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Introduction: Lentiviral vectors (LV) represent a key tool for gene and cell therapy applications. The production of these vectors in sufficient quantities for clinical applications remains a hurdle, prompting the field toward developing suspension processes that are conducive to large-scale production. The HEK293 stable producer cell line employed in our present study addresses this by growing in suspension, thus offering direct scalability, and produces a green fluorescent protein (GFP)-expressing lentiviral vector in the $10^6$–$10^7$ transducing units (TU)/mL range in batch culture without optimization. We describe a LV production strategy using this stable inducible producer cell line grown in suspension using a novel perfusion process.

Objective: We sought to develop a scalable LV production process using a stable inducible HEK293 cell line, significantly outperforming routine LV production protocols in batch mode.

Method: In the process that we have developed, cells are retained in a 3.5L Chemap bioreactor operated in perfusion mode using either a BioSep acoustic cell filter (Applisens) or a VHUÔ Perfusion Filter (Artemis Biosystems). The process that was developed employs perfusion mode to reach higher cell densities and to drive productivity. Cultures were grown up to $1–1.5 \times 10^6$ cells/mL in batch mode. Perfusion was started at 0.5 volume of medium per reactor volume per day (VVD), and increased to up to 1 VVD after induction. The culture was induced after reaching the target cell density ($5 \times 10^6$ cells/mL). After induction, perfusion mode (1 VVD) was started with fresh medium containing inducers for 3-4 days. In all perfusion runs, harvests were collected, and the LV-containing supernatant was kept on ice or at 4 °C until clarification (once daily) and subsequently stored at -80 °C until quantification using the GTA assay.

Conclusion: Our study demonstrates that LV production in perfusion mode using the VHU filter is outperforming our routine perfusion approach using an acoustic cell filter. Using this novel device, the cumulative functional LV titers were increased by up to 30-fold compared to batch mode, reaching a cumulative total yield of $>2 \times 10^{11} \text{ TU/L}$ of bioreactor culture (Figure 1). This approach is easily amenable to large scale production and commercial manufacturing. Next steps will include applying this process to non-model LV products, such as LV expressing chimeric antigen receptors (LV-CAR).
Merocytic dendritic cell: a new subset of conventional dendritic cells

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Introduction: Conventional dendritic cells (cDC) are potent antigen-presenting cells that induce the activation of naïve T cells in response to pathogens. cDC activity is mediated primarily by two cDC subsets, namely cDC1 and cDC2, each bearing unique properties. Recently, another DC subset, termed merocytic dendritic cells (mcDC), was defined. In contrast to both cDC1 and cDC2, mcDC are able to reverse T cell anergy, even in non-inflammatory conditions, properties that could be exploited to potentiate cancer treatments.

Objectives: Here, we further characterize mcDC to determine their relationship to cDCs.

Methods and results: First, we demonstrate that mcDC express key cDC traits, namely they express the cDC-restricted transcription factor, Zbtb46, and are very potent inducers of mixed lymphocyte reactions. Second, transcriptomic studies reveal that mcDC are more closely related to cDC1 than to cDC2. In contrast, similar to cDC2, mcDC are dependent on IRF4, but not IRF8 and BATF3, two major transcription factors required for cDC1 differentiation. Third, investigating mcDC population dynamics in reconstitution kinetics studies and in parabiotic mice, we demonstrate that, as for cDC1 and cDC2, mcDCs are terminally differentiated cells.

Conclusion: Altogether, these data demonstrate that mcDC compose novel cDC subset. Defining the properties of mcDC in mice may help identify a functionally equivalent subset in humans leading to the development of novel cancer immunotherapies.
**7 - Gene therapy and tissue engineering: a strategy to treat recessive dystrophic epidermolysis bullosa**

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**Introduction:** Recessive dystrophic epidermolysis bullosa (RDEB) is a rare and severe genetic disease in which minor mechanical stress in the skin causes the formation of blisters and erosions. RDEB is caused by a wide variety of mutations in the \(COL7A1\) gene – encoding type VII collagen. Type VII collagen is normally secreted into the extracellular space by dermal fibroblasts and epidermal keratinocytes and forms anchoring fibrils at the dermal-epidermal junction (DEJ). \(COL7A1\) mutations leads to defective anchoring fibrils ultimately resulting in a loss of adhesion between the epidermis and the dermis. Production of autologous skin substitutes from genetically corrected patient’s cells, and their subsequent graft, has the potential to be a suitable treatment to the permanent skin lesions of RDEB patients.

**Objectives:** We have generated a high-titer \(293\text{Vec}\) retrovirus cell clone to produce a self-inactivating (SIN) vector that allows the delivery of the \(COL7A1\) gene into keratinocytes and fibroblasts. The objectives were to obtain a high transduction efficacy for both keratinocytes and fibroblasts, while maintaining the stem potential of keratinocytes, to restore type VII collagen production, and to produce skin substitutes from corrected cells.

**Methods:** RDEB cells producing no type VII collagen were amplified and transduced using a SIN \(COL7A1\) vector. Transduction efficacy was assessed through immunostaining and flow cytometry. Transduced stem keratinocytes were quantified using keratin 19 as a stem marker. These corrected cells were used to produce skin substitutes using the self-assembly approach, and were analyzed by immunofluorescence. The adhesion of the epidermis to the dermis was measured by mechanical peeling tests.

**Results:** We observed that viral particles infected the keratinocytes and fibroblasts of this RDEB patient with a transduction rate up to 40% and 70% respectively (single transduction). Up to 80% of keratin 19-expressing keratinocytes were successfully transduced and maintained in culture. Skin substitutes were produced from the transduced cell populations and we observed the restoration of type VII collagen at the DEJ. The adhesion of the epidermis to the dermis was also restored compared to skin substitutes produced from RDEB mutated cells.

**Conclusion:** We developed an efficient method to restore type VII collagen production in a RDEB patient’s cells using a SIN \(COL7A1\) retroviral vector. Stem cell keratinocytes were effectively transduced and observed in the epidermal layer of the skin substitutes. Adhesion of the epidermis to the dermis was also restored compared to controls. In conclusion, our results indicate that this method might be suitable for the permanent treatment of RDEB skin lesions.
**INTRODUCTION:** Adeno-associated virus (AAV) is proving to be a powerful tool for gene transfer in gene therapy applications. As of January 2018, 204 clinical trials used AAV as a tool for gene transfer which ranks it as number 4 amongst all the vectors used for gene therapy applications.

**METHODOLOGY:** One of the most common methods of AAV production is by adenovirus-free transient transfection of HEK293 cells with plasmids carrying necessary components for vector assembly and gene of interest (GOI). Adenovirus components are supplied by the stably integrated E1A and E1B genes present in the 293 cell line, and other components, such as, E4, E2A and VAI, are provided by transient transfection along with Rep/Cap and GOI. The Rep gene encodes four Rep proteins (Rep 78, 68, 52, 40) under p5 and p19 promoters which are activated by the proteins expressed by E1A gene present in 293 cells. The relative importance of each Rep for AAV production is not fully understood.

We report here the key Rep proteins that are necessary for the AAV production by first generating four plasmids expressing individual Rep proteins (Figure 1) and later producing AAV with various combinations of these plasmids along with Capsid and Adeno-helper plasmids. The p19 is located within the coding region of Rep78 and Rep68, therefore, this sequence was modified without changing the amino acid sequence to prevent expression of Rep52 and Rep40 (Figure 2). Because high level of expression of Rep could be toxic to the cells, we control their expression using the Cumate inducible promoter (CMV5CuO), which is repressed by the presence of the cumate repressor (CymR).

To determine which Rep proteins are essential, a transient transfection was done using a combination of plasmids encoding Rep78, 68, 52, and 40. HEK293 cells and HEK293 cells expressing the CymR repressor (HEK293-CymR) were used for these experiments.
9 - New Cell Type-Specific Promoter Enable Gene Therapy Using Chimeric Antigen Receptor in Hematopoietic Stem Cells to Have Long-Lasting Anti-Tumor Effect

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Introduction: Although efficient, Chimeric Antigen Receptor (CAR)-T cells therapy still presents several drawbacks including exhaustion and loss of engineered cells, and the cytokine release syndrome. To circumvent these issues, we envision to engineer hematopoietic stem cells (HSCs) to provide a continuous replenishment of CAR-T and CAR-NK cells. However, this would result in a potentially dangerous pan-hematopoietic expression of the CAR transgene.

Objective: To avoid this, we designed a T-cell specific and a NK-cell specific synthetic promoter in order to restrict the CAR expression only to the cytotoxic cells progeny issued from CAR-modified HSCs. We assessed the efficacy of our promoters in vitro and in vivo in humanized mice.

Methods: Potential sequences for T-cell or NK-cell specific expression were designed in silico and then cloned in a GFP-reporter vector to test their specificity. We assessed in vitro the expression of the reporter gene using cell lines from various lineages and primary cells isolated from peripheral blood. We then transduced CD3⁴⁺ cell and humanized NSG mice engrafted with a human thymus.

Results: Upon transfection with GFP under the control of our synthetic promoter, only Jurkat (T cells) for our T-specific promoter and NK-92 (NK cells) for the NK-specific promoter, expressed GFP. Consistently, nucleofection of PBMC with the T-cell specific promoter resulted in a GFP expression in T cells but not in B cells or monocytes. The promoter specificity was validated in vivo as progeny from engineered human HSCs injected in NSG showed the same T-cell specific expression pattern (Fig 1). Using an in vitro artificial organoid thymic differentiation system, we observed that our synthetic promoter was active as early as CD7⁺CD1 progenitor cells. We also showed that T cells transduced with our specific promoter retained CAR T-cell toxicity when facing their targets, despite a lower CAR expression than with classic strong promoter.

Conclusion: Our results show that we were able to generate new synthetic promoters with a lineage specificity. We are currently testing our strategy in vivo against human B-ALL cells lines and primary patients’ blasts. We are also validating our new NK-specific promoter in vivo. This new strategy could help overcome side effects while improving CAR-T cells persistence, and could be used for both hematological malignancies and solid tumors after autologous transplantation.
5 - LA MUTATION D’UN GENE REGULANT LA RESPIRATION MITOCHONDRIALE IMPACTE L’HOMEOSTASIE IMMUNITAIRE.

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Le syndrome de Leigh Franco-Canadien (LSFC) est une maladie génétique caractérisée par la survenue de crise d’acidose métabolique soudaine qui augmente considérablement la gravité et la mortalité. Ce syndrome est causé par la mutation du gène LRPPRC. Ce gène régule la stabilité de l’ARNm de nombreuses protéines mitochondriales impliquées dans l’assemblage des chaînes respiratoires. La mutation du gène LRPPRC module donc la respiration mitochondriale qui est connue pour orienter la réponse immunitaire. De façon surprenante les crises d’acidoses métaboliques chez les patients LFSC surviennent souvent à la suite d’infections. Ces résultats mettent en évidence la probable contribution du système immunitaire dans le déclenchement des crises d’acidoses métaboliques.

OBJECTIF: Comprendre le rôle du gène LRPPRC sur les fonctions immunitaires, à l’aide de différents modèles murins.

MÉTHODES: Nous avons généré divers modèles murins. Le phénotype embryonnaire léthal des souris LRPPRC\textsuperscript{-/-} nous a amené à générer un modèle de déplétion conditionnel : LRPPRC\textsuperscript{fl/fl} x Mx1-Cre. Nous avons aussi généré des souris LRPPRC-A354V-KI+/-, qui porte la mutation orthologue à celle identifiée chez les patients LSFC.

RÉSULTATS: La délétion du gène LRPPRC dans la souris LRPPRC\textsuperscript{fl/fl} x Mx1-Cre adulte résulte à une déplétion de nombreuses cellules immunitaires. Chez les souris LRPPRC-A354V-KI+/- nous avons aussi observé des dérégulations immunitaires.

CONCLUSIONS: Nos données préliminaires suggèrent que la délétion de LRPPRC, qui perturbe la respiration mitochondriale, influence l’homéostasie immunitaire. Nous étudions actuellement son rôle dans le déroulement des réponses immunitaires. Reste à savoir si ces observations pourront être généralisées à d’autres syndromes métaboliques.
Enhancement of Natural Killer (NK) Cell Cytotoxic Activity by CRISPR-targeted Gene Knockout Platforms

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Introduction: Cancer is a major health problem throughout the world and traditional treatments faces resistances issues. Thus there is an emergence of treatments based on immunotherapy which consists in amplifying the immune response of patients to eliminate cancer cells. Natural Killer (NK) cells have the inherent ability to kill infected and malignant cells and thus represent an attractive cell population for cancer cellular immunotherapy. Unfortunately, several cancers may escape NK cell elimination by expressing NK-cell inhibitory ligand.

Aim: We proposed to invalidate, by CRISPR, genes coding for NK inhibitory proteins, in order to increase NK cell cytotoxic activity against cancer cells. The non-classical human leukocyte antigen E (HLA-E) expression is frequently overexpressed in tumor diseases and represents an immune suppressive molecule by binding to NKG2A on NK and T cells. We therefore targeted first the NKG2A gene in NK cells.

Methods: Feldan Therapeutics developed an innovative and novel peptide-based technology, named Feldan shuttle (FS), that allows the direct administration of gRNA-CRISPR nuclease complexes in NK cells, without any viral integration into the host genome. We first applied the CRISPR methods to knockout NKG2A gene with the nuclease Cpf1 on amplified human NK cells. Then we tested the efficacy of this strategy on a breast cancer cell line (MDA-MB231) on which we forced the expression of NKG2A ligand: HLA-E and its peptide.

Results: We were able to obtain a ~23% decrease of NKG2A expression compared to control cells, resulting in a ~60% NKG2A expressing cells among the NK cell population. This NK cell population was able to kill HLA-E+ as efficiently as HLA-E breast cancer cells, contrary to control NK cells which displayed a reduced cytotoxic activity toward HLA-E+ cells (in a ratio-dependent manner). We then sorted NKG2A+ and NKG2A-negative NK cells from control and CRISR-NK cells. Both NKG2A+ cell population, from control and CRISPR-NK cell populations, kept a 100% expression of NKG2A. Concerning NKG2A-negative cell population, the one from control cells were re-expressing ~40% of NKG2A among the population but the CRISPR-NK cell one were only re-expressing ~15% of NKG2A. We observed a correlation between the lytic activity against HLA-E- or HLA-E+ target cells and the percentage of NKG2A-negative NK cells. In addition, we noted that this correlation was observed under the ratio of 40% of NKG2A-negative cells while a plateau was reached after 40%, suggesting that a total knock-out of NKG2A may not be necessary to observe the maximum lytic capacity of the bulk of NK cells.

Conclusion: NKG2A shut down by CRISPR technology via a new method of shuttling in NK cells was associated with an increased cytotoxicity towards HLA-E+ cancer cells. Moreover, our data suggest that a pure NKG2A- NK cell population is not needed to outdo the HLA-E shield of cancer cells. This data could open new avenue in cancer cellular immunotherapy with NK cells.
3 - Base editing strategy allows high frequency insertion of the protective A673T mutation in the APP gene to prevent the development of Alzheimer's disease

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There are currently 47.5 million cases of Alzheimer's disease (AD) in the world and there will be 75.6 million cases in 2030 according to the World Health Organization. Amyloid precursor protein (APP) is usually cut by the alpha-secretase, however an abnormal cut by beta-secretase leads to the accumulation of beta-amyloid peptides, which form plaques in Alzheimer patient brain. Numerous APP gene mutations favour the accumulation of plaques. However, it was discovered that a variant of the APP gene (A673T) in Icelanders reduces by 40% beta-secretase cutting and prevents the development of AD in older person (more than 95 years). We hypothesized that the insertion of this mutation in the patient genome would be an effective and sustainable treatment to slow down the progression of sporadic and familial Alzheimer’s disease forms (FAD).

The objective of our project was in a first time to show the protective effect of A673T in a FAD APP gene and determine against which mutation the treatment was the most effective. Secondly, we wanted to achieve a permanent correction by base editing to insert the A673T mutation and obtain evidence of the reduced formation of amyloid plaque.

A Plasmid containing the APP gene mutated for a FAD was transfected in neuroblastoma SH-SY5Y and the supernatant was harvested 72 hours later. Another plasmid containing this mutation in addition of the A673T mutation was transfected in parallel. Every known FAD mutation in exon 16 and 17 of APP were tested. The concentrations of Aβ peptides were quantified with double antibody sandwich Elisa.

Next, we introduced A673T mutation by base editing with the CRISPR/Cas9 system in HEK 293T cells and SH-SY5Y neuroblastoma. The results were characterized and quantified by Deep Sequencing.

The Aβ peptides concentration was decreased in most of the cases when the A673T mutations was inserted up to 80%. We also succeeded to edit the A673T mutation in up to 57% of the APP genes. Our approach aims to attest the protective effect of A673T and the efficiency of base editing in the development of an Alzheimer’s disease treatment.
2 - A Baboon Envelope to transduce NK cells

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Introduction: NK cells, with their intrinsic ability to recognize and kill tumor cells represent an interesting tool for immunotherapy. Although infusions of activated NK cells are promising immunotherapy that are safe and well tolerated, they need to be improved. The modification of NK cells with chimeric antigen receptors (CAR) could improve their functions.

Objectives: The aim of this project was to develop an efficient transduction technique and produce NK cells expressing CARs.

Methods: Freshly isolated NK and NK obtained from the NK cell Activation and Expansion System (NKAES) were transduced with lentiviral vectors pseudotyped with different envelope glycoproteins. NK cells were then expanded using the NKAES system for 14-21 days. GFP or CAR expression was assessed by flow cytometry. Viral receptor expressions were evaluated by RT-PCR and flow cytometry. The effect of CAR expression was tested in cytotoxicity against a NK resistant pre-B-ALL leukemia cell line. VSV-G-LVs resulted in poor NK transduction rate (0,28±0,12%) while RD114- and MV-LVs performed better (21±2,8% and 13±4,7%, respectively). The use of BaEV-LVs outperformed them all with a transduction rate mean of 30±2,2% in freshly isolated NK and 87±6.7% in NKAES, even at low MOI. Transgene expression was sustained for at least 21 days. BAEV receptor (ASCT1/ASCT2) expression patterns were consistent with transduction efficacy. The expression of a third generation anti-CD22 CAR or the expression of two CARs simultaneously allowed the killing of pre-B-ALL cells.

Conclusions: This BAEV-LVs will allow the development of NK-based immunotherapies such as CAR-NK cells which are a promising cancer therapy.
4 - Characterization of the implication of the Idd2 locus in type 1 diabetes development

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Introduction:

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of the beta cells of the pancreas. Genetic susceptibility to T1D is conferred by complex traits. More than 20 genetic loci, termed Idd for “insulin dependent diabetes”, are linked to T1D in the NOD mice, a model which spontaneously develops T1D. The second genetic locus, namely Idd2, was identified in the 1980s, but its contribution to T1D susceptibility was never validated.

Objectif et méthode: To assess the specific contribution of genetic variants of the Idd2 locus to T1D, we generated a NOD.B10-Chr9 congenic mouse, where the Chr9 locus encompasses part of the Idd2 locus. Comparing our newly generated congenic strain (NOD.B10-Chr9) to the NOD strain enables us to assess the specific contribution of Idd2 in T1D development.

Conclusion:

Using this congenic strain, we validate that Idd2 confers resistance to insulitis and, consequently, to T1D in the NOD mouse. By generating bone marrow chimeras and spleen cell transfers, we find that the resistance is conferred in a bone marrow-intrinsic manner. Moreover, we demonstrate that Idd2 contributes to the formation of germinal centers as well as the expression of the major histocompatibility complex (MHC) on B cells. Together, these data not only validate Idd2 as a genetic locus contributing to T1D susceptibility, it strongly supports recent evidence of the contribution of the humoral response in T1D susceptibility.
INTRODUCTION: Gene therapy is emerging to become one of the most promising therapies to effectively treat multiple types of diseases. This type of treatment allows to replace/repair and/or knock out genes that are responsible for certain disorders as well as to insert new genes. Viral vectors with their ability to accurately deliver nucleic acid into the cell have been a tool of choice for efficient gene therapy. The recently FDA approved gene therapy from Spark therapeutics, Luxturna, is a viral vector based gene therapy and many biotech are following in its wake.

DISCUSSION: As a consequence, manufacturers are having a difficult time to fulfill the demands of viral vector biomanufacturing compliant with Good Manufacturing Practices (cGMP – recommended for clinical trials) and many companies and researchers alike will face more than 1 year delay to obtain their cGMP vector. Recognizing this need and to accelerate cell and gene therapies in Quebec, the Center of Excellence for Cellular Therapy (CETC), located at Hospital Maisonneuve Rosemont (Montréal), is currently developing a cGMP Gene Therapy Platform (GTP) to meet the needs of clinical grade viral vectors.

The CETC is a fully operational state-of-the art cGMP manufacturing facility and is one of the bio-units represented by the Center for Commercialisation of Cancer Immunotherapy (C3i) located within the Hospital Maisonneuve-Rosemont. As a partner, C3i is aiming at accelerating the cell therapy field and clinical applications from researchers to small biotech and pharma companies. The CETC facility has received the highest certification from the Foundation for the Accreditation of Cell Therapies (FACT, USA) for “more than minimally manipulated cells” and is the only academic GMP-compliant manufacturing center in Canada that meets expectations from Health Canada, EMA and the FDA regulatory agencies.

CONCLUSION: C3i is offering a privileged access to GMP-compliant viral vector manufacturing using our local Gene Therapy Platform supported with excellent expertise. Our Commercialisation Center is able to provide fast and efficient small to medium viral vectors scale production, with the clear goal of accelerating translation of research into clinical trials to position Canada as a leader in Cell and Gene Therapy.
CRISPR-Cas9-mediated edition of the human TRIM5 gene increases the resistance of Jurkat T lymphocytic cells to HIV-1 infection

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Introduction: TRIM5α is a cytoplasmic antiviral effector induced by type I interferons (IFN-I) present in many mammals. It intercepts retroviruses soon after entry by interacting with the capsid core, inducing the uncoating of the core and the degradation of their genome. Inhibition of retroviral replication by TRIM5α is efficient, therefore a successful retroviral infection necessitates that the virus escapes recognition by TRIM5α; this is achieved by mutations in the capsid protein. Human TRIM5α generally does not interact efficiently with HIV-1 capsids. We previously showed, however, that two points mutations, R332G/R335G, in the capsid-binding region confer human TRIM5α the capacity to target and strongly restrict HIV-1 upon over-expression of the mutated gene by gene transfer.

Objectives: Here, we explored the possibility to introduce these two mutations in the endogenous human TRIM5α gene by CRISPR-Cas9-mediated gene editing. This approach has several advantages over gene transfer, primarily the fact that the therapeutic gene would be expressed at physiological levels and would be inducible by IFN-I.

Methods: We electroporated CRISPR ribonucleoproteins (RNPs) and the donor DNA into Jurkat T lymphocytic cells and isolated clones by limiting dilution. In a single experiment, we analyzed 47 clones using a PCR assay specific for TRIM5α bearing the desired mutations. In a second round of analysis, we performed PCR followed by digestion with a restriction enzyme that cuts only the correctly mutated alleles. We found that 6 clones (13 %) contained at least one gene-edited allele, and one clone (clone 6) seemed to have both alleles edited. Upon challenge with an HIV-1 vector, clone 6 was significantly less permissive (8-fold) compared to unmodified cells, whereas the cell clones with monoallelic modifications were only slightly less permissive. Following IFN-β treatment, inhibition of HIV-1 infection in clone 6 was even more striking: a 40-fold decrease in the percentage of HIV-1 infected cells was measured in comparison to unmodified cells.

Conclusion: In summary, we demonstrate the feasibility of potently inhibiting HIV-1 through the edition of TRIM5α, but our results also emphasize the importance of biallelic modification in order to reach significant levels of inhibition.
Gene edition of CCR5 locus for T-cell based immunotherapy

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Introduction: To further expand the field of T-cell based immunotherapy, we have developed an “in-house” platform which allows the safe knock-in of a transgene in the chemokine receptor 5 (CCR5) gene with the use of the CRISPR/Cpf-1 nuclease and an adenovirus-associated virus (AAV) bringing the recombination template. The CCR5 locus was chosen as a target since it appears to have no disadvantages in terms of health if not expressed and protect from HIV-1 infection and disease progression as well.

Objective: To develop a platform of CRISPR gene editing at the CCR5 locus for expanding T-cell based immunotherapy.

Methods: We have designed a single guide RNA (sgRNA CCR5) for the Cpf1 nuclease targeting the second exon of the CCR5 gene with great efficiency (above 70% of nuclease activity in different cells lines and primary cells such as HSCs). Interestingly, usual algorithms (COSMID and TEFOR) predict only 4 potential off-target sites for the chosen sgRNA that are all located in intergenic sequences that are devoid of DNase I hypersensitivity sites, suggesting that they don’t participate to gene regulation. We have screened potential off-target sites through Tracking of Indels by Decomposition (TIDE) in THP1 (ATCC) and Jurkat E6.1 cells. We only found a low frequency (2.5%) deletion of 1 nucleotide at the position 89024124 of the chromosome 7 in THP1 cells but not Jurkat E6.1 cells suggesting a good specificity of our sgRNA (as compared to previous report on the off-target effect of the Cas9 nuclease in the context of gene therapy). We also generated on a pAAV backbone a homology donor template comprising 5' and 3' homology arms for the CCR5 locus flanking a multiple cloning site (MCS). From this MCS backbone, we have generated four pAAV donor templates: (i) pAAV- P2AmaC46 containing the membrane anchored C46 transgene – an HIV inhibitor – under the control of the CCR5 endogenous promoter, (ii) pAAV-P2AGFP containing a GFP cassette under the control of the CCR5 endogenous promoter, (iii) pAAV-sp-GFP containing a GFP cassette under the control a synthetic promoter and (iv) pAAV-pIL2-GFP containing a GFP cassette under the control a synthetic IL2 promoter. As a proof of principle, we tested our gene editing strategy in human T-cell isolated by magnetic separation (EasySep™ Human T Cell Isolation Kit - STEMCELL Technologies). The T-cells were stimulated with CD3/CD28 Dynabeads\textsuperscript{®} (ratio 1:1) in RPMI 10% FBS supplemented with 100 UI/mL IL-2. After 3 days of stimulation, we electroporated the T-cells alongside the Cpf-1 nuclease, the CCR5 guide and the various pAAV plasmides. We detected transgene expression at significant level by both flow cytometry and specific PCR.

Conclusion: We plan to use our strategy in early T-cell progenitors generated from hematopoietic stem cells differentiated on OP9-DL4. Hopefully, this approach will permit to provide a long-lasting source of gene-modified T-cells. Furthermore, the conception of our recombination template offers a wide range of therapeutic possibilities thanks the plasticity its MCS desig