IL-21-induced autophagy governs superior polyfunctional CTL responses in elite controllers

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

. **Key role of CD8 in HIV control**. The cellular immune response plays a critical role in controlling viral replication during HIV-1 infection and needs to be a part of any successful vaccine approach [1-5]. The qualitative feature of the cellular responses most closely associated with immunological control of HIV infection is CD8 T-cell cytotoxic (CTL) potential, which is responsible for mediating the elimination of infected CD4 T-cells. One approach to assessing CTL potential in HIV-1-infected patients is to measure several of their functions simultaneously including perforin production [6]. In this context, defective CTL potential is observed in the majority of patients despite years of suppressive antiretroviral therapy (ART), and occurs as early as a few weeks after HIV-1 infection [7, 8] **[9]**. Since effective CTL potential is required to ensure successful outcomes with both HIV vaccination and reservoir purge [2, 10], **an urgent need exists to devise strategies to enhance antiviral CTL responses in patients under ART**.

. **EC, right model to investigate high quality CD8.** The rare ability of some individuals, called elite controllers (EC), to naturally control HIV-1 infection can be exploited to identify molecular mechanisms involved in host protection that may function as potential therapeutic targets [11, 12] **[13]**. In this context, our previous works identified the transcription factor Foxo3a as a critical player in the survival of both memory CD4 T- and B-cells in EC **[14-17]**. We also identified the pro-inflammatory IL-32 cytokine as a novel biomarker for control failure among this group **[18]**. Compelling evidence show that natural control of EC is even more closely associated with their superior Gag-specific CTL responses [19-22]. However, the molecular mechanisms responsible for superior CTL responses in EC are still unclear. **Understanding the mechanisms of superior CD8 protection capacity in EC will surely unveil new molecular therapeutic targets for the improvement of antiviral CTL responses in the other patients**. We are particularly interested in investigating autophagy since it has been associated with HIV containment [23].

. **Our preliminary data.** We confirmed superior antiviral CTL potential in EC when compared to the other patients after polyclonal, CEF- (cytomegalovirus, Epstein-Barr virus and influenza virus) and HIV-1 specific activations (Fig. 1). We further show that all activations induce higher autophagy in CD8 T-cells from EC (Fig. 2). Interestingly, our preliminary data indicate that higher autophagy in stimulated CD8 T-cells from EC (*i*) plays a critical role in governing their superior CTL responses (Fig. 3) and (*ii*) is associated with the frequencies of IL-21-producing CD4 T-cells (Fig. 4).

2. HYPOTHESIS

Since IL-21 production is known to be impaired during HIV-1 infection except in EC [24] **[25, 26]** and modulates perforin expression [27], **we hypothesize that IL-21 treatments may be considered to increase antiviral CTL responses in non-EC patients in an autophagy-dependent manner**.

3. OBJECTIVES

Specifically, the following three aims are proposed in CD8 T-cells (Fig. 5):
Aim 1. Determine why CD8 activation in EC leads to higher autophagy;
Aim 2. Assess the role of autophagy in superior CTL responses in EC;
Aim 3. Investigate the impact of IL-21 on autophagy-dependent CTL responses during HIV-1 infection.

4. METHODOLOGY

. **Study population:** We will collect total PBMC from patients under ART (ART$^+$), EC and HIV-1-uninfected donor controls, thanks to the cohorts from the *AIDS and Infectious Diseases network* and Dr. Routy. Similarly to previously done **[15, 17]**, the inclusion criteria of untreated EC and ART$^+$ patients are: middle-aged subjects (same for uninfected controls), presumed HIV-1 infection for a minimum of 6 years, infected with non-deleted virus, absence of protective gene polymorphisms, CD4 counts over 400 cells /µl of blood and undetectable viral load (< 50 copies /ml) for 3 years or more. The rationale for comparing EC

to ART$^+$, which maintain undetectable viral loads for years, is to focus on the host mechanisms and eliminate the side effects that would be caused by high viremia and viral protein production. Indeed, several HIV-1 proteins are reported to interfere with autophagy [28]. Of note, we will systematically add AZT and ritonavir in all ART$^+$ cultures to maintain medical viral suppression (confirmed by p24 ELISA using supernatants).

. Polyclonal and antigen-specific activations: We will activate or not total PBMC from ART$^+$, EC and HIV^{free} subjects for 6 hours either with beads coated with anti-CD3 and anti-CD28 antibodies (polyclonal activation), with CEF control peptide pool or with Gag p55/p24 peptides in the presence of anti-CD28 antibodies. We will add GolgiPlug/GolgiStop at the onset of cell cultures for the assessment of cytokine and cytotoxic molecule expressions. Following peptide stimulations, antigen-specific CD8 T-cells will be determined by their positive staining for IFN-γ (Fig. 1A). Of note, we will also include HIV^{free} donors as negative controls for HIV-1-specific activation. Uninfected controls are used for setting gating regions and discerning positive from negative cells **[Xavier Dagenais-Lussier *et al.*, PLoS Pathogens, in Press]**.

. **Autophagy assessment:** Autophagy is a multi-step and complex degradative process, which is regulated by many autophagy-related genes (ATG) from the formation of autophagosomes up to the proteolytic degradation by lysosomal proteases [29]. Therefore, it will be optimal to monitor autophagy by investigating the expression of several ATG and using complementary experimental methods (Fig. 2A). In our project, we will assess autophagy in CD8 T-cells by measuring **a.** the expression of ULK_1, Beclin-1, ATG_5, ATG_{12} and ATG_{14} using flow cytometry (FACS). Flux through the pathway will be investigated by blocking lysosomal acidification with bafilomycin-A1 and observing **b.** the accumulation of selective autophagy substrates like p62. We will also determine by Image Stream analysis **c.** the lysosomal localisation of LC3 [30]. Finally, in the context of polyclonal activation, we will use electron microscopy to evaluate, on purified CD8 T-cells, **d.** the number and frequencies of cells containing autophagic vacuoles (AV), and the average number of AV per cell [23]. CD8 purification will be achieved by negative selection. Similarly to CD4 isolation, our protocol allows us to get more than 95.4% purification with any cell stimulation and apoptosis (confirmed by FACS) **[17, 31, 32]**. Image Stream and microscopic analyses will be done in collaboration with Drs. Stäger and Veyrier, who have proven expertise with these approaches **[33]** [34].

Aim 1. Determine why CD8 activation in EC leads to higher autophagy. We aim to conduct sets of experiments to address several questions. Autophagy will be assessed in CD8 T-cells using methods "**a-c**". *1. Is high autophagy in EC explained by earlier induction of the pathway?* Following polyclonal or specific activations, autophagy will be assessed in CD8 T-cells every hour starting at 1-hour post-culture. *2. Is high autophagy in EC explained by lower mTor activity?* mTor is the master regulator of autophagy by preventing the formation of autophagosomes [35]. If reduced autophagy in activated CD8 T-cells from ART$^+$ patients is due to higher intrinsic mTor activity, we should be able to enhance their autophagy induction during cell activation by adding rapamycin or Torin-1 (mTor inhibitors). Briefly, we will purify CD8 T-cells, treat them with mTor inhibitors for 1 hour, wash them twice, and finally culture them with their autologous CD8^{neg}PBMC (at CD8:autologous PBMC ratio = 2:10) in the presence cell activations. *3. Is high autophagy in EC explained by stronger TcR signaling?* TcR signaling status will be monitored by assessing the expression of active phospho-LcK (LcK pY505), ZAP70 pY319 and $ERK_{1/2}$ pT202/Y319 by PhosFlow as well as total protein levels at 15- and 30-minute post-activations. Since EC are also reported to display Gag-specific CTL responses of high functional avidity [21], autophagy will be assessed following HIV-1-specific activation using lower antigen concentrations (down to 2ng/mL).

Aim 2. Assess the role of autophagy in superior CTL responses in EC. Our preliminary data vouch for a critical role of autophagy in superior CTL potential in EC (Fig. 3). We aim to confirm this observation with larger number of subjects per group and, following antigen-specific activations, investigate whether lysosomal phases of autophagy are involved or not. We also plan to include *in vitro* suppression assay (*i*VSA) as an additional method to monitor CTL responses.

First, we will specifically target Beclin-1 expression in purified CD8 T-cells using small interfering RNA before co-culturing them with their autologous CD8^{neg}PBMC in the presence of cell activations (Fig. 3A). CTL potential will be monitored by flow cytometry by investigating the co-expressions of several antiviral

players such as perforin, granzymes A and B, IL-2 and TNF-α as shown in Fig. 1. To investigate the involvement of late phase of autophagy, we will also pre-treat purified CD8 T-cells for 1 hour with chloroquine or bafilomycin-A1 (both lysosomal inhibitors). This will be done prior to CD8 activation using the co-culture assays and CTL assessment. Finally, we will perform *i*VSA using infected CD4 T-cell targets as previously reported [22, 36]. In brief, to generate CD4 target cells (T), we will remove CD8 from PBMC with positive-selection magnetic beads **[17, 31, 32]**, activate cells with IL-2 and phytohaemagglutinin (PHA) for 72 hours, infect them for 4 hours with HIV-1_{NL4-3} (MOI = 0.001), and wash them twice. In the meantime, we will purify CD8 T-cells at 6-hour post-activations to collect the effector cells (E). We will co-cultured E with autologous T at multiple E:T ratios (range 0:1 to 5:1) and finally collect supernatants on days 0, 1, 3, 5 and 7 to assess p24 levels by ELISA. The ability of CD8 effectors to inhibit viral replication will be evaluated by applying the formula: $-\text{Log}_{10}$ (p24 with CD8/p24 without CD8). We will perform *i*VSA with Dr. Cohen's lab, which performs routinely *in vitro* HIV-1 infections.

. Aim 3. Investigate the impact of IL-21 on autophagy-dependent CTL responses. First, we aim to confirm the correlations between autophagy in activated CD8 and IL-21 production by CD4 (defined by both % of producing cells and average cytokine level per cell) with a larger number of subjects per group and following antigen-specific activations. We will monitor the status of IL-21 signaling in activated CD8 T-cells by assessing the expression of active JAK1 pY1022, JAK3 pY785, STAT3 pY705, STAT5 pY694 and IL-21 receptor (IL-21 R) by flow cytometry at 30-minute, 1- and 6-hour post-activations. Once it is done, we will investigate potential correlations between IL-21 signaling and autophagy for all conditions. For the next sets of experiments, we aim to modulate IL-21 signaling directly on purified CD8 cells prior to their cell activations. Briefly, we will purify CD8 T-cells, treat them with either recombinant IL-21 or with neutralizing antibodies against IL-21 R for 1 hour to respectively activate and inhibit the signaling pathway **[25, 26]**. Since we also aim to investigate if IL-21-induced autophagy during HIV-1 infection acts in a JAK- and STAT- dependent manner, *in vitro* CD8 modulations of IL-21 signaling will also be done in the presence or absence of selective chemical inhibitors for JAK1, JAK3, STAT3 and STAT5 proteins. Thereafter, we will wash CD8 T-cells and co-culture them with their autologous CD8^{neg}PBMC in the presence of coated beads or specific antigens for 6 hours. For all conditions, phospho-protein expression levels, autophagy, CTL responses will respectively be determined at 30-minute, 1- and 6-hour post-activations. Finally, we are interested to investigate whether autophagy and antiviral CTL responses in activated CD8 from EC are impaired if we remove CD4 T-cells (our main source of IL21 production) from cultures. To do so, we will deplete CD4 subset from autologous CD8^{neg}PBMC with positive-selection magnetic beads.

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 **[15, 17, 31]**, 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 IL-21 will enhance autophagy-dependent CTL activity in ART^{+} patients in the range of naturally protected EC**. **We also expect to provide a complete picture of the autophagy-dependent mechanisms that are modulating antiviral CD8 protection during HIV-1 infection**. Finally, the collaborations between junior and senior investigators from several FRQS research sites will also provide synergy and foster student trainings.

6. TIMELINE

All resources, infrastructures (cytometry devices and electronic microscope), biological samples and collaborations are in place to ensure success and study completion in the next year. Almost all experiments will be performed in the IAF by two of my Ph.D. students, Hamza Loucif and Xavier Dagenais-Lussier who have already optimized the majority of the methods.

Aim 1, including microscopic observations, will be completed in six months (January-june 2020) and **Aims 2-3** after another 6 months (july-december 2020). **One publication** is expected in the next few months after study completion (around march-april 2021).

7. FINANCIAL BUDGET

. The proposed budget will **cover the supply costs and user fees that are requested to ensure the completion of the project and to start generating new data (for CIHR programs)**. The principal applicant will also support this project when required through his other research funds. In this context, the principal applicant is also recipient of NSERC research grant.

. 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	Cell culture media, plates and reagents	RPMI, antibiotics, FBS, plastics, antivirals, coated beads, p24 ELISA kits, antigens and specific peptides, PHA, cytokines (IL-21, IL-2) autophagy chemical modulators (Bafilomycin-A1, Torin-1, etc.)	$6,200	Consumables requested for cell cultures **(Aims 1-3 and Future directions)**
2	Isolation kits (Stem Cell Biotech.)	CD8 negative isolation (X 3) and CD4 positive isolation kits (X 1)[1] . $700/kit X 4 = $2,800	$2,800	For CD8 purifications and *i*VSA assays (**Aims 1-3**)
3	FACS antibodies and reagents	. For all anti-human monoclonal antibodies that are requested for the project (autophagy, CTL, anti-phospho-proteins, etc.) . Intracellular staining kits, Lyso-ID, Zenon conjugation kits *(some antibodies have to be conjugated with flurochromes before use)*	$7,000	Consumables requested for FACS (**Aims 1-3 and Future directions**)
4	Access to Flow Cytometry acquisition and analysis stations	. Fees for **LSRII Fortessa**[2] ($50/h X 100h) = $5,000 . Fees for **Amnis Image Stream**[2] ($100/h X 30h) = $3,000	$8,000	Funds requested for user fees **(Aims 1-3 and Future directions)**
5	Reagents and fees for microscopy	. Reagents and sample/slide preparations ($100/sample X 40) = $4,000 . Fees for **Zeiss microscope**[2] and sample analysis ($50/sample X 40) = $2,000	$6,000	for scanning electron microscopy analyses **(Aim 1)**
			total: $30,000	

[1]; *kits requested in complement of those already in our lab.*

[2]; *Internal fees proposed by the IAF-INRS*

8. REFERENCES

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ANNEX 1: FIGURES 1 AND 2

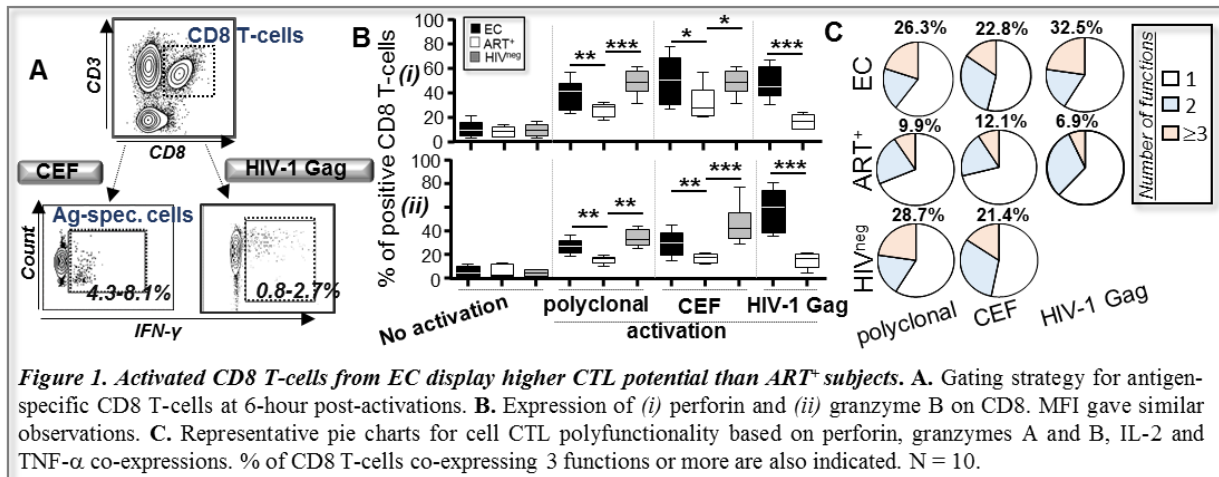

Figure 1. Activated CD8 T-cells from EC display higher CTL potential than ART+ subjects. **A.** Gating strategy for antigen-specific CD8 T-cells at 6-hour post-activations. **B.** Expression of *(i)* perforin and *(ii)* granzyme B on CD8. MFI gave similar observations. **C.** Representative pie charts for cell CTL polyfunctionality based on perforin, granzymes A and B, IL-2 and TNF-α co-expressions. % of CD8 T-cells co-expressing 3 functions or more are also indicated. N = 10.

Figure 2. Activated CD8 T-cells from EC display higher autophagy than ART+ subjects. **A.** Schematic representation of the experimental design to assess the autophagy process by targeting different key molecules involved in different phases. **B.** Flow cytometry assessment of ULK-1 and Beclin-1 expressions on total and Ag-specific CD8 T-cells following cell activation. MFI gave similar observations. **C.** Lysosomal LC3 localisation (by Image Stream). At 6-hour post-activations in the presence of bafilomycin-A1, cells were collected, stained with the lysosomal marker Lyso-ID (EnZo Life Sciences), surface stained with anti-CD8 antibodies, fixed/permeabilized and labeled with anti-LC3 and anti-IFN-γ antibodies. ***(i)*** Representative images of single CD8 T-cells (for polyclonal) or Ag-specific IFN-γ+CD8 T-cells (for Gag stimulation) merged for fluorescent signals from LC3 and Lyso-ID. BF, Bright Field. ***(ii)*** % of BDS^{high}CD8 T-cells for all conditions. BDS (bright Detail Similarity) represents the co-localisation index of fluorescently-stained LC3 and Lyso-ID. N = 10.

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ANNEX 2: FIGURES 3-5

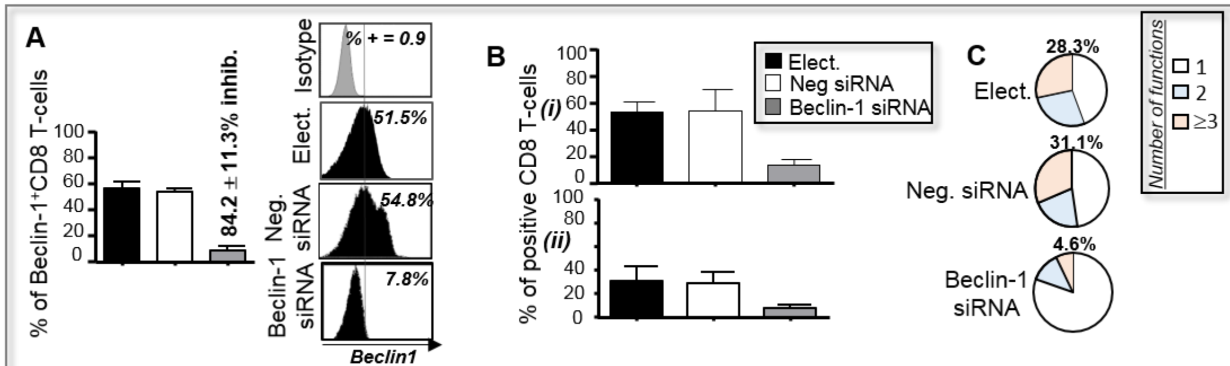

Figure 3. Autophagy inhibition in EC impairs their CTL potential. Purified CD8 T-cells from 3 EC have been electroporated alone (elect.) or transfected with neg. or Beclin-1 siRNA for 24 hours in RPMI complemented with 40% FBS. Transfected cells were then co-cultured for 6 hours with autologous CD8^{neg}PBMC in the presence of coated beads (for polyclonal activation). **A.** Beclin-1 expression on CD8 T-cells at 24-hour post-transfection (with the percentage of Beclin1 inhibition in bold). Representative histograms including isotype control are also shown on the right. **B,C.** CTL assessment in activated CD8 T-cells that have been pre-transfected or not with Beclin-1 siRNA. **B.** Expression of ***(i)*** perforin and ***(ii)*** granzyme B. **C.** Representative pie charts for cell polyfunctionality. % of CD8 T-cells co-expressing 3 functions or more are also indicated.

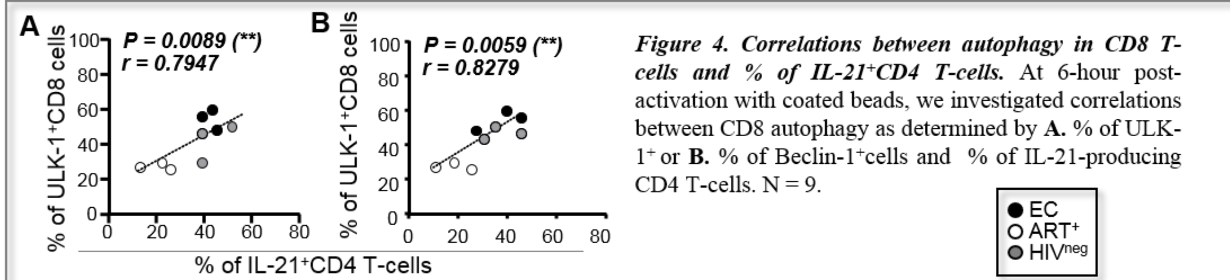

Figure 4. Correlations between autophagy in CD8 T-cells and % of IL-21^{+}CD4 T-cells. At 6-hour post-activation with coated beads, we investigated correlations between CD8 autophagy as determined by **A.** % of ULK-1^{+} or **B.** % of Beclin-1^{+}cells and % of IL-21-producing CD4 T-cells. N = 9.

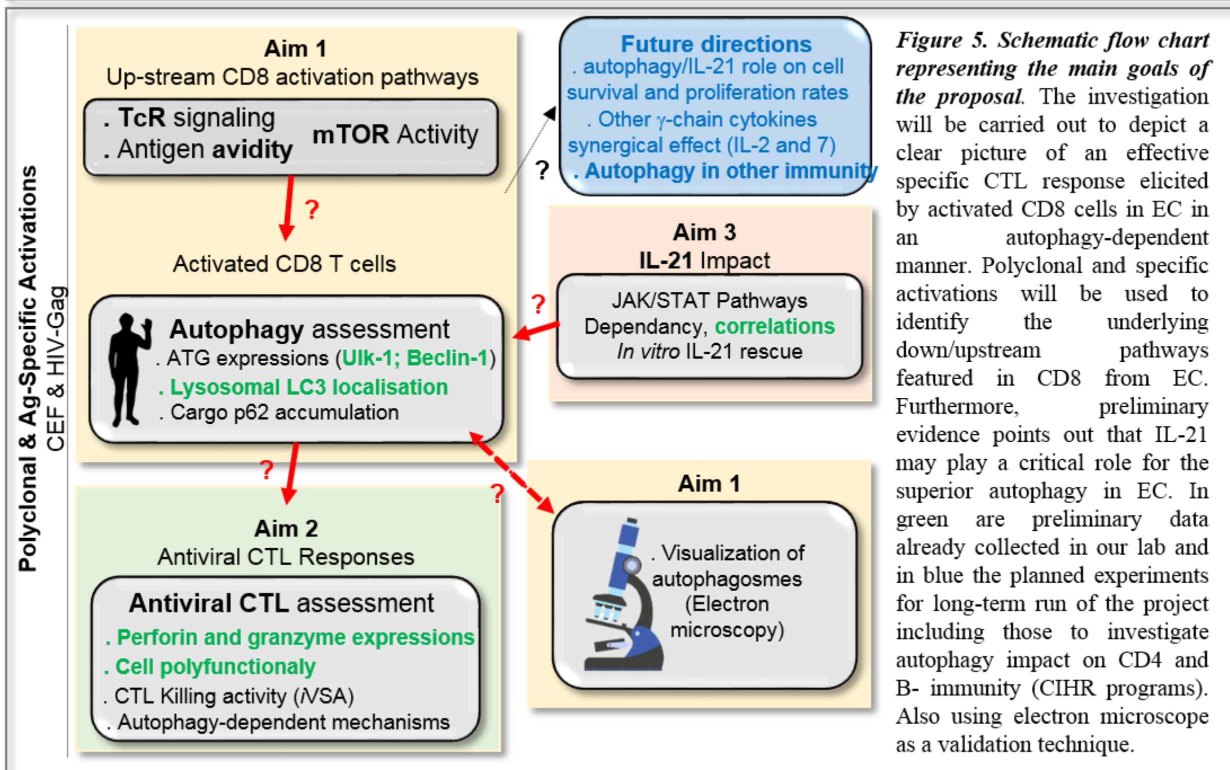

Figure 5. Schematic flow chart representing the main goals of the proposal. The investigation will be carried out to depict a clear picture of an effective specific CTL response elicited by activated CD8 cells in EC in an autophagy-dependent manner. Polyclonal and specific activations will be used to identify the underlying down/upstream pathways featured in CD8 from EC. Furthermore, preliminary evidence points out that IL-21 may play a critical role for the superior autophagy in EC. In green are preliminary data already collected in our lab and in blue the planned experiments for long-term run of the project including those to investigate autophagy impact on CD4 and B- immunity (CIHR programs). Also using electron microscope as a validation technique.

POUR USAGE INTERNE

Date d'évaluation :

Acceptation ☐ **Refus :** ☐

Raisons du refus :

Signature :

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CRITÈRES DE SÉLECTION - GREVENYNGHE → 24

Modèle des Instituts de recherche en santé du Canada / IRSC			
	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	
1. RATIONNEL *(1.1 Mise en contexte et* *1.2 importance de l'étude proposée)*	*4,0*
2. HYPOTHÈSE *(Veuillez énoncer clairement* *2.1 l'hypothèse principale du projet et* *2.2 les hypothèses secondaires, le cas échéant).*	*3,8*
3. OBJECTIFS *(Veuillez spécifier* *3.1 les objectifs principaux, et* *3.2 les objectifs secondaires, le cas échéant).*	*4,2*
4. MÉTHODOLOGIE *(Veuillez spécifier* *4.1 si des outils ou des méthodologies innovantes seront utilisées,* 4.2 *Veuillez* préciser la faisabilité, *4.3 les difficultés potentielles et* *4.4 les mesures d'atténuation, lorsque pertinent)*	*4,8*
5. ANALYSES ET RÉSULTATS ANTICIPÉS *(Veuillez préciser* *5.1 les méthodes statistiques qui seront utilisées* *5.2 et énoncer les résultats attendus).*	*4,5*
6. ÉCHÉANCIER *(Veuillez identifier* *6.1 les étapes à suivre pour l'atteinte des objectifs.)*	*3,5*

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Grevenynghe → 2a

Modèle des Instituts de recherche en santé du Canada / IRSC			
	Impact potentiel	**Intervalle**	**Mérite scientifique**
NOM DU PARTICIPANT : GREVENYNGHE			**TOTAL** = 4,6 → 24,8 /6 = **4,1**3

FORCES :

- **SUJET INTÉRESSANT ET IMPORTANT POUR LE VIH.**
- **PROJET TRÈS DÉTAILLÉ.**
- **COMPÉTENCE DE L'ÉQUIPE.**
- **UTILISATION DE COHORTES AVEC UN NOMBRE DE PATIENTS STATISTIQUEMENT SATISFAISANT.**

FAIBLESSES :

L'UTILISATION DE PATIENTS SOUS ART, BIEN QUE JUSTIFIÉE DANS LA DEMANDE, N'EST PAS TOUT À FAIT CONVAINQUANT ET IL SERAIT JUDICIEUX D'AJOUTER UNE COHORTE DE PATIENTS NON SOUS ART COMME TÉMOIN.

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CRITÈRES DE SÉLECTION - GREVENYNGHE → 26

Modèle des Instituts de recherche en santé du Canada / IRSC			
	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	
1. RATIONNEL *(1.1 Mise en contexte et* *1.2 importance de l'étude proposée)*	*Important dans le contexte des connaissances actuelles* ***4.5***
2. HYPOTHÈSE *(Veuillez énoncer clairement* *2.1 l'hypothèse principale du projet et* *2.2 les hypothèses secondaires, le cas échéant).*	*Hypothèse très pertinente basée sur des données préliminaires récentes* ***4.3***
3. OBJECTIFS *(Veuillez spécifier* *3.1 les objectifs principaux, et* *3.2 les objectifs secondaires, le cas échéant).*	*3 objectifs bien définis* ***4.3***
4. MÉTHODOLOGIE *(Veuillez spécifier* *4.1 si des outils ou des méthodologies innovantes seront utilisées,* *4.2 Veuillez préciser la faisabilité,* *4.3 les difficultés potentielles et* *4.4 les mesures d'atténuation, lorsque pertinent)*	*Les méthodes proposées ont été développées récemment dans le laboratoire et elles sont maitrisées par l'étudiant responsable de ce projet, assurant ainsi une bonne faisabilité. De plus des collaborations sont mises en place afin d'accéder à certaines méthodologies (image Stream, analyses microscopiques, iVSA).* ***4.2***
5. ANALYSES ET RÉSULTATS ANTICIPÉS *(Veuillez préciser* *5.1 les méthodes statistiques qui seront utilisées* *5.2 et énoncer les résultats attendus).*	*Analyses classiques de comparaison de groupes. Augmentation la réponse CTL, autophagie dépendante, chez les patients traités par IL-21 afin d'atteindre le niveau naturel observés chez les elites controllers.* ***4.3***

Réseau
SIDA/MI

Grevenynghe → 2b

Modèle des Instituts de recherche en santé du Canada / IRSC			
	Impact potentiel	**Intervalle**	**Mérite scientifique**
6. ÉCHÉANCIER *(Veuillez identifier 6.1 les étapes à suivre pour l'atteinte des objectifs.)*			*Les ressources et les méthodologies sont déjà en place, les deux étudiants sélectionnés pour ce projet ont optimalisés les méthodes. Les échantillons cliniques sont déjà disponibles grâce aux cohortes dirigées par le Dr Routy.* ***4.2***
NOM DU PARTICIPANT : GREVENYNGHE			**TOTAL = 4.32**

FORCES : CE PROJET EST IMPORTANT DANS LE CONTEXTE DES CONNAISSANCES ACTUELLES, EN PARTICULIER DANS LE CONTEXTE D'UNE GUÉRISON FONCTIONNELLE. L'HYPOTHÈSE DE TRAVAIL EST PERTINENTE ET LES RÉSULTATS OBTENUS PEUVENT AVOIR UN IMPACT IMPORTANT SUR LE DÉVELOPPEMENT DE PRODUITS THÉRAPEUTIQUE VISANT À AMÉLIORER LA RÉPONSE ANTIVIRALE CTL.

FAIBLESSES :

Réseau SIDA/MI

CRITÈRES DE SÉLECTION - GREVENYNGHE → JC

Modèle des Instituts de recherche en santé du Canada / IRSC			
	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

Critère	Note
1. RATIONNEL *(1.1 Mise en contexte et* *1.2 importance de l'étude proposée)*	4.2
2. HYPOTHÈSE *(Veuillez énoncer clairement* *2.1 l'hypothèse principale du projet et* *2.2 les hypothèses secondaires, le cas échéant).*	4.2
3. OBJECTIFS *(Veuillez spécifier* *3.1 les objectifs principaux, et* *3.2 les objectifs secondaires, le cas échéant).*	4.2
4. MÉTHODOLOGIE *(Veuillez spécifier* *4.1 si des outils ou des méthodologies innovantes seront utilisées,* 4.2 *Veuillez* préciser la faisabilité, *4.3 les difficultés potentielles et* *4.4 les mesures d'atténuation, lorsque pertinent)*	4.2
5. ANALYSES ET RÉSULTATS ANTICIPÉS *(Veuillez préciser* *5.1 les méthodes statistiques qui seront utilisées* *5.2 et énoncer les résultats attendus).*	4.2
6. ÉCHÉANCIER *(Veuillez identifier* *6.1 les étapes à suivre pour l'atteinte des objectifs.)*	3.4

Réseau SIDA/MI

Grevenynghe 57 DC

Modèle des Instituts de recherche en santé du Canada / IRSC			
	Impact potentiel	**Intervalle**	**Mérite scientifique**
NOM DU PARTICIPANT : GREVENYNGHE			**TOTAL** = 4.07

nf

FORCES : An interesting perspective as to the potential regulatory function of IL-21 in modulating CTL effectiveness in HIV patients. The link to autophagy is certainly worth investigating. The PI hypothesizes that exogenous administration of IL-21 may improve CTL responses in HIV patients. The basis of this is that EC have normal IL-21 production.

FAIBLESSES : The effects of IL-21 are highly pleiotropic and it may be challenging to properly isolate or attribute improved CD8+ function solely to autophagy. Furthermore, it is well known that IL-21 therapy helps to maintain CD8+ function for HIV and SIV infections. Therefore, the hypothesis as it is currently postulated, does not appear to be very novel as the effects of IL-21 therapy on HIV patients have been previously described. The hypothesis also appears to relate primarily to Aim 3 of the proposal.

In contrast, this proposal appears to focus heavily on dissecting the mechanisms and contributions of autophagy on CTL responses primarily in ECs - something different to the hypothesis, and definitely more novel and interesting. However, the proposal is written like an abridged CIHR grant proposal. The workload proposed is simply unrealistic for a 1-year project and lacks focus.

The PI should focus on answering a simple and clearly defined question with one or maybe two realistic objectives. This funding opportunity is designed to enhance the capabilities and breadth of a research project or research program and not constitute one in itself.