High USP18 and PTEN expressions in immune non-responders prevent proper CD4 T-cell recovery through mitochondria damage and defective cell maintenance

1. RATIONAL (note: citations of our own work are in bold)

The introduction of suppressive antiretroviral therapy (ART) had a major impact on the morbidity and mortality of HIV-1-infected patients **[1]**. Nonetheless, despite effective control of HIV-1 replication with ART, a minority of treated patients called immune non-responders (INRs) fails to show increased CD4 T-cell counts to the level observed in uninfected control donors [2, 3]. INRs remain at greater risk for health complications and non-AIDS diseases including cardiovascular disease, liver disease, renal disease, and malignancies when compared to immune responders (IRs) in whom the CD4 T-cell counts are properly restored [4, 5]. **It is critical to identify new molecular disturbances in INRs that may be considered as therapeutic targets for improving immune reconstitution of HIV-1-infected patients.**

Although the precise determinants of immune failure are not fully defined, several characteristics of immune cells characterize this syndrome and may provide insight into these mechanisms. For instance, it is well-acknowledged that INRs display elevated levels of systemic inflammation that are illustrated by increased bacterial translocation [2], release of pro-inflammatory cytokines and chemokines such as IL-1β and IL-6 [2, 6-8], gut dysbiosis [9], and inflammasome activation [10]. In fact, elevated inflammation in INRs is currently believed to be the principal correlate of poor immunological response to ART, which affects CD4 T-cell function and maintenance. In this context, several studies show that inflammation in INRs drives T-cell immune activation and exhaustion, as well as dysfunction of T-regulatory (T_{reg}) cells [2, 11-13]. Interestingly, evidence shows that inflammation in INRs is also associated with impaired responsiveness to interleukin 7 (IL-7), increased levels of apoptosis following T-cell activation, and mitochondria impairments in CD4 populations including memory and T_{reg} subsets [7, 11, 13-15]. **The intrinsic mechanisms that are responsible for defective CD4 T-cell maintenance and survival in INRs are not yet fully elucidated.**

Type I interferons (IFN-I) are one of the primary immune defenses against viruses. However, in the case of established persistent viral infection including HIV-1, sustained elevation of IFN-I expression bears deleterious effects to the host and is today considered as a significant driver of immune defects including cell loss **[1, 16]**. Among INRs, it has been suggested that CD4 T-cell recovery and IL-7 response may be adversely affected by the effects of sustained expression of interferon-stimulated genes (ISGs) such as OAS1, MxA, ISG56 and IFI127 in CD4 T-cells [13, 17-19]. Our preliminary results also show increased expression of USP18, another ISG, in CD4 T-cells from INRs when compared to those from both IRs and uninfected control donors (Fig. 1a). Interestingly, our group has just completed a study, which demonstrates that high USP18 expression in memory CD4 T-cells during primary HIV-1 infection is responsible for stabilizing PTEN, thereby hindering AKT activation after cytokine and T-cell receptor (TcR) stimulations **[Dagenais-Lussier X. *et al*, in current revision in PLoS Pathogens]**. Therefore, we propose here a new model of immunological failure in which USP18 in CD4 T-cells is responsible for negatively interfering with AKT activation through a PTEN-dependent mechanism.

2. HYPOTHESIS

We hypothesize that targeting USP18 in CD4 T-cells from INRs is an effective strategy to rescue mitochondrial damage and cell survival by improving AKT activation.

3. OBJECTIVES

Specifically, the following three aims are proposed in CD4 T-cells:
Aim 1. To assess the impact of high USP18 expression on AKT activation in INRs;
Aim 2. To investigate whether targeting USP18 in INRs reduces mitochondrial damage;
Aim 3. To determine the impact of USP18 in INRs on cell survival and long-lasting maintenance.

4. Methodology

Study population. PBMCs and plasma will be collected from INRs, IRs and HIV-1-uninfected control donors, thanks to the cohorts from the AIDS and Infectious Diseases network. Similarly to others [2, 7, 13, 15], INR subjects will be defined as having CD4 T-cell counts below 350/µl and IRs as having CD4 T-cell counts above 350 cells/µl after two years of ART with viral control. INRs and IRs will be selected as middle-aged patients. The study will also include age-matched and HIV-1-uninfected control donors (HIV^{free}). Among the 287 patients under ART, we have been able to identify 10 INRs with available cells and plasma samples. <u>AZT and ritonavir are systematically added to all HIV^{+} cultures to maintain viral suppression</u> **[20]**.

Aim 1. To assess the impact of high USP18 expression on AKT activation in INRs. Since our data show that CD4 T-cells from INRs display increased USP18 expression that drives PTEN stability (Fig. 1), we assume that AKT activation will be impaired in this group when compared to IRs and HIV^{free} subjects. Therefore, PBMCs from all groups will be first cultured overnight in the presence or absence of SF1670 (PTEN inhibitor), or inhibitory USP18 binding proteins (USP BP). Cells will be then stimulated or not with cytokines (IL-2, IL-7, and IL-15), or polyclonally-activated with anti-CD3 and anti-CD28 antibodies for 15 and 30 minutes. Levels of intracellular AKT pS473 and PTEN will be finally determined by PhosFlow on the total CD4 population, as well as on specific CD4 subsets known to be impaired in INRs such as $CD45RA^{neg}$ memory cells, $CD71^{+}$ cycling cells, and $FoxP3^{+}CD25^{+}CD127^{neg}$ T_{reg} cells [13]. Since tissues, especially the gut-associated lymphoid tissues, display the highest levels of inflammation and immune impairments in treated patients **[1, 21]**, AKT activation will also be monitored in gut-homing $CCR6^{+}$ CD4 T-cells. We will confirm that reduced AKT activation in INRs is also observed when using purified CD4 T-cells. CD4 purification will be achieved from PBMCs using the EasySep™ human $CD4^{+}$ T-cell enrichment kit (Stem Cell Technologies). Our protocol allows us to collect CD4 T-cells with more than 96% purity with any cell stimulation and apoptosis **[20, 22, 23]**. Similarly to PBMCs, levels of activated AKT will be determined by flow cytometry on all subsets including cycling, memory, T_{reg} and gut-homing cells. Our group is particularly interested to assess whether the defective AKT activation in INRs is observed in HIV-1-specific CD4 T-cells. Briefly, PBMCs from HIV-1-infected subjects will be pre-treated overnight with SF1670 or USP BP, and then stimulated with overlapping gag peptides (clade B; NIH) and anti-CD28 antibodies for 6 hours with Monensin and Brefeldin-A. Responsive HIV-1-specific CD4 T-cells will be characterized by their positive staining for IFN-γ (Fig. 2a). To appreciate AKT activation levels on HIV-1-specific clones, cells will be subjected to another 15 minute-long HIV-1-specific activation. Levels of intracellular AKT pS473 and PTEN will finally be assessed on $IFN\text{-}\gamma^{+}$ CD4 T-cells (Fig. 2b). Of note, our group will collaborate with Drs. Jenabian and Ancuta, who have expertise in T_{reg} and gut-homing CD4 T-cells respectively [24-27]. Finally, we aim to determine whether reduced AKT activation in INRs correlates with the induction of microbial translocation and inflammation. Plasma levels of the soluble receptor for LPS (sCD14) and pro-inflammatory cytokines (IL-1β, IL-6 and IL-18) will be done in Dr. Routy's lab, which performs routinely ELISA quantifications.

Aim 2. To investigate whether targeting USP18 in INRs reduces mitochondrial damage. Our preliminary data shows that activated CD4 T-cells from INRs display mitochondrial impairments when compared to IRs and HIV^{free} subjects. Mitochondrial impairments in INRs were illustrated by increased membrane depolarisation (Fig. 3) and reduced mitochondrial spare respiratory capacity (or SRC) after 3 days of TcR stimulation (Fig. 4). Of note, SRC is defined as the extra capacity available in cells to produce energy in response to increased stress, and as such is directly associated with T-cell survival [28]. Similarly to **Aim 1**, total PBMCs and purified CD4 T-cells from all groups will be pre-treated or not with SF1607 or USP BP, and then stimulated with cytokines or anti-CD3 and anti-CD28 antibodies for 24, 48 or 72 hours. Mitochondria membrane potentials for all conditions will be assessed in total, memory, cycling, T_{reg}, gut-homing CD4 T-cells for all conditions by flow cytometry using $DiOC_6(3)$ and Mitotracker Deep Red FM dyes as previously done **[29]**. Intracellular levels of activated caspase-9 and caspase-3, as well as Bcl-2, Bim and Bad pS136 expressions will be determined by flow cytometry. With the help of flow cytometry, the state of mitochondria will also be monitored in INRs and IRs after HIV-1-specific stimulations. As

additional surrogate marker of HIV-1-mitochondrial damage, mitochondrial DNA content (mtDNA) will be measured in purified CD4 T-cells by qPCR [30, 31]. SRCs in purified CD4 T-cells will be measured in real-time using the Seahorse technology (Agilent). We have acquired the Seahorse XFe96 Analyzer. The device is installed in our BCL2 area that will allow my group to work with live infected samples.

Aim 3. To determine the impact of USP18 in INRs on cell survival and long-lasting maintenance. Our group will show that targeting USP18, PTEN and activated caspase-9 impacts two survival phenotypes that our group has found impaired in INRs, such as IL-7-mediated cell protection (against Fas-mediated apoptosis) and long-lasting maintenance (Fig. 5). First, PBMCs and purified CD4 T-cells from all groups will be pre-treated overnight or not with SF1670, USP BP, or the caspase-9 inhibitor Z-LEHD-FMK (R&D Systems). Then, anti-Fas CH11 antibodies to trigger Fas-mediated apoptosis will be added in cultures for another 24 hours with or without cytokines such as IL-2, IL-7 and IL-15, as previously done **[20, 22, 23, 32]**. Expressions of PTEN, AKT pS473, active caspase-9, and CD95 (Fas receptor) as well as levels of apoptosis using Annexin-V staining will be determined in total and gated memory, cycling, T_{reg} and gut-homing CD4 T-cells. Percentages of cytokine-protection will be determined by the formula: (Fas-mediated apoptosis without cytokine – Fas-mediated apoptosis with cytokine) / Fas-mediated apoptosis without cytokine. To appreciate the long-lasting CD4 T-cell maintenance, a 28 day-long co-culture assay where cells are subjected to multiple rounds of TcR activation has been optimized in our lab **[20, 23]**. Briefly, purified CD4 T-cells will be first polyclonally-activated with or without SF1670, USP BP or Z-LEHD-FMK, and then co-cultured for 7 days with autologous CD4-depleted PBMCs (ratio CD4 / CD4-depleted PBMC = 1/3). At day 7, 14 and 21 days of co-cultures, we will re-stimulate the cells with or without inhibitors. Number of viable cells by trypan blue exclusion counts, as well as apoptosis levels, intracellular expression of AKT pS473, PTEN, active caspase 9, Bcl-2, Bim, Bad pS136, and mitochondria depolarisation will be determined by flow cytometry on all CD4 subsets at days 7, 14, 21 and 28 days. CD4 T-cells will also be purified during co-cultures to assess SRCs with our Seahorse device. Finally, to ensure sustained USP18 targeting during co-cultures, we will also transduce CD4 T-cells with specific CRISPR-Cas9 lentiviral particles that are already produced and tested in Dr. Labonté's lab in CD4 T-cells (Fig. 6).

5. ANALYSES AND EXPECTED RESULTS

10 subjects per study group and per experiment will achieve a significant statistical power based on the observed changes. As previously done **[20, 22, 33]**, Mann-Whitney, paired *t*, and Pearson tests will be used to analyze differences between study groups, *in vitro* treatments within defined group, and correlations respectively. Taking into account our preliminary data, we expect that targeting USP18 or PTEN in INRs will result in significant improvements of AKT activation in CD4 T-cells. We also expect that targeting USP18 will rescue mitochondrial impairments and cell survival by a PTEN- and caspase-dependent mechanism. Although we don't have definitive proof, we strongly believe that high USP18 in INRs will also affect HIV-specific CD4 T-cells. Overall, **our proposed study unveils a new mechanism of immune failure that may be considered for therapeutic interventions**. In addition to research, the collaborations between junior and senior investigators from several FRQ-S research sites will provide synergy and foster training of graduate and postgraduate students.

6. TIMELINE

All resources, biological samples and collaborations are in place to ensure success and study completion in the next year and half. The majority of experiments will be performed in the IAF by two of my Ph.D. students, Dagenais-Lussier and Hamza Loucif, who have already optimized all methods and cell cultures described in the proposal. These students have also expertise in working with infected samples that were provided from the network **[22] [Dagenais-Lussier X. *et al*, in current revision in PLoS Pathogens]. Aim 1** will be completed in three months (april-june 2019) and **Aims 2-3** after another 15 months (june 2019-september 2020). **One publication** is expected in the next few months after study completion (around october-november 2020).

FINANCIAL BUDGET

. Some of the materials are already in our lab, such as LVP constructs, cytokines and TcR triggering antibodies, gag peptides, EasySep isolation kits, as well as primers and reagents for quantifying mtDNA.

. The proposed budget will **cover the remaining supply costs and user fees that are requested to ensure the completion of our proposal**. The principal applicant will also support this project when required through his other research funds and as Professor of the INRS. In this context, the principal applicant is recipient of current Banting and CRSNG research grants.

. The salaries of his two PhD. students are already covered for the next three years (with a FRQS fellowship awarded to X. Dagenais-Lussier).

The budget details along with justifications are given below:

No.	Reagents and Instrument access	Description	Estimated cost	Justification
1	ELISA kits	ELISA kits (96 well plates) for IL-1β, IL-6, IL-18 and sCD14	$3,000	For the measurement of inflammatory cytokines in triplicate (Aim 1)
2	Seahorse kits	Seahorse XF Cell mito stress kits; specific reagents such Agilent solutions and plates	$3,000	For the assessment of mitochondrial SRC in purified CD4 T-cells (Aims 2, 3)
3	FACS antibodies and reagents	. all anti-human monoclonal antibodies requested for phenotyping CD4 T-cell subsets . Intracellular staining kits, Annexin-V, DiOC$_6$(3) dye; anti-PTEN, IFN-γ, AKT pS473, USP18 Abs, etc.	$6,000	Consumables requested for flow cytometry (Aims 1-3)
4	Access to Flow Cytometry acquisition and analysis stations	estimated 50 hours x $20 per hour	$1,000	Funds requested for user fees (Aims 1-3)
5	Cell culture media, plates and reagents	RPMI media, FBS, antibiotics, culture plates, SF1670, USP BP, other inhibitors, plastics (tubes, etc.)	$2,000	Consumables requested for cell cultures (Aims 1-3)

total: $15,000

POUR USAGE INTERNE

Date d'évaluation :

Acceptation ☐ **Refus :** ☐

Raisons du refus :

Signature:

References (note: citations of our own work are in bold)

1. **Loucif, H., et al., *Deciphering natural control of HIV-1: A valuable strategy to achieve antiretroviral therapy termination.* Cytokine Growth Factor Rev, 2018. 40: p. 90-98.**
2. Lederman, M.M., et al., *Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells.* J Infect Dis, 2011. **204**(8): p. 1217-26.
3. Piconi, S., et al., *Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy.* AIDS, 2010. **24**(13): p. 1991-2000.
4. Baker, J.V., et al., *CD4+ count and risk of non-AIDS diseases following initial treatment for HIV infection.* AIDS, 2008. **22**(7): p. 841-8.
5. Lewden, C., et al., *HIV-infected adults with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population.* J Acquir Immune Defic Syndr, 2007. **46**(1): p. 72-7.
6. Prebensen, C., et al., *High MIP-1beta Levels in Plasma Predict Long-Term Immunological Nonresponse to Suppressive Antiretroviral Therapy in HIV Infection.* J Acquir Immune Defic Syndr, 2015. **69**(4): p. 395-402.
7. Shive, C.L., et al., *Inflammatory cytokines drive CD4+ T-cell cycling and impaired responsiveness to interleukin 7: implications for immune failure in HIV disease.* J Infect Dis, 2014. **210**(4): p. 619-29.
8. Stiksrud, B., et al., *Plasma IP-10 Is Increased in Immunological NonResponders and Associated With Activated Regulatory T Cells and Persisting Low CD4 Counts.* J Acquir Immune Defic Syndr, 2016. **73**(2): p. 138-48.
9. Lu, W., et al., *Association Between Gut Microbiota and CD4 Recovery in HIV-1 Infected Patients.* Front Microbiol, 2018. **9**: p. 1451.
10. Bandera, A., et al., *The NLRP3 Inflammasome Is Upregulated in HIV-Infected Antiretroviral Therapy-Treated Individuals with Defective Immune Recovery.* Front Immunol, 2018. **9**: p. 214.
11. Marziali, M., et al., *T-cell homeostasis alteration in HIV-1 infected subjects with low CD4 T-cell count despite undetectable virus load during HAART.* AIDS, 2006. **20**(16): p. 2033-41.
12. Saidakova, E.V., et al., *Lymphopenia-induced proliferation of CD4 T-cells is associated with CD4 T-lymphocyte exhaustion in treated HIV-infected patients.* Indian J Med Res, 2018. **147**(4): p. 376-383.
13. Younes, S.A., et al., *Cycling CD4+ T cells in HIV-infected immune nonresponders have mitochondrial dysfunction.* J Clin Invest, 2018. **128**(11): p. 5083-5094.
14. Marchetti, G., et al., *Comparative analysis of T-cell turnover and homeostatic parameters in HIV-infected patients with discordant immune-virological responses to HAART.* AIDS, 2006. **20**(13): p. 1727-36.
15. Shive, C.L., et al., *Inflammation Perturbs the IL-7 Axis, Promoting Senescence and Exhaustion that Broadly Characterize Immune Failure in Treated HIV Infection.* J Acquir Immune Defic Syndr, 2016. **71**(5): p. 483-92.
16. **Dagenais-Lussier, X., et al., *Sustained IFN-I Expression during Established Persistent Viral Infection: A "Bad Seed" for Protective Immunity.* Viruses, 2017. 10(1).**
17. Cha, L., et al., *IFN-alpha exerts opposing effects on activation-induced and IL-7-induced proliferation of T cells that may impair homeostatic maintenance of CD4+ T cell numbers in treated HIV infection.* J Immunol, 2014. **193**(5): p. 2178-86.
18. Fernandez, S., et al., *CD4+ T-cell deficiency in HIV patients responding to antiretroviral therapy is associated with increased expression of interferon-stimulated genes in CD4+ T cells.* J Infect Dis, 2011. **204**(12): p. 1927-35.
19. Nguyen, T.P., et al., *Responsiveness to IL-7 but not to IFN-alpha is diminished in CD4+ T cells from treated HIV infected patients who experience poor CD4+ T-cell recovery.* AIDS, 2016.

30(13): p. 2033-42.

20. **van Grevenynghe, J., et al., *Transcription factor FOXO3a controls the persistence of memory CD4(+) T cells during HIV infection.* Nat Med, 2008. 14(3): p. 266-74.**
21. **van Grevenynghe, J., et al., *Lymph node architecture collapse and consequent modulation of FOXO3a pathway on memory T- and B-cells during HIV infection.* Semin Immunol, 2008. 20(3): p. 196-203.**
22. **Dagenais-Lussier, X., et al., *Kynurenine Reduces Memory CD4 T-Cell Survival by Interfering with Interleukin-2 Signaling Early during HIV-1 Infection.* J Virol, 2016. 90(17): p. 7967-79.**
23. **Olagnier, D., et al., *HTLV-1 Tax-mediated inhibition of FOXO3a activity is critical for the persistence of terminally differentiated CD4+ T cells.* PLoS Pathog, 2014. 10(12): p. e1004575.**
24. Gosselin, A., et al., *HIV persists in CCR6+CD4+ T cells from colon and blood during antiretroviral therapy.* AIDS, 2017. **31**(1): p. 35-48.
25. Jenabian, M.A., et al., *Soluble CD40-ligand (sCD40L, sCD154) plays an immunosuppressive role via regulatory T cell expansion in HIV infection.* Clin Exp Immunol, 2014. **178**(1): p. 102-11.
26. Jenabian, M.A., et al., *Regulatory T cells negatively affect IL-2 production of effector T cells through CD39/adenosine pathway in HIV infection.* PLoS Pathog, 2013. **9**(4): p. e1003319.
27. Planas, D., et al., *HIV-1 selectively targets gut-homing CCR6+CD4+ T cells via mTOR-dependent mechanisms.* JCI Insight, 2017. **2**(15).
28. van der Windt, G.J., et al., *Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development.* Immunity, 2012. **36**(1): p. 68-78.
29. **Sze, A., et al., *Host restriction factor SAMHD1 limits human T cell leukemia virus type 1 infection of monocytes via STING-mediated apoptosis.* Cell Host Microbe, 2013. 14(4): p. 422-34.**
30. de Mendoza, C., et al., *Changes in mitochondrial DNA copy number in blood cells from HIV-infected patients undergoing antiretroviral therapy.* AIDS Res Hum Retroviruses, 2004. **20**(3): p. 271-3.
31. Peraire, J., et al., *HIV-1-infected long-term non-progressors have milder mitochondrial impairment and lower mitochondrially-driven apoptosis in peripheral blood mononuclear cells than typical progressors.* Curr HIV Res, 2007. **5**(5): p. 467-73.
32. **Riou, C., et al., *Convergence of TCR and cytokine signaling leads to FOXO3a phosphorylation and drives the survival of CD4+ central memory T cells.* J Exp Med, 2007. 204(1): p. 79-91.**
33. **van Grevenynghe, J., et al., *Loss of memory B cells during chronic HIV infection is driven by Foxo3a- and TRAIL-mediated apoptosis.* J Clin Invest, 2011. 121(10): p. 3877-88.**

ANNEXE 1: Figures 1-4

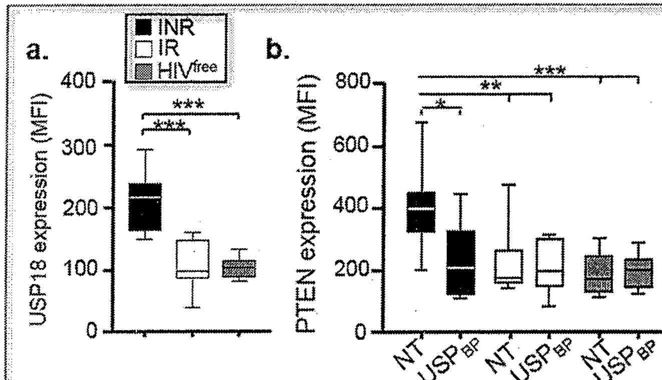

Fig. 1. USP18 stabilizes PTEN expression in CD4 T-cells in INRs. a. *Ex vivo* USP18 expression in mean fluorescence intensity (MFI). **b.** PTEN expression in CD4 T-cells that have been cultured overnight with or without USP BP. N = 10 (Flow cytometry).

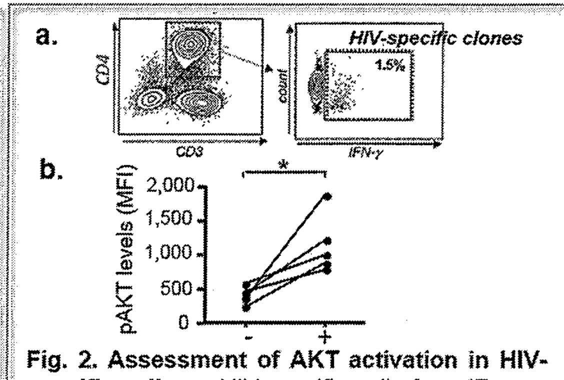

Fig. 2. Assessment of AKT activation in HIV-specific cells. a. HIV-specific cells from IRs are determined by IFN-γ positive expression after 6 hours of gag stimulation. **b.** AKT pS473 levels are determined on gated HIV-specific cells with (+) or without (-) an additional 15 minute-long gag stimulation. N = 5 (Flow cytometry).

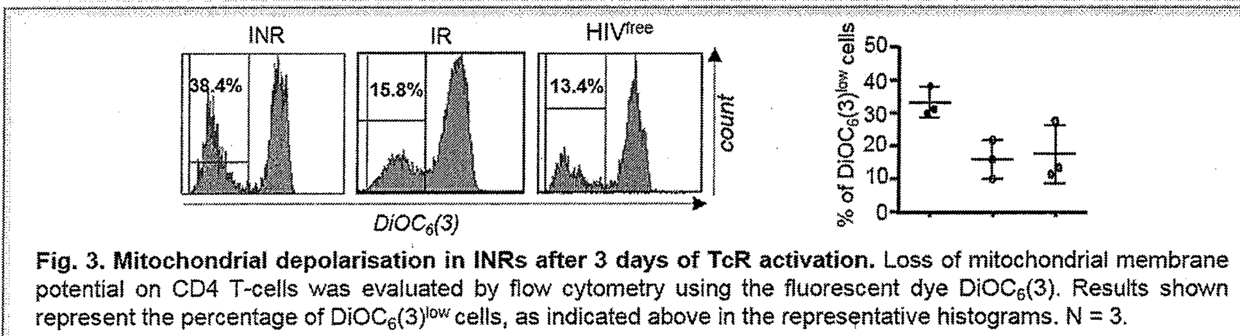

Fig. 3. Mitochondrial depolarisation in INRs after 3 days of TcR activation. Loss of mitochondrial membrane potential on CD4 T-cells was evaluated by flow cytometry using the fluorescent dye $DiOC_6(3)$. Results shown represent the percentage of $DiOC_6(3)^{low}$ cells, as indicated above in the representative histograms. N = 3.

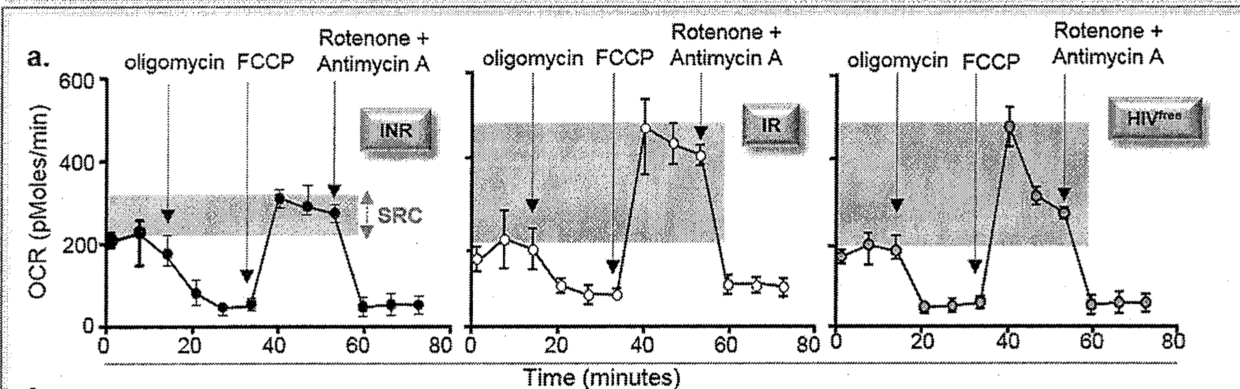

Fig. 4. Reduced spare respiratory capacity (SRC) in activated CD4 T-cells from INRs. After 3 days of TcR activation, mitochondrial respirations were measured in CD4 T-cells according to the Seahorse manufacturer's protocol (Seahorse XF cell Mito Stress Test). **a.** Test profiles in INR, IR and HIV^{free} subjects with the spare respiratory capacities that are illustrated by the blue areas. OCR = O_2 consumption rates. **b.** SRC as indicated by maximum OCR calculated as percentage of baseline OCR. N = 3.

ANNEXE 2: Figures 5, 6

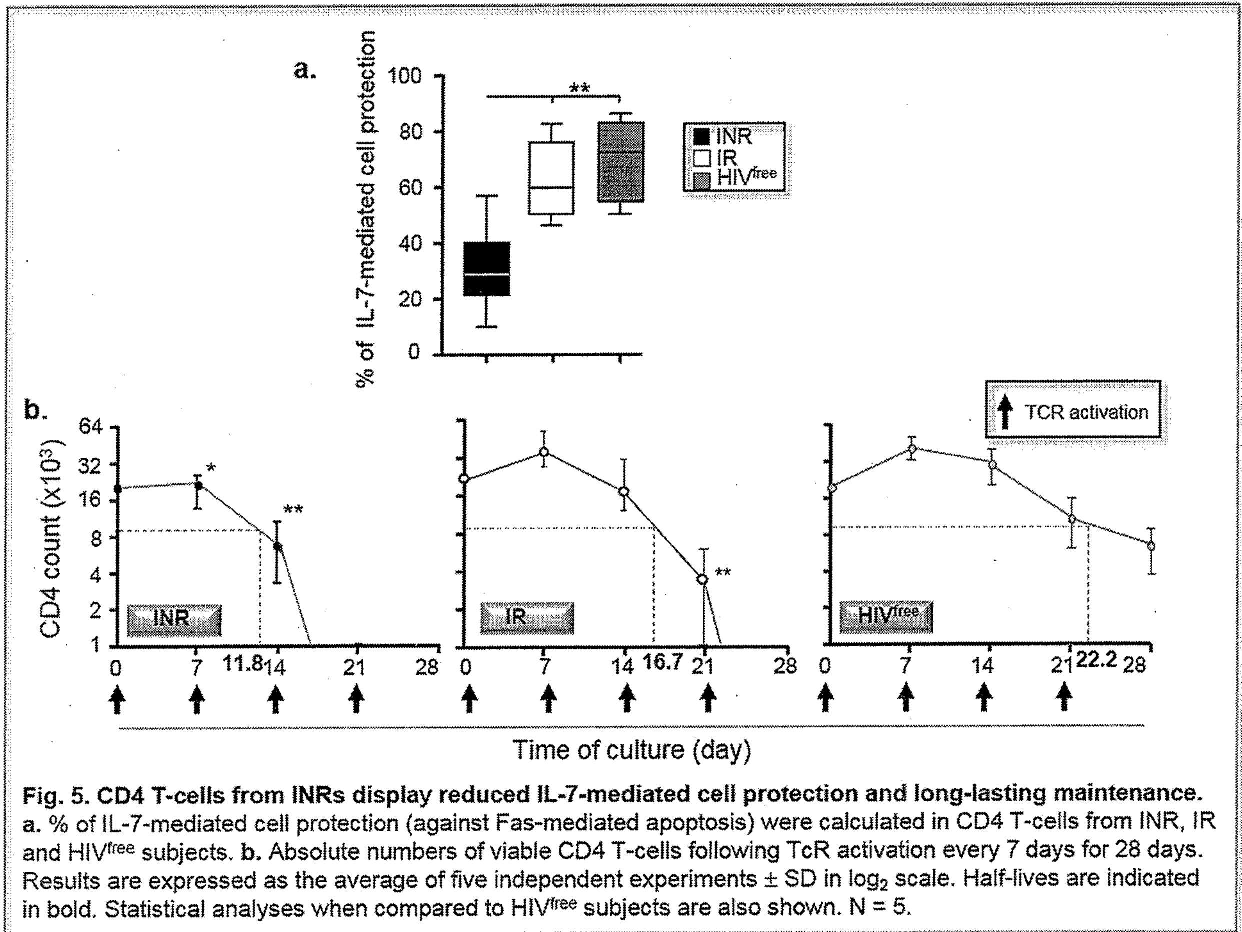

Fig. 5. CD4 T-cells from INRs display reduced IL-7-mediated cell protection and long-lasting maintenance. **a.** % of IL-7-mediated cell protection (against Fas-mediated apoptosis) were calculated in CD4 T-cells from INR, IR and HIV^{free} subjects. **b.** Absolute numbers of viable CD4 T-cells following TcR activation every 7 days for 28 days. Results are expressed as the average of five independent experiments ± SD in $\log_2$ scale. Half-lives are indicated in bold. Statistical analyses when compared to HIV^{free} subjects are also shown. N = 5.

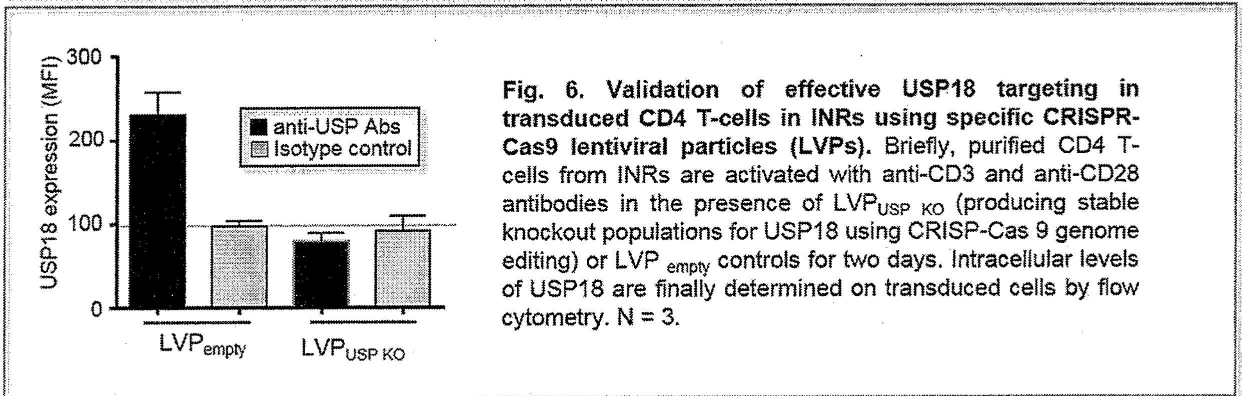

Fig. 6. Validation of effective USP18 targeting in transduced CD4 T-cells in INRs using specific CRISPR-Cas9 lentiviral particles (LVPs). Briefly, purified CD4 T-cells from INRs are activated with anti-CD3 and anti-CD28 antibodies in the presence of $LVP_{USP\ KO}$ (producing stable knockout populations for USP18 using CRISP-Cas 9 genome editing) or LVP_{empty} controls for two days. Intracellular levels of USP18 are finally determined on transduced cells by flow cytometry. N = 3.

Réseau SIDA/MI

CRITÈRES DE SÉLECTION

	Impact potentiel	Intervalle	Mérite scientifique
Subventionnable	Extrêmement important	4,5 – 4,9	Exceptionnel
	Très important	4,0 – 4,4	Excellent
	Important	3,5 – 3,9	Excellent, mais peut nécessiter une révision
Non subventionnable	Modéré	3,0 – 3,4	Très bien, mais nécessite une révision pour être subventionnable
	Limité	2,5 – 2,9	Révision importante nécessaire
	Négligeable	0,0 – 2,4	Laisse beaucoup à désirer

Il est proposé aux évaluateurs d'accorder une note (0,0 à 4,9) pour chacun des critères, en vue de les additionner et de les diviser par 5.

1. RATIONNEL *(Mise en contexte et importance de l'étude proposée)*	4
2. HYPOTHÈSE *(Veuillez énoncer clairement l'hypothèse principale du projet et les hypothèses secondaires, le cas échéant).*	4
3. OBJECTIFS *(Veuillez spécifier les objectifs principaux, et les objectifs secondaires, le cas échéant).*	4
4. MÉTHODOLOGIE *(Si des outils ou des méthodologies innovantes seront utilisées pour ce projet, veuillez les spécifier ici. Veuillez préciser la faisabilité, les difficultés potentielles et les mesures d'atténuation, lorsque pertinent)*	4
5. ANALYSES ET RÉSULTATS ANTICIPÉS *(Veuillez préciser les méthodes statistiques qui seront utilisées et énoncer les résultats attendus).*	4
6. ÉCHÉANCIER *(Veuillez identifier les étapes à suivre pour l'atteinte des objectifs.)*	4
INSCRIRE LE CODE RS DU PARTICIPANT : RS004	**TOTAL** = 4,8

FORCES :

- Problem of immune non-responders remains a question with a lack of clear answers.
- Rational and preliminary data are convincing.
- Methodology adapted to aim of the study.
- Human forces and technological skills available.

FAIBLESSES :

I am not sure that the number of cells available will be sufficient to perform th high number of experiments that the authors have planned.

Réseau SIDA/MI

CRITÈRES DE SÉLECTION

	Impact potentiel	Intervalle	Mérite scientifique
Subventionnable	Extrêmement important	4,5 – 4,9	Exceptionnel
	Très important	4,0 – 4,4	Excellent
	Important	3,5 – 3,9	Excellent, mais peut nécessiter une révision
Non subventionnable	Modéré	3,0 – 3,4	Très bien, mais nécessite une révision pour être subventionnable
	Limité	2,5 – 2,9	Révision importante nécessaire
	Négligeable	0,0 – 2,4	Laisse beaucoup à désirer

Il est proposé aux évaluateurs d'accorder une note (0,0 à 4,9) pour chacun des critères, en vue de les additionner et de les diviser par 6.

Critère	Note
1. RATIONNEL *(Mise en contexte et importance de l'étude proposée)*	4.8
2. HYPOTHÈSE *(Veuillez énoncer clairement l'hypothèse principale du projet et les hypothèses secondaires, le cas échéant).*	4.8
3. OBJECTIFS *(Veuillez spécifier les objectifs principaux, et les objectifs secondaires, le cas échéant).*	4.8
4. MÉTHODOLOGIE *(Si des outils ou des méthodologies innovantes seront utilisées pour ce projet, veuillez les spécifier ici. Veuillez préciser la faisabilité, les difficultés potentielles et les mesures d'atténuation, lorsque pertinent)*	4.8
5. ANALYSES ET RÉSULTATS ANTICIPÉS *(Veuillez préciser les méthodes statistiques qui seront utilisées et énoncer les résultats attendus).*	4.8
6. ÉCHÉANCIER *(Veuillez identifier les étapes à suivre pour l'atteinte des objectifs.)*	4.8
INSCRIRE LE CODE RS DU PARTICIPANT : RS-004	**TOTAL = 4.4**

FORCES :

THIS IS A VERY WELL-CRAFTED STUDY THAT IS OF HIGH INTEREST FOR THE FIELD. IT GAINS FROM HAVING PATIENT COHORTS TO PROVIDE THE EXCLUSIVE SAMPLES AND ROBUST PRELIMINARY DATA WRITTEN-UP INTO A MANUSCRIPT THAT IS CURRENTLY UNDER PEER REVIEW IN A TOP TIER JOURNAL IN THE FIELD. THIS PROJECT IS LOW RISK AND HIGH REWARD. THE METHODS ARE RELATIVELY SIMPLE AND STRAIGHT FORWARD: MAINLY BASED ON FLOW CYTOMETRY AND ELISA; AND THE TOOLS AND REAGENTS HAVE ALL BEEN ACQUIRED AND DEVELOPED FOR THE MANUSCRIPT THAT HAS BEEN SUBMITTED.

FAIBLESSES :

THE WAY THE PROJECT IS WRITTEN MAKES IT SOUND LIKE IT IS TO RECOVER COSTS AND NOT TO SEEK SEED FUNDING FOR A NOVEL PROJECT 'PROJET PILOTE'. THE AUTHORS ALSO USE WORDS AND DEFINITIVE SENTENCES LIKE 'OUR GROUP WILL SHOW', 'ONE PUBLICATION IS EXPECTED', WHICH IMPART THE NOTION THAT THE EXPERIMENTS HAVE ALREADY BEEN DONE

AND THAT THE RESULTS ARE ALREADY KNOWN. THIS IS WHY THAT DESPITE DESERVING 4.8 AS A SCORE, I MANUALLY EDITED IT TO 4.4. I DO NOT BELIEVE THAT COST-RECOVERY IS IN LINE WITH THE SPIRIT OF THIS FUNDING COMPETITION.

I LEAVE IT TO THE COMMITTEE TO DECIDE WHETHER I AM RIGHT OR WRONG ON THIS ISSUE. IN EITHER CASE, THIS IS AN OUTSTANDING AND TECHNICALLY SOUND PROJECT.