)	692 (447-996)	549 (389-781)	798 (470-1326)
	Mean fold change (95% CI)	0.41 (0.35, 0.49)	0.44 (0.36, 0.52)	0.37 (0.27, 0.49)	0.34 (0.28, 0.42)	0.53 (0.40, 0.71)	0.43 (0.27, 0.67)
	P value	<0.001					
MIG	Baseline (pg/ml)	1532 (980-2804)	1534 (1004-2804)	1431 (938-2562)	1908 (1093-3799)	1182 (920-2201)	1611 (887-2444)
	Mean fold change (95% CI)	0.26 (0.19, 0.33)	0.25 (0.18, 0.35)	0.27 (0.17, 0.42)	0.16 (0.10, 0.24)	0.39 (0.30, 0.51)	0.39 (0.22, 0.70)
	P value	<0.001					

TDF, Tenofovir; FTC, Emtricitabine; ABC, Abacavir; 3TC, Lamivudine; EFV, Efavirenz; ATV/r, Atazanavir/ritonavir; LPV/r, Lopinavir/ritonavir; IL-6, Interleukin-6; hs-CRP, Highly sensitive C-reactive protein; sCD14, soluble CD14; 1P10, Interferon-γ-Inducible Protein 10; MIG, Monokine induced by interferon-γ; CI, Confidence Interval.
*One-sample Student's t test of the change of each marker in the overall study sample.

Table 3 Regression analyses comparing the impact of antiretroviral therapy components on biomarker changes

		Univariable		Multivariable*	
Marker	**Antiretrovirals**	**Mean fold change percentage difference (95% CI)**	**P value**	**Mean fold change percentage difference (95% CI)**	**P value**
IL-6	TDF/FTC	Ref.			
	ABC/3TC	1 (-67 to 41)	0.97	4 (-80 to 49)	0.90
	EFV	Ref.			
	ATV/r	-39 (-120 to 12)	0.16	-20 (-101 to 28)	0.48
	LPV/r	38 (-8 to 63)	0.09	43 (-23 to 74)	0.15
hs-CRP	TDF/FTC	Ref.			
	ABC/3TC	18 (-77 to 62)	0.60	7 (-155 to 66)	0.88
	EFV	Ref.			
	ATV/r	-16 (-139 to 43)	0.68	-34 (-203 to 41)	0.47
	LPV/r	16 (-97 to 63)	0.67	41 (-29 to 82)	0.39
sCD14	TDF/FTC	Ref.			
	ABC/3TC	7 (-13 to 3)	0.48	14 (-8 to 31)	0.18
	EFV	Ref.			
	ATV/r	-14 (-36 to 4)	0.13	-19 (-43 to 1)	0.06
	LPV/r	-6 (-31 to 14)	0.57	-4 (-36 to 21)	0.77
IP10	TDF/FTC	Ref.			
	ABC/3TC	16 (-22 to 42)	0.36	30 (-6 to 54)	0.09
	EFV	Ref.			
	ATV/r	-55 (-118 to -12)	0.01	-57 (-120 to -11)	0.011
	LPV/r	-26 (-92 to 17)	0.27	-4 (-80 to 39)	0.87
MIG	TDF/FTC	Ref.			
	ABC/3TC	-8 (-103 to 42)	0.80	24 (-62 to 65)	0.47
	EFV	Ref.			
	ATV/r	-146 (-306 to -45)	0.001	-136 (-339 to -27)	0.007
	LPV/r	-151 (-395 to -31)	0.006	-48 (-297 to 45)	0.44

TDF, Tenofovir; FTC, Emtricitabine; ABC, Abacavir; 3TC, lamivudine; EFV, Efavirenz; ATV/r, Atazanavir/ritonavir; LPV/r, Lopinavir/ritonavir; IL6, Interleukin-6; hs-CRP, Highly-sensitive C-reactive protein; sCD14, soluble CD14; IP10, Interferon-γ-inducible protein 10; MIG, Monokine induced by interferon-γ; CI, Confidence Interval.
*Adjusted for age, smoking, prior AIDS, baseline CD4 cell count, baseline HIV viral load, HCV or HBV coinfection, and viral blips.

and inflammation. As a result, the approach used in randomized trials, which includes all patients independently of their virological response, may not be appropriate. Furthermore, removal of patients with uncontrolled viral load from a randomized trial will result in a simple observational study. Despite the lack of randomization in our study, baseline characteristics were well balanced across the treatment groups, and factors that might have influenced the choice of cART regimen or the time course of the markers of interest were systematically included in the analyses. In addition, all the patients under care in the Infectious Diseases department of Pitié-Salpêtrière Hospital were screened for eligibility criteria, thus avoiding any major selection bias. However, one cannot exclude that unrecognized confounding regarding treatment selection could be present. In our study, the strict inclusion criteria regarding the viral replication resulted in a limited sample size. However, even with this sample size, significant differences were detected for some of the comparisons. In addition, most p-values of non-significant tests were above 0.15 indicating that for most comparisons, power was not an issue. It is possible that the freezing of plasma sample could have modified the marker levels and diminished our ability to detect differences. However, all the groups of antiretroviral regimens were studied similarly and this should not have biased the comparison between groups. Moreover, as in most published studies, studying thawed plasma allowed to study all marker dosages simultaneously in the same experiment, limiting the inter-individual variability. The high variability of inflammatory markers between individuals could have precluded seeing differences between treatment groups.

Elevated IL-6 and CRP levels before antiretroviral treatment initiation or after treatment interruption have

been linked to a higher risk of AIDS-defining events and death [23,34]. In our study, IL-6 levels fell during the first two years of effective antiretroviral therapy, and the decline did not differ significantly between the NRTI backbones or across the different third agents. These results are consistent with those of the HEAT and ACTG A5224 trials [28,29]. With regard to hs-CRP, we found that its levels fell slightly although not significantly, with marked inter-individual differences and no differential effect of the studied antiretroviral drugs. In the ACTG A5224, HEAT and NICE trials, CRP levels either rose or fell after cART initiation, while a differential effect of antiretroviral drugs was found in ACTG A5224 trial but not in the HEAT or the NICE trials [28-30]. The high inter-individual variability, differences in study designs, such as discussed above, and in baseline characteristics may explain the discordant results.

Elevated plasma sCD14 levels are an independent predictor of death among HIV-infected patients [35]. SCD14, a marker of monocyte activation that correlates with HIV viremia, and is also considered to be a marker of microbial translocation across the intestinal mucosa, correlating positively with plasma lipopolysaccharide levels as being its soluble receptor [36,37]. Despite 2 years of effective antiretroviral therapy, sCD14 levels did not change significantly, in keeping with previous reports, suggesting that regimens used in our study may not restore the intestinal barrier function, resulting in persistent microbial translocation and immune activation [36,38]. Previous study has reported that among treated HIV-infected patients, persistent HIV-DNA in the gut correlates with levels of microbial translocation and immune activation [39]. Interestingly, Taiwo B et al. have recently reported that an NRTI-sparing regimen consisting of boosted darunavir plus raltegravir led to a decline in sCD14, IL-6 and IP-10 levels [40], suggesting that integrase inhibitors may be more effective in this respect than other antiretroviral drugs.

Persistent immune activation despite virologically effective therapy has been linked to immunological failure [41,42]. While IL-6, hs-CRP, sCD14 and IP-10 have been studied in other studies, MIG has not been evaluated in the context of naïve patients initiating cART. IP-10 (CXCL-10) and MIG (CXCL-9), two chemokines induced by interferon gamma, specifically target lymphocytes, particularly activated T cells, as well as macrophages, and are critical mediators of T cell migration during T-cell-dependent immune responses [43]. High levels of these chemokines reflect immunological activation in HIV-infected patients [44]. A recent study has shown that elevated plasma IP-10 levels in primary HIV-1 infection are strongly predictive of rapid HIV disease progression [45]. In our study, IP-10 and MIG levels showed the largest decline among the studied biomarkers during the first two years of effective cART, running parallel to the fall in plasma viremia. IP-10 and MIG were also the only markers for which a differential effect of the studied antiretroviral drugs was observed. The fall in both markers was larger with EFV than with ATV/r. To investigate whether these differences could be explained by the change in CD4 cell count, analyses were made adjusting for the change of CD4 cell count and differences were still significant and could not be explained by the change in CD4 cell count. We hypothesize that other ART-mediated mechanisms such as the more rapid decay in HIV-RNA in the first 14 days of treatment initiation associated with EFV than with ATV/r observed in a randomized study [46] could explain, at least partly, the observed difference between EFV and ATV/r. However, it is unclear whether this has any clinical implications. To our knowledge, the differential impact of antiretroviral drugs on IP-10 and MIG has not previously been studied in naïve HIV-infected patients.

The differential effect of the studied antiretroviral drugs on changes of two immune activation markers such as IP-10 and MIG levels suggests that these markers could be worthwhile when evaluating new antiretroviral drugs. The presence of sCD14 despite two years of viral suppression reflects the persistence of immune activation, and may contribute to the maintenance of residual viremia and HIV DNA reservoirs [47]. With the growing interest for finding a cure for HIV infection and with the preoccupation of long-term management of HIV-infected individuals, it is key to assess the capacity of new drugs and new combinations of drugs to drive immune activation and inflammation down.

Competing interests

No members of the study team have any financial or personal relationships with people or organizations that could inappropriately influence this work. Dominique Costagliola and Christine Katlama have received at some stage in the past travel grants, consultancy fees, honoraria and study grants from various pharmaceutical companies including Bristol-Myers-Squibb, Gilead Sciences, Janssen-Cilag, Merck-Sharp & Dohme-Chibret and ViiV Healthcare.

Authors' contributions

Conception and design: SH, AG, MG, GC, A-GM, DC, CK. Plasma samples collection: SH, SF, A-GM. Biomarkers measurements: SH, AG, GC, BA. Collection and assembly of data: SH, AG, MG, SF, FC. Statistical analyses: SH, MG, DC. Critical revision of the article for scientific accuracy: all. Final approval of the article: all.

Acknowledgements

We thank Damien Egault and Rachid Agher for the technical assistance.

Author details

[1]INSERM, UMR_S 1136, Pierre Louis Institute of Epidemiology and Public Health, Paris F-75013, France. [2]Sorbonne Universities, UPMC Univ Paris 06, UMR_S 1136, Pierre Louis Institute of Epidemiology and Public Health, Paris F-75013, France. [3]INSERM, UMR_S 1135, CIMI, Paris F-75013, France. [4]Sorbonne Universities, UPMC Univ Paris 06, UMR_S 1135, CIMI, Paris F-75013, France. [5]AP-HP, Hôpital Pitié-Salpêtrière, Département d'immunologie, Paris F-75013, France. [6]AP-HP, Hôpital Pitié-Salpêtrière, Service de virologie, Paris F-75013, France. [7]AP-HP, Hôpital Pitié-Salpêtrière, Service des maladies infectieuses et tropicales, Paris F-75013, France.

Received: 19 November 2013 Accepted: 26 February 2014
Published: 4 March 2014

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doi:10.1186/1471-2334-14-122
Cite this article as: Hattab *et al.*: Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study. *BMC Infectious Diseases* 2014 **14**:122.

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3. Supplementary data

a) Comparison of patients characteristics between those who had stored plasma and those who did not have it

Characteristics of patients who had stored plasma were not different from those did not have it except for the sex distribution. Comparisons are shown in the following table:

	Patients with plasma	Patients without plasma	p-value
Age	41 (34-47)	38 (32-47)	0.13
Sex (male)	120 (81%)	90 (69%)	0.02
Baseline CD4+ cell count	286 (181-373)	275 (165-391)	0.72
Baseline HIV-1 RNA level	4.9 (4.3-5.3)	4.8 (3.8-5.3)	0.16
Prior AIDS events	20 (13%)	17 (13%)	0.78
Prescribed ART, n (%)			
TDF/FTC	120 (81%)	97 (74%)	0.19
ABC/3TC	29 (19%)	34 (26%)	0.19
EFV	60 (40%)	43 (33%)	0.21
ATV/r	38 (26%)	43 (33%)	0.18
LPV/r	39 (26%)	31 (24%)	0.63
FPV/r	12 (8%)	14 (10%)	0.45

b) Interaction terms between the NRTI and the third agent for markers

Marker	p-value of interaction terms
IL-6	0.19
hs-CRP	0.26
IP-10	0.34
MIG	0.91
sCD14	0.72

c) Sensitivity analyses: regression analyses comparing the impact of antiretroviral therapy components on biomarker changes excluding patients with hepatitis C coinfection

		Multivariable analyses*	
Marker	Antiretrovirals	Mean fold change percentage difference (95% CI)	P value
IL-6	TDF/FTC		
	ABC/3TC	4(-80 to 49)	0.895
	EFV		
	ATV/r	-20(-101 to 28)	0.476
	LPV/r	43(-22 to 74)	0.148
hs-CRP	TDF/FTC		
	ABC/3TC	8(-153 to 66)	0.881
	EFV		
	ATV/r	-34(-203 to 41)	0.469
	LPV/r	41(-101 to 82)	0.394
sCD14	TDF/FTC		
	ABC/3TC	14(-7 to 31)	0.184
	EFV		
	ATV/r	-19(-43 to 0)	0.054
	LPV/r	-4(-36 to 21)	0.771
IP10	TDF/FTC		
	ABC/3TC	30(-6 to 55)	0.094
	EFV		
	ATV/r	-57 (-120 to -11)	0.011
	LPV/r	-4 (-80 to 39)	0.874
MIG	TDF/FTC		
	ABC/3TC	24 (-62 to 63)	0.469
	EFV		
	ATV/r	-136 (-339 to -27)	0.007
	LPV/r	-48 (-297 to 45)	0.437

* adjusted for age, smoking, prior AIDS, baseline CD4 cell count, baseline HIV viral load, HBV coinfection, and viral blips.

VIII. DISCUSSION

The study of immune activation and inflammation has emerged as a key element in the research on HIV infection. By comparison with HIV-uninfected individuals, HIV-infected patients have higher levels of immune activation and inflammation even when the viral load is controlled under cART (Reingold J *et al*, 2008; Neuhaus J *et al*, 2010; Alcaide ML *et al*, 2013). Elevated levels of immune activation and inflammation among HIV-infected patients receiving cART have been associated with increased risk of mortality (Kuller LH *et al*, 2008; Tien PC *et al*, 2010; Sandler NG *et al*, 2011), non-AIDS events (Duprez DA *et al*, 2012; Borges AH *et al*, 2013) as well as with an insufficient restoration of CD4+ T-cell count under therapy (Lederman MM *et al*, 2011; Zhang X *et al*, 2013).

Immune activation and inflammation can be evaluated either by the quantification of cellular markers expressed on the cell membrane of activated cells such as the expression of HLA-DR and CD38 in the case of T-lymphocytes, or by the measurement of soluble markers which are produced by these activated cells and are detectable in plasma. Reliable measurement of cellular activation on frozen samples requires special expertise in cell preparation and storage. Otherwise, cellular markers should be measured on fresh blood to avoid the loss of activated cells upon freezing (Reimann KA *et al*, 2000).

Soluble markers have the advantage that their measurement is reliable using frozen plasma, which does not require special expert in storage. In addition, the use of soluble markers in longitudinal studies might help evade the variability of used techniques as well as the inter-individual variability over time through the simultaneous measurement of markers on plasma obtained at different time points. Furthermore, the predictive value of several soluble markers in relation with clinical outcomes in HIV-infected patients has been reported in several studies. The ALLRT study which included patients who initiated cART and experienced early and sustained virological control, showed significant associations between non-AIDS-defining events (stroke, myocardial infarction, malignancy, serious non-AIDS-defining bacterial infections) and higher levels of soluble markers of inflammation and coagulation (IL-6, sTNFR-I, sTNFR-II, and D-dimer) in specimens obtained at baseline, at year 1 post-cART and at the pre-event time points. In this study, cellular

markers of T-cell activation were not significantly related to clinical non-AIDS defining outcomes (Tenorio AR *et al*, 2014).

The evaluation of the impact of antiretroviral therapy on changes of immune activation and inflammation markers is valuable as it helps identify the factors associated with the persistence of markers post-therapy and allows the development of interventions that target these factors. The objective of the first part of my PhD work was to assess immune activation and inflammation marker levels in HIV-infected patients before ART initiation and to identify factors associated with high levels of these markers. Then, to evaluate changes of markers over two years of virologically cART and to assess factors associated with the persistence of elevated levels.

To evaluate appropriately the impact of antiretroviral therapy on changes of immune activation and inflammation markers, certain methodological aspects were considered. The first concern is; when and in which population? As the control of viremia and the restoration of immunity occur in the first periods that follow the initiation of antiretroviral therapy (Lok JJ *et al*, 2010), then, the selection of naïve patients initiating therapy will help evaluate the neat impact of a given therapy on changes of markers. In addition, we selected patients who experienced rapid and persistent virological control in order to control the impact of residual viral replication, the main driver of immune activation and inflammation (Cohen Stuart JW *et al*, 2000), on changes of markers. This why the intent-to-treat approach used in the randomized trials may not be appropriate as it includes all participants independently of their virological response.

The second concern is the choice of valuable markers that have predictive value in HIV-infected patients receiving antiretroviral therapy as well as the use of appropriate ways to measure these markers. We chose to evaluate IL-6, Monokine induced by interferon-γ (MIG), interferon-γ inducible protein 10 (IP-10) and soluble CD14 (sCD14). Using Enzyme-Linked ImmunoSorbent Assay (ELISA), we quantified IL-6 and sCD14 while IP-10 and MIG levels were determined with Cytometric Bead Array kits. These techniques were chosen taking into account their sensitivity in measuring these markers (the ability to detect the marker at lower threshold).

IL-6 is a pro-inflammatory cytokine which is secreted by different cell types and is responsible for the acute phase response in the liver (Rose-john S *et al*, 2006). Elevated levels of IL-6, along with D-dimer, were associated with increased risk of mortality and cardiovascular disease in the SMART study (Kuller LH *et al*, 2008; Duprez DA *et al*, 2012). The association between elevated IL-6 levels and increased cardiovascular disease risk was also reported in HIV-uninfected individuals (Danesh J *et al*, 2008).

IP-10 and MIG are two small cytokines secreted by several cell types including T-lymphocytes, monocytes and macrophages. They belong to the CXC chemokine family that is also known as monokine induced by interferon gamma (O'Donovan N *et al*, 1999). They are critical mediators of T cell migration during T cell-dependent immune responses and elicit their chemotactic functions by interacting with the chemokine receptor CXCR3 (Piali L *et al*, 1998). Patients who had elevated plasma IP-10 levels during the primary phase of HIV-1 infection experienced earlier decline in the CD4+ cell count before the initiation of antiretroviral therapy compared to patients who had lower levels (Liovat A-S *et al*, 2012). In a case-control study, higher levels of IP-10, in addition to other markers were found in HIV-infected patients who developed AIDS-related non-Hodgkin lymphoma from the MACS cohort in the years preceding the diagnosis, compared to their controls (Vendrame E *et al*, 2014).

Soluble CD14 is one of two forms of the cluster differentiation (CD14), a myeloid differentiation antigen expressed primarily on monocytes and macrophages. sCD14 was found as an independent predictor of mortality in HIV-infected patients in the SMART study (Sandler NG *et al*, 2011). In a recent study, sCD14 was found to be independently associated with coronary artery calcification after adjustment for traditional risk factors (Longenecker CT *et al*, 2014). Besides being a marker of monocyte activation, sCD14 binds to bacterial lipopolysaccharide (LPS) in plasma and it is sometimes referred to as a marker of bacterial translocation across the intestinal mucosa (Cassol E *et al*, 2010).

Marker levels at the time of cART initiation

This study showed that levels of immune activation and inflammation markers were higher in viremic HIV-infected patients than in HIV-uninfected controls. Higher levels of IL-6 and sCD14 were found among patients who had experienced AIDS-defining events. With regard to IP-10 and MIG, higher levels of IP-10 were associated with a low CD4/CD8 ratio and with high viral load, while higher levels of MIG were associated with high viral load. The correlation between HIV viremia and elevated levels of inflammation markers was reported in patients from the SMART study who were matched for age with participants from the Multi-Ethnic Study of Atherosclerosis (MESA) and participants in the Coronary Artery Development in Young Adults (CARDIA). HsCRP and IL-6 levels were higher among HIV-infected participants than among CARDIA and MESA participants. In HIV-infected participants receiving ART and had controlled HIV RNA levels, marker levels decreased but remained higher than those in the general population (Neuhaus J *et al*, 2010). Another study compared immune activation markers between viremic HIV-infected patients from South-Africa with HIV-uninfected controls and found that IP-10, MCP-1 and sCD14 levels correlate positively with HIV-1 viremia (Cassol E *et al*, 2010). Simmons RP *et al* have reported that HIV-1 infection induces strong production of IP-10 through TLR7/9-dependent pathways (Simmons *RP*, 2013).

The association between high levels of sCD14 and IL-6 with AIDS events might reflect the role of inflammation in the pathogenesis of HIV infection. Previous work has shown that elevated levels of Inflammation (CRP, IL-6), coagulation (D-dimer), and tissue fibrosis (hyaluronic acid) measured in viremic patients before the initiation of ART were associated with higher risk of subsequent AIDS-defining events and deaths in a case-control study where cases and controls were matched on baseline CD4 cell count, hepatitis status, and randomization date (Boulware DR *et al*, 2011). The fact that HIV-replication induces large increases in IFN-γ (Stacey AR *et al*, 2009) explains the association between high viral load and higher levels of IP-10 and MIG, chemokines induced by IFN-γ.

Changes of markers over two years of effective cART

During the first two years of virologically effective cART, IL-6, IP-10 and MIG declined significantly to values that were not significantly different from those of healthy controls. In contrast, sCD14 levels did not decrease and remained higher than levels observed in the healthy controls. Similar decreases in IL-6 and IP-10, but not in sCD14, levels have been recently reported in a study conducted in the ALLRT cohort, in which HIV viremia was constantly controlled (Tenorio AR *et al*, 2014). While IL-6 level remained elevated in only 3% of patients, IP-10 and MIG levels remained elevated in one fifth of patients post-cART. The discrepancy in the proportion of patients with elevated IL-6 level in one hand, and the proportion of patients with elevated IP-10 and MIG in the other hand could be explained in different ways. First, the majority of the patients had IL-6 levels within the normal range at treatment initiation, even if values were in the upper range of normal values. Second, the variability in IL-6 levels in the control group was more important than the variability of IP-10 and MIG levels, as illustrated by the higher coefficient of variation. Furthermore, it is possible that immune activation markers need longer time to subside to normal levels than inflammation markers. Even if we observed that the level of IL-6 returned to normal level in most patients, it does not contradict the fact that high levels of IL-6 are associated with increased risk of cardiovascular disease in HIV-infected patients (Duprez DA *et al*, 2012) as well as in the general population (Danesh J *et al*, 2008).

There are several possible explanations for the stably high sCD14 levels despite two years of virologically effective cART. First, factors other than HIV viremia may be responsible for persistent sCD14 elevation. In particular, two years of suppressive cART may not be sufficient to restore the intestinal barrier function and attenuate monocytes activation allowing bacterial translocation to persist (Cassol E *et al*, 2010). Second, the cART regimens used in our study may not be effective to attenuate monocytes activation. This hypothesis is supported by the finding that raltegravir-containing regimens have been shown to reduce sCD14 levels (Taiwo B *et al*, 2013; Pallikkuth S *et al*, 2013). In our study, we were not able to test this hypothesis as the number of patients who changed their initial regimen to raltegravir containing regimens was very small precluding any specific investigation.

The persistence of high levels of immune activation and inflammation markers despite 2 years of virologically effective cART suggest that rapid and durable control of HIV viremia is useful but might not be sufficient to drive immune activation to normal levels. Thus, other interventions might be needed. Whether the early initiation of cART at higher CD4+ cell count, as now recommended by many recent guidelines, could normalize immune activation levels remains to be assessed. In addition, the impact of new antiretroviral drugs on immune activation and inflammation markers is a clinically relevant question that merits investigation. With regard to sCD14, the beneficial role of prebiotic oligosaccharide, which was shown to improve the microbiota composition substantially and reduced plasma sCD14 levels when administered in untreated HIV-infected patients, might be assessed in cART-suppressed HIV-infected patients (Gori A *et al*, 2011).

Factors associated with elevated marker levels after two years of effective cART

The small proportion of patients who had elevated IL-6 levels post-cART was small precluding the assessment of factors associated with its persistence. However, in the study of Bastard JP *et al (2012),* which evaluated levels of inflammatory markers among patients receiving cART with viral loads ranging between 1-500 copies/ml, IL-6 values correlated positively with HIV viral load and the viral load threshold value for significantly increased IL-6 was 31 copies/mL suggesting that high circulating interleukin-6 levels under cART correlates with residual HIV viremia (Bastard JP *et al,* 2012).

Interestingly, levels of IP-10 and MIG were not related to age before cART initiation, but once HIV viremia was controlled, older patients were more likely to retain elevated levels of these markers after treatment. Each 10-year age increment was associated with at least 60% increase in the risk of retaining elevated levels of markers of immune activation post-cART. These results are compatible with the proposed link between immune activation and premature aging (Appay V *et al*, 2011) and may partly explain the slower CD4 cell recovery in older patients observed in a previous work of our team (Grabar S *et al*, 2004). In the same way, in a study which evaluated levels of immune activation markers, microbial translocation and biomarkers of cardiovascular disease (sCD25, sCD14, sCD163, LPS, sVCAM-1,

sICAM-1 and IP-10) in post-menopausal HIV-infected women receiving antiretroviral treatment with documented viral suppression, levels of all markers were significantly elevated in HIV-infected women suggesting that HIV-infected antiretroviral-treated aging women who achieved viral suppression are in a generalized status of immune activation (Alcaide ML *et al*, 2013).

If age was the only variable associated with elevated immune activation levels post effective cART, it is possible that the small number of patients with elevated immune activation levels could have precluded the detection of such association with other variables such as the CD4+ cell count and viral blips. In the literature, it is documented that higher levels of immune activation and inflammation are associated with lesser restoration of the CD4+ cell count in patients receiving effective cART for long periods of time (Lederman MM *et al*, 2011; Zhang X *et al*, 2013). Thus, the increase of CD4+ cell count might be a protective factor against the persistence of immune activation.

Transient low-level viremia (blips) are common in the usual care of HIV infection and the magnitude of virologic blips is associated with a higher risk for virologic rebound (Grennan JT *et al*, 2012). This study included patients who experienced isolated viral blips, in order to investigate the possible impact of blips on residual immune activation and inflammation. Even if such association was not observed in this study, Taiwo B *et al* have evaluated the association between transient low-level viremia of 50-400 HIV RNA copies/mL and immune activation levels. They showed an association between T cell activation and the risk of subsequent viral blips, but no causal relationship was established (Taiwo B *et al*, 2013).

In order to control the impact of other coinfections on changes of markers, hepatitis C virus (HCV) coinfection was considered as non-inclusion criteria. The association between HCV coinfection and the persistence of immune activation was assessed in a study that compared immune activation levels among HIV/HCV coinfected patients with chronic hepatitis C with their levels in HIV monoinfected patients or HIV/HCV seropositive patients with cleared HCV. In this study where patients were matched for age and sex, immune activation was significantly increased in HIV/HCV coinfected patients compared to patients with HIV monoinfection or those HIV/HCV coinfected with cleared HCV. Their results suggest that immune

activation in HIV/HCV coinfection with well-controlled HIV may arise from chronic HCV viremia (Hodowanec AC *et al*, 2013). With regard to hepatitis B virus (HBV) coinfection, all patients in our study have controlled viral load under cART and no association was found between HBV and the persistence of immune activation. Due to the fact that the sample was selected retrospectively, other co-infections such as cytomegalovirus and sexually transmitted diseases, non-infectious conditions such as drug abuse and other non-infectious inflammatory conditions that might influence biomarker levels were not assessed, as these variables were not available for all patients. In the ANRS CO3 Aquitaine Cohort, different CMV-induced immune responses were associated with chronic immune activation in patients receiving cART with long-term virological suppression independently of age, CD4+ T-cell count, 16S ribosomal DNA load, and regulatory T-cell count (Wittkop L *et al*, 2013). In this way, another study reported that the use of valganciclovir among ART-treated HIV-infected and CMV-seropositive individuals decreased significantly immune activation markers (Hunt PW *et al*, 2011).

The impact of different cART components on changes of immune activation and inflammation markers

The evaluation of the impact of different antiretroviral regimens on changes of immune activation and inflammation markers is valuable as this allows favoring the use of regimens that have favorable impact and the avoidance of those which have the potential to increase these markers. In the second part of my work, I compared the impact of different antiretroviral drugs on markers of immune activation and inflammation during the first two years of virologically effective cART. This analysis was performed in a sub-group of patients who kept the same regimen over the study period to control the impact of treatment switching. Changes of all markers were not different between the two NRTI backbones (TDF/FTC and ABC/3TC) while the choice of the third drug influenced changes of immune activation markers IP-10 and MIG. The decline of IP-10 and MIG was smaller among patients received ATV/r compared to patients received EFV while no difference was observed between patients received LPV/r compared to patients received EFV. In addition, the overall changes of all markers (IL-6, IP-10, MIG and sCD14) were similar to changes observed in the first part of the study. Beside these markers, hs-CRP was evaluated; its decline was slight and did not reach statistical significance.

To date, few studies have compared the impact of different antiretroviaral drugs on changes of immune activation and inflammation markers in cART-naïve patients. Of these, the ACTG A5224 study was the largest trial assessing the dynamics of different inflammatory markers after 96 weeks of cART. In this study, levels of all markers decreased with no differential impact of the ART components while hs-CRP remained stable in the TDF/FTC arm and increased in the ABC/3TC arm (McComsey GA *et al*, 2012). By contrast, hs-CRP decreased with both TDF/FTC and ABC/3TC along with other markers in the HEAT study, which evaluated the efficacy, safety and tolerability of these combinations combined to boosted lopinavir (Smith KY *et al*, 2009). As I mentioned in the first part of the discussion, the intent-to-treat approach used in these randomized trials may not be appropriate as the inclusion of all participants independently of their virological response troubles the interpretation of the obtained results. My work might be the first to compare the

impact of different antiretroviral drugs in patients with well-controlled viral load in a real-life population.

Beside the strengths, potential limitations regarding the observational design of this comparative study and the lack of randomization could arise. However, the removal of patients with uncontrolled viral load from a randomized trial will result in a simple observational study. Regarding the lack of randomization, baseline characteristics were well balanced across the treatment groups and factors that might have influenced the choice of cART regimen or the time course of the markers of interest as well as factors known to influence inflammation markers such as the age and the smoking were systematically included in the analyses. In addition, all the patients under care in our department were screened for eligibility criteria, thus avoiding any major selection bias. However, one cannot exclude that unrecognized confounding regarding treatment selection could be present.

The second potential limitation is the relatively small sample size. In our study, the strict inclusion criteria regarding the viral replication and the stability of the initial cART regimen resulted in a limited sample size. However, even with this sample size, significant differences were detected for some of the comparisons. In addition, most p-values of non-significant tests were well above 0.15 indicating that for most comparisons, power was not an issue.

The decline of immune activation markers IP-10 and MIG was smaller among patients received ATV/r compared to patients received EFV. To investigate whether these differences could be explained by the change in CD4 cell count under cART, analyses were made adjusting for the change of CD4 cell count and differences were still significant and could not be explained by the change in CD4 cell count. We hypothesize that other ART-mediated mechanisms could explain the observed difference between EFV and ATV/r. This hypothesis is supported by the finding of a more rapid decay in HIV-RNA in the first 14 days of treatment initiation associated with EFV than with ATV/r in a randomized study. This observation could explain, at least partly, the observed difference between EFV and ATV/r (Edén A *et al*, 2010).

The differential impact of the two recommended NRTI backbones, which are widely used in routine care (TDF/FTC and ABC/3TC), was previously evaluated in

switch studies. In the BICOMBO and the STEAL studies, virologically suppressed patients were randomized to switch their NRTI backbone to either TDF/FTC or ABC/3TC. At 48 weeks, there were no significant differences in the mean change of markers of inflammation, coagulation, endothelial function or renal function (Martínez E *et al*, 2010; Martin A *et al*, 2010). With regard to the third agent, a switch from protease-based tri-therapy to a raltegravir- containing tri-therapy in patients with suppressed viremia led to a decrease in biomarkers associated with inflammation, insulin resistance and hyper-coagulability in the SPIRAL study (Martínez E *et al*, 2012). Similarly, a switch from Efavirenz-based tri-therapy to a raltegravir-containing tri-therapy was associated with a significant decrease in markers of inflammation, monocyte activation, bone mineral metabolism and renal function (Gupta SK *et al*, 2013). These results are in favor of a differential impact of the different molecules combined as a third agent on markers of inflammation and activation but not for the two NRTI backbones (TDF/FTC and ABC/3TC).

The stability of hs-CRP levels despite two years of effective cART and despite the profound decline of IL-6, one of the cytokines that triggers its synthesis by the liver, might be explained in two ways. First, the induction of CRP synthesis through other pro-inflammatory cytokines such as IL-1 might be partially responsible for the stability of CRP (Pepys MB *et al*, 2003). Second, the high inter-individual variability of this marker observed in this study. This finding might be clinically important given the association between elevated levels of CRP and mortality in HIV-infected patients observed in the Women's Interagency HIV Study (WIHS) and the Fat Redistribution and Metabolic change in HIV (FRAM) (Feldman JG *et al*, 2003; Tien PC *et al*, 2010). In the JUPITER study, rosuvastatin use was associated with a decrease in CRP levels in HIV-uninfected individuals leading to decreases in cardiovascular disease risk (Ridker PM *et al*, 2010). In line with these studies, a French study (CESAR trial) is now evaluating the effect of rosuvastatin in patients receiving suppressive cART with no indication for a statin treatment on markers of immune activation and inflammation. If the findings of this trial are encouraging, then, the use of rosuvastatin might be considered among patients with high CRP under effective cART.

IX. CONCLUSIONS AND PERSPECTIVES

This study of the impact of cART on changes of immune activation and inflammation markers showed that:

- Among untreated HIV-infected patients, markers of immune activation and inflammation (IL-6, IP-10, MIG and sCD14) levels were higher than those observed among the HIV-seronegative controls.
- After two years of virologically effective cART, IL-6, IP-10 and MIG levels declined significantly from baseline to levels that were not different from controls while no change of sCD14 levels was observed. Hs-CRP declined slightly but the decrease did not reach statistical significance.
- Age was the only factor associated with elevated levels of the immune activation markers IP-10 and MIG after two years of virologically effective cART.
- Changes of all markers were not different between the two NRTI backbones (TDF/FTC and ABC/3TC) while the choice of the third drug influenced changes of immune activation markers; the decline of IP-10 and MIG was smaller with ATV/r than with EFV.

The persistence of immune activation and inflammation markers despite two years of virologically effective cART suggest that early and persistent virological control is useful but might not be sufficient to drive immune activation to normal levels. As the age of individuals at HIV diagnosis is increasing over time, the finding that older patients are more likely to keep elevated immune activation levels, while being at higher risk of morbidities, might be clinically relevant. Beside effective cART, older patients should be prioritized when considering future interventions to attenuate immune activation.

The differential impact of the third agent on changes of immune activation markers suggests that the use of IP-10 and MIG could be useful along with classical criteria (HIV-1 RNA and CD4 cell count) when evaluating new antiretroviral drugs.

Several questions remain, after this work:

- First, if two years of virologically effective cART are not sufficient to drive immune activation to normal levels, it would be important to evaluate this impact after longer period of virological suppression (e.g. 5 years).
- Second, as the study population we evaluated was composed of naïve patients who initiated cART with moderate immunodeficiency, it remains to be assessed whether the initiation of cART at higher CD4+ cell count (>500/mm^3), as now recommended by recent guidelines, could drive immune activation to normal levels more quickly.
- Third, the impact of new antiretroviral drugs on immune activation and inflammation markers should be evaluated. Of these, *Dolutegravir* which has shown superior virological efficacy to efavirenz in the SINGLE study merits investigation.
- Fourth, it would be interesting to evaluate the impact of virologically effective cART among patients who achieve normal CD4+ cell count (>500/mm^3), as the mortality rates in this population are no longer different from general population in several studies (Costagliola D, 2014).

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XI. ANNEXES

A. Information note on the study

INFLAVIR

Impact de différentes stratégies de traitements antirétroviraux sur les marqueurs d'inflammation et/ou d'activation plasmatiques chez les patients initiant un traitement antirétroviral (INFLAVIR)

NOTE D'INFORMATION AUX PATIENTS

Merci de lire attentivement ce document d'information

Madame, Monsieur,

Au cours de l'infection par le VIH, il existe une très forte stimulation du système immunitaire qui provoque un état d'inflammation chronique et persistent. L'activation du système immunitaire et l'inflammation pourrait jouer un rôle dans la pathogenèse des comorbidités qui surviennent même sous traitement efficace et à faible charge virale. Plusieurs facteurs pourraient expliquer cet état d'inflammation chronique et persistent (persistance de la réplication VIH, existence de réservoirs, coinfections). A ce jour la, le rôle propre de traitement antirétroviral n'est pas connu. C'est pourquoi nous réalisons l'étude "INFLAVIR" dont le nom complet est " **Impact de différentes stratégies de traitements antirétroviraux sur les marqueurs d'inflammation et/ou d'activation plasmatiques chez les patients initiant un traitement antirétroviral (INFLAVIR)**". Le but de cette recherche est d'évaluer l'impact de la stratégie antirétrovirale sur l'évolution des marqueurs de l'inflammation au cours des deux premières années de traitement et en présence d'une réponse virologique complète, et d'identifier des caractéristiques cliniques qui pourraient modifier cette évolution.

Dans le cadre de cette étude, une analyse de marqueurs d'inflammation et/ou d'activation sera effectuée en utilisant le sang qu'on vous a prélevé avant le début du traitement antirétroviral puis au cours de votre suivi (prélèvements faits à 1 et 2 ans après l'initiation du traitement). La participation à cette étude n'a pas de contraintes pour vous. Votre participation à cette étude ne nécessite aucune modification de votre prise en charge, ni aucun autre prélèvement de sang.

Compte tenu de la nécessité de la recherche, les données recueillies vous concernant feront l'objet d'un traitement informatisé à l'INSERM unité 943 à l'hôpital de la Pitié Salpêtrière, à Paris. Le secret médical sera respecté et toutes les données vous concernant resteront confidentielles.

En accord avec la loi "Informatique et Libertés" du 6 janvier 1978 modifié par la loi 2004-801 du 6 août 2004, vous avez, à tout moment, auprès de votre médecin, un droit d'accès et de rectification des données enregistrées. Par l'intermédiaire du COREVIH auquel est rattaché le Service Clinique dans lequel vous consultez, vous serez informé des résultats de l'étude dès que ceux-ci seront disponibles.

Vous pouvez librement et à tout moment, vous opposez au recueil et à l'utilisation des données vous concernant en vous adressant à votre médecin. Votre décision sera sans conséquence sur votre prise en charge médicale.

Le Comité de Protection des Personnes (CPP) a autorisé cette recherche et la Commission Nationale Informatique et Libertés (CNIL) en a été informée.

Après avoir lu cette note d'information, n'hésitez pas à poser toutes les questions que vous désirez à votre médecin.

Cette note d'information doit être conservée par le patient.

B. Consent for participating in the study

Impact de différentes stratégies de traitements antirétroviraux sur les marqueurs d'inflammation et/ou d'activation plasmatiques chez les patients initiant un traitement antirétroviral (INFLAVIR)

FORMULAIRE DE CONSENTEMENT ECLAIRE

Je soussigne(e) (nom, prénom)………………………………………………………

Ne(e) le………………………………………………………………………………

Certifie,

- Avoir reçu la note d'information concernant l'étude (INFLAVIR)
- Avoir la possibilité de poser toutes les questions que je souhaite au Dr (nom, prénom)……………… …………, comprendre la nature et les objectifs de cette étude.
- Avoir eu un délai de réflexion

J'ai compris que je suis libre d'interrompre ma participation à cette étude à tout moment sans motiver ma décision et sans qu'elle n'entraine de conséquences dans la qualité de ma prise en charge.

J'ai pris connaissance que l'analyse de marqueurs sera effectuée en utilisant le sang prélevé dans le cadre de ma prise en charge et que la participation à cette étude ne nécessite aucune modification de la prise en charge, ni aucun autre prélèvement de sang.

J'accepte que mes données cliniques enregistrées dans mon dossier clinique soient utilisées dans cette étude.

J'ai compris que les données collectées à l'occasion de la recherche seront protégées dans le respect de la confidentialité. Elles pourront être consultées uniquement par les personnes soumises au secret professionnel appartenant à l'équipe du médecin investigateur.

J'accepte le traitement informatisé des données à caractère personnel me concernant dans les conditions prévues par la loi informatique et liberté. J'ai été informé de mon droit d'accès et de rectification des données me concernant.

J'ai été informé que le CPP Ile de France IV a donné un avis favorable pour la réalisation de cette recherche le 16 novembre 2011.

Par l'intermédiaire du COREVIH Ile de France Centre, je pourrai être informé des résultats de l'étude dès que ceux-ci seront disponibles.

J'accepte librement de participer à l'étude (INFLAVIR) dans les conditions précisées dans la note d'information.

Le |__|__|/|__|__|/|__|__|__|__|

C. Paper form for clinical data collection

INFLAVIR

INCLUSION INFLAVIR ❒**Oui** ❒**Non**

Si inclusion : numéro patient |__|__|__|

Numéro dossier/virologie : |__|__|__|__|__|__|__| Initiales Nom/Prénom |__|__|

Date de prescription du traitement : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Lieu d'initiation du traitement : SMIT Pitié ❒ Autre ❒…………………………

1er traitement ARV…………………… Groupe…1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Etat à l'initiation du traitement

VIH-1 : Oui ❒ Non ❒

VIH-2 : Oui ❒ Non ❒

Naif : Oui ❒ Non ❒

Si non ARV pour grossesse Oui ❒ Non ❒

derniere date de traitement |__|__|/|__|__|/|__|__|__|__|
jour mois année

dernier traitement ………………………………………

Evénement clinique dans les 3 derniers mois Oui ❒ Non ❒

Si oui : Infection opportuniste Oui ❒ Non ❒ ………………………………….

Cancer Oui ❒ Non ❒…………………………………

Autre Oui ❒ Non ❒…………………………………

Date |__|__|/|__|__|/|__|__|__|__|
jour mois année

Corticostéroïdes depuis au moins 3 mois Oui ❒ Non ❒

Chimiothérapie Oui ❒ Non ❒

Charge virale VIH

Critère de CV<50 à 2 ans Oui ❒ Non ❒

Pas de échec viral âpres être contrôlé Oui ❒ Non ❒

Durée du traitement antirétroviral

Traitement en cours à M24 Oui ❒ Non ❒

Arrêt temporaire entre J0 et M24 : Oui ❒ Non ❒

Si oui : arrêt de moins d'un mois ❒ arrêt de plus d'un mois ❒

J0 : Date de prescription du traitement : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Ou Date de début du traitement si différent :|__|__|/|__|__|/|__|__|__|__|
jour mois année

Médecin consultant : ……………………………………….

Date de dernière visite : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Date de prochaine visite programmée : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Caractéristiques du patient

Date de naissance : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Sexe : ❒ Homme ❒ Femme ❒ Transgenre

Taille(m) : |__|.|__|__| Poids (kg) à J0 :|__|__|__| ou BMI : |__|__|. |__|

Groupe d'exposition au VIH :
❒ Homo Bisexuel ❒ Toxicomanie IV ❒ Hétérosexuel ❒ Inconnu
❒ Transfusion sanguine ❒ Materno-fœtale ❒ Autre Préciser……

Tabac : ❒ actuel ❒ arrêt ❒ non-fumeur ❒ Ne sait pas

Toxicomanie actuelle/cocaine : ❒ Oui ❒ Non ❒ Ne sait pas

Infection VIH

Date de 1ère sérologie VIH1 positive : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Génotype de résistance : Fait ❒ Non fait ❒

Si génotype fait : Date |__|__|/|__|__|/|__|__|__|__|
jour mois année

Résultat …………………………………………………..

Stade CDC à l'initiation du traitement antirétroviral ❒ A ❒ B ❒ C
Evenement classant ❒ Oui ❒ Non ………………………………………………

2

Charge virale VIH, CD4+, CD8+ entre initiation du traitement (J0) et M24			
Delai J0	**JO***		
Date	__ __/__ __/__ __	__ __/__ __/__ __	__ __/__ __/__ __
CV copies/ml	__ __ __ __ __ __ __ __	__ __ __ __ __ __ __ __	__ __ __ __ __ __ __ __
CD4	__ __ __ __ __	__ __ __ __ __	__ __ __ __ __
CD4%	__ __ %	__ __ %	__ __ %
CD8	__ __ __ __ __ __	__ __ __ __ __ __	__ __ __ __ __ __
CD8%	__ __ %	__ __ %	__ __ %
CD4/CD8	__ __, __ __	__ __, __ __	__ __, __ __
Tubes conservés	Oui ☐ Non ☐	Oui ☐ Non ☐	Oui ☐ Non ☐

*** Valeur préthérapeutique la plus proche de JO**

Delai J0			
Date	__ __/__ __/__ __	__ __/__ __/__ __	__ __/__ __/__ __
CV copies/ml	__ __ __ __ __ __ __ __	__ __ __ __ __ __ __ __	__ __ __ __ __ __ __ __
CD4	__ __ __ __ __	__ __ __ __ __	__ __ __ __ __
CD4%	__ __ %	__ __ %	__ __ %
CD8	__ __ __ __ __ __	__ __ __ __ __ __	__ __ __ __ __ __
CD8%	__ __ %	__ __ %	__ __ %
CD4/CD8	__ __, __ __	__ __, __ __	__ __, __ __
Tubes conservés	Oui ☐ Non ☐	Oui ☐ Non ☐	Oui ☐ Non ☐

Les co infections par les virus hépatites

Sérologie VHB :

Antigène HBs	Positif ❐	Négatif ❐	Non Fait ❐
Anticorps anti-HBs	Positif ❐	Négatif ❐	Non Fait ❐
Anticorps anti-HBc	Positif ❐	Négatif ❐	Non Fait ❐
Antigène HBe	Positif ❐	Négatif ❐	Non Fait ❐
Anticorps anti-HBe	Positif ❐	Négatif ❐	Non Fait ❐

Statut VHB: Non-infecté ❐ Hépatite chronique ❐ Hépatite aigue ❐ Hépatite ancienne

Date de sérologie : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Sérologie VHC :

Anticorps anti-VHC Positif ❐ Négatif ❐ Non Fait ❐

Date de sérologie : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Charge virale ARN-VHC Positif ❐ Négatif ❐ Non Fait ❐

Si charge virale VHC positive : valeur |__|__|__|__|__|__|__| copies/ml

Evénements cliniques entre J0 et M24

Evénement clinique	date (jj/mm/aa)
	|__|__|/|__|__|/|__|__|
	|__|__|/|__|__|/|__|__|
	|__|__|/|__|__|/|__|__|
	|__|__|/|__|__|/|__|__|
	|__|__|/|__|__|/|__|__|
	|__|__|/|__|__|/|__|__|

Autre traitement entre J0 et M24

Traitement / vaccin	**Indication**	**date de début** jj/mm/aa	**date de fin** jj/mm/aa
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|

Antirétroviral traitement entre J0 et M24

		N° ligne	Date début	Date fin
2NRTI+1PI				
TDF-FTC ou TDF-3TC + LPV/r	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
TDF-FTC ou TDF-3TC + ATV/r	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
TDF-FTC ou TDF-3TC + FPV	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
TDF-FTC ou TDF-3TC +Daru/r	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
ABC-3TC + LPV/r	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
ABC-3TC + ATV/r	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
ABC-3TC + FPV/r	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
AZT-3TC + LPV/r	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
2NRTI+1NNRTI				
TDF-FTC +EFV	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
ABC-3TC + EFV	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
TDF-FTC +NEVI	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
ABC-3TC+NEVI	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
AZT-3TC +NEVI	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
3TC+d4T +NEVI	❒	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
Autres combinaisons				
		|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|
		|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|

Le patient suit un protocole : Oui ❒ Non ❒

Si oui, lequel ……………………………………

Date début : |__|__|/|__|__|/|__|__|__|__|
jour mois année

Date fin : |__|__|/|__|__|/|__|__|__|__|
jour mois année

D. Attended formations during the PhD period

1. **Ecole d'été de santé publique et d'épidémiologie 2012**
   - Méthodologie de base en Statistique et en Epidémiologie
2. **Ecole d'été de santé publique et d'épidémiologie 2013**
   - Régression multiple en épidémiologie : modèle logistique et modèle de Cox

3. **Methods en Pharmaco- Epidemiologie, Ecole Doctorle 393.**

4. **Cycle «Gestion de l'information scientifique »**
   - Séminaire : Maitrisez l'information scientifique
   - Atelier : Gestion bibliographique niveau 1 : principes de la bibliographie et prise en main du logiciel Zotero

5. **Cycle « Pratiques managériales »**
   - Séminaire: Découvrez les grands principes du management
   - Atelier: Pilotez un projet : animer et gérer
   - Atelier: Décidez pour prioriser, gérer votre temps et votre stress

6. **Cycle « Communication »**
   - Séminaire: Communication écrite et orale
   - Atelier: Conduisez efficacement vos entretiens et vos réunions

7. **Cycle « Projet professionnel et gestion de carrière »**
   - Séminaire : Projet professionnel et recherche d'emploi
   - Atelier : Expliciter son projet professionnel personnalisé et élaborer des stratégies d'évolution
   - Atelier : Concrétiser son intégration professionnelle ; CV et lettres
   - Atelier : Concrétiser son intégration professionnelle ; Entretiens

8. **Cours intensive de langue français -30 heures.**

9. **PowerPoint 2010 prise en main**