

RNA-based strategies for dendritic cell reprogramming and uses thereof.**Technical field**

5 The present invention relates to compositions and methods for reprogramming cells to type 1 conventional dendritic cells or antigen-presenting cells.

Background

Cellular reprogramming relies on rewiring the epigenetic and transcriptional network of
10 one cell state to that of a different cell type. Transcription factor (TF)-overexpression experiments have highlighted the plasticity of adult somatic or differentiated cells, providing new technologies to generate any desired cell type. Through forced expression of TFs, it is possible to reprogram somatic or differentiated cells into induced pluripotent stem cells (iPSCs) that are remarkably similar to embryonic stem
15 cells (Takahashi and Yamanaka 2006; Takahashi et al. 2007). Alternatively, a somatic cell can also be directly converted into another specialized cell type (Pereira, Lemischka, and Moore 2012; Wang et al. 2021). Direct lineage conversion has proven successful to reprogram mouse and human fibroblasts into several cell types, such as neurons, cardiomyocytes and hepatocytes, using TFs specifying the target-cell identity.
20 Direct cell conversions were also demonstrated in the hematopoietic system, where forced expression of TFs induced a macrophage fate in B cells and fibroblasts (Xie et al. 2004) and the direct reprogramming of mouse fibroblasts into clonogenic hematopoietic progenitors was achieved with Gata2, Gfi1b, cFos and Etv6 (Pereira et al. 2013). Traditionally, cell reprogramming experiments have relied on the use of
25 lentiviral vectors to deliver transcription factors to target cells. However, the use of lentiviral vectors is associated with high costs and safety concerns due to their integrative nature (Schlimgen et al. 2016). RNA has emerged as a promising non-viral vector for delivery of transcription factors as it allows the highest cell reprogramming efficiency when compared with other non-viral non-integrative delivery systems (Bailly, Milhavet, and Lemaitre 2022). Although linear modified mRNA (modRNA) has been
30 previously used to induce pluripotency in human fibroblasts (Mandal and Rossi 2013; Warren et al. 2010), its low and transient transgene expression and the requirement for multiple transfections still limit the applicability of this approach (Yoshioka et al. 2013). To circumvent these challenges, self-replicative/self-amplifying RNAs (srRNAs or saRNAs) were developed based on self-replicating single-stranded RNA viruses such
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as the Venezuelan equine encephalitis virus (EEV) RNA replicon. saRNA was shown to allow extended and higher transgene expression when compared to modRNA, allowing at the same time higher reprogramming efficiency to pluripotency, particularly in the presence of interferon inhibitors (Yoshioka et al. 2013). More recently, trans-amplifying RNA (taRNA) has been developed to bypass challenges of associated with saRNA size, production and scalability (Lundstrom 2023). taRNA is composed of a split-vector derivative of saRNA combining a non-replicating mRNA encoding an alphaviral replicase and a transreplicon RNA encoding for the therapeutic gene. Beside modRNA, saRNA and taRNA, circular RNA (circRNA) has also recently emerged as a gene delivery platform (Chen et al. 2023). CircRNA is a single-stranded RNA in which the 5' and 3' ends join to form a covalently closed loop. The circular structure confers multiple functional benefits, including improved stability, longer expression duration and easier upscaling. Although multiple RNA-based platforms have been shown to allow delivery of therapeutics genes, only modRNA and saRNA have been used for induction of reprogramming to pluripotency (Warren et al. 2010; Mandal and Rossi 2013; Yoshioka et al. 2013; Yoshioka and Dowdy 2017), and only modRNA has been shown to allow direct cell fate reprogramming (Preskey et al. 2016; Murthy et al. 2015; Van Pham et al. 2017; Connor et al. 2018).

Reprogrammed cells are very promising therapeutic tools for regenerative medicine, and cells obtained by differentiation of iPSCs are already being tested in clinical studies (Pires et al. 2019). It has been demonstrated that antigen-presenting dendritic cells (DCs) can be programmed from fibroblasts, stromal cells and cancer cells, after overexpression the transcription factors PU.1, IRF8 and BATF3 mediated by lentiviral vectors (Rosa et al. 2022; 2018), opening the opportunity to apply cell reprogramming to modulate immune responses and develop new immunotherapies. More recently, the same combination of transcription factors was shown to allow reprogramming of mouse and human cancer cells, including primary cancer samples, to functional cDC1-like cells able to present endogenous tumor antigens and mount efficient anti-tumor immunity in immune-checkpoint inhibitor (ICI)-resistant models upon in-vivo transfer (Zimmermann et al. 2023). These studies opened the opportunity to apply cell reprogramming to modulate immune responses and develop new immunotherapies.

Classically, the DC compartment can be divided in two functionally different DC subsets: conventional DCs (cDCs), which are professional antigen-presenting cells (APCs), and plasmacytoid DCs (pDCs) (Wculek et al. 2019). While pDCs are professional producers of type I interferons during viral infection, cDCs drive antigen-

specific immune responses by presenting exogenous antigens to T cells. The cDC subset can be further divided in myeloid/conventional DC type 1 (cDC1 or DC1s) and myeloid/conventional DC type 2 (cDC2). While cDC1 are specialized in antigen cross-presentation and priming of antigen-specific cytotoxic CD8⁺ T cells, cDC2s excel on their ability to present antigens to CD4⁺ T cells, priming antigen-specific T helper 2 (Th2), Th17 and regulatory T cell responses. This expands the flexibility of the immune system to react appropriately to a wide range of different pathogens and danger signals.

Human cDC1s, characterized by surface expression of CD141, CLEC9A, XCR1 and CD226 (Wculek et al. 2019; Heidkamp et al. 2016), are defined functionally by secreting immune-modulatory cytokines, including IL-12, and interferons (IFN), and chemokines such as CXCL10, and by cross-presenting antigens to CD8⁺ T cells (Lauterbach et al. 2010; Poulin et al. 2010). In the context of anti-tumor immunity, *Batf3*^{-/-} animals lacking cDC1s fail to reject immunogenic tumours (Hildner et al. 2008). This effect was shown to depend on tumor-resident cDC1s, highlighting the importance of this DC subset at the tumor site to mediate immune-rejection of established tumours (Böttcher et al. 2018) (Bottcher et al., 2018) and response to therapy (Salmon et al. 2016; Spranger et al. 2017). Accordingly, cDC1 abundance in human tumours was associated with patient survival and responsiveness to checkpoint inhibitors (Barry et al. 2018; Broz et al. 2014). Human primary cDC1s are very rare *in vivo*, so their study and translational applications require methods to generate functional cDC1s *in vitro*. Human CD34⁺ bone marrow (BM) progenitors have been used to derive CD141⁺ DC1s *in vitro* in the presence of FLT3L with SCF, GM-CSF and IL-4 (Poulin et al. 2010). More recently, FLT3L was combined with co-culture with Notch-expressing stromal cell lines to favour cDC1 differentiation (Kirkling et al. 2018; Balan et al. 2018). The generation of cDC1-, cDC2- and pDC-like cells from induced pluripotent stem cell (iPSC) cultures was also demonstrated (Sontag et al. 2017). However, these protocols are complex, require feeder layers and result in low yields as well as a mixture of different DC subsets with conflicting functions.

Although transcription factors are recognized as master regulators of cell fate and represent an exciting avenue to generate cDC1s for therapy, other non-coding factors, such as microRNAs (miRNAs), are also interesting reprogramming factors due to their described role in cell differentiation and reprogramming (Pascale et al. 2022). miRNAs are short RNAs approximately 22 nucleotides long, which repress target mRNA

transcripts with seed sequence complementarity through either mRNA cleavage or translational repression (O'Brien et al. 2018). Their canonical biogenesis starts in the nucleus, where Drosha ribonuclease cleaves the primary (pri)-miRNA RNA sequence, resulting in a pre-miRNA hairpin which is exported to the cytoplasm and cleaved again

5 by the RNase III enzyme Dicer, whereupon one or both hairpin strands are preferentially loaded into Argonaute proteins, which mediate their function. miRNAs have been successfully used to promote reprogramming towards induced pluripotent stem cells (iPSCs) with the miR-302/367 cluster (Anokye-Danso et al. 2011), neurons with miR-9/124 (Yoo et al. 2011), and cardiomyocytes with miR-1/133a/208a/499

10 (Jayawardena et al. 2012). Additionally, as short non-coding RNAs, miRNAs can easily be delivered by viral vectors, as well as by non-viral vectors (e.g., RNA) alongside transcription factors and small molecules (Kogut et al. 2018; Mueller et al. 2020). miRNAs can allow or facilitate reprogramming by recapitulating their known roles in development (DeVeale, Swindlehurst-Chan, and Blelloch 2021; Ivey and Srivastava

15 2015), by targeting specific reprogramming barriers (Pascale et al. 2022; Abernathy et al. 2017; Lee et al. 2018) between others.

The biogenic pathway of miRNAs can generate non-archetypal variants called isomiRs from the same pre-miRNA strand, including 5' isomiRs with 5'-offsets resulting in an altered seed sequence and target set (Morin et al. 2008; Wagner et al. 2024). isomiRs

20 are increasingly recognized for their role in cancer, diabetes, and immune disorders (Wagner et al. 2024). For example, the -1 isomiR of miR-142-3p (a hematopoietic regulator (Sun et al. 2015) is often expressed at higher levels than its canonical strand (Karlsen et al. 2019; Manzano et al. 2015; Aparicio-Puerta et al. 2023). While tools for isomiR detection and annotation are now available (Wagner et al. 2024), validation

25 studies in the context of development remain challenging due to the difficulty of strand-specific perturbation in complex culture systems and in vivo models. Direct reprogramming offers a unique possibility for isomiR characterization.

However, canonical miRNAs or isomiR mimics enabling the programming of immune cell fates have not yet been identified.

30 Thus, new RNA-based strategies are necessary to further optimize and increase the translatability, including the scalability, of direct cell fate reprogramming approaches towards immune cell fates, in particular cDC1 reprogramming to generate homogeneous populations of differentiated human cDC1s *in vitro* and *in vivo*.

Summary

Provided herein are compositions and methods for reprogramming cells into dendritic or antigen-presenting cells. The inventors have also discovered that co-expression of PU.1, IRF8 and BATF3 mediated by RNA allows direct reprogramming of cells and that efficiency can be significantly improved by co-transfection with miRNA mimics and by incubation with inhibitors of IFN signalling. The inventors have discovered that direct reprogramming of cells by expression of transcription factors PU.1, IRF8 and BATF3 can be significantly improved by co-expression of miRNAs or miRNA mimics. Further, the present invention also provides compositions of isomiRs for use in cell reprogramming, and methods thereof.

Therefore, a first aspect of the invention relates to a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of: PU.1, IRF8, and BATF3, wherein the RNA construct is selected from the group consisting of: self-amplifying RNA (saRNA), circular RNA (circRNA), non-modified RNA, modified RNA (modRNA) and trans-amplifying RNA (taRNA).

A second aspect of the invention relates to a composition comprising one or more miRNA mimics, wherein said one or more miRNA mimics are isomiRs, for use in cell reprogramming.

A third aspect of the invention relates to a method for reprogramming or inducing a cell, said method comprising the steps of:

- a. Contacting a cell with a composition comprising one or more constructs or vectors encoding a combination of at least two transcription factors;
 - b. Expressing the at least two transcription factors;
 - c. Contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;
- whereby the cell is reprogrammed or induced.

A fourth aspect of the invention relates to a method for reprogramming or inducing a cell into a dendritic cell or antigen-presenting cell, the method comprising the steps of:

- 5 a. Contacting a cell with a composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
- b. Expressing the at least two transcription factors;
- 10 c. Contacting the cells with a composition comprising one or more miRNAs, or primary miRNAs (pri-miRNAs) thereof, or miRNA mimics, or a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, or miRNA mimics;

whereby a dendritic cell or antigen-presenting cell is obtained.

15 A fifth aspect of the invention relates to a method for reprogramming or inducing a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of:

- a. Contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
- 20 b. Expressing the at least two transcription

 A sixth aspect of the invention relates to a method of improving cell reprogramming efficiency and/or fidelity of a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of the method for reprogramming or inducing a cell into a dendritic cell or antigen-presenting cell as described herein, and
25 wherein said method further comprises the steps of:

- a. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, HLA-ABC, and/or MHC-II in the cell, after the step of
30 expressing the at least two transcription factors;
- b. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, HLA-ABC, and/or MHC-II in the cell after the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, or miRNA mimics, or with a
35 composition comprising one or more construct(s) or vector(s)

encoding the one or more miRNAs, or pri-miRNAs thereof, or miRNA mimics;

- c. comparing the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, HLA-ABC, and/or MHC-II assessed in steps a. and b;

wherein a higher level of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II, preferably a higher level of expression of CD45 and MHC-II, even more preferably a higher level of expression of CD45 and HLA-DR after contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, or miRNA mimics, indicates increased cell reprogramming efficiency; and,

wherein a higher level of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II, preferably a higher level of expression of XCR1, even more preferably a higher level of expression of CD226 after contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, indicates increased cell reprogramming fidelity.

A seventh aspect of the present invention relates to a cell comprising the one or more RNA construct(s) as described herein.

An eighth aspect of the present invention relates to a reprogrammed or induced cell obtained by the method as described herein, preferably wherein the cell expresses CLEC9A, CD45, CD141, XCR1, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II.

A ninth aspect of the present invention relates to the composition, the cell, or the reprogrammed or induced cell as described herein, for use in medicine.

A tenth aspect of the present invention relates to composition, the cell, or the reprogrammed or induced cell as described herein, for use in the treatment of cancer or infectious diseases.

Description of Drawings

Figure 1. miRNA screening identifies miR-124, miR-126, miR-142 and miR-150 as enhancers of cDC1 reprogramming.

- 5 (A) 15 miRNA candidates were chosen based on their role in hematopoiesis or cell reprogramming and cloned into lentiviral vectors. miRNAs were co-transduced with PU.1, IRF8 and BATF3 (PIB) reprogramming factors, and induced dendritic cells (iDCs) screened for Clec9a-tdT⁺ reporter activation, and expression of CD45, MHC-II and XCR1. (B) Quantification of Clec9a-tdT reporter activation. (C) Reprogramming
- 10 efficiency measured by the percentage of Clec9a-tdT⁺ cells expressing antigen-presenting-cell markers CD45 and MHC-II. (n=3, mean + SD). (D) Reprogramming fidelity measured by the percentage of Clec9a-tdT⁺ cells expressing DC1-restricted marker XCR1. (n=2, mean + SD). (E) Flow cytometry plots showing Clec9a-tdT reporter activation and expression of CD45, MHC-II and XCR1 upon co-transduction
- 15 with miR-124 and miR-142. *P < 0.05, **P < 0.01, ****P < 0.0001 one-way ANOVA with Dunnett's multiple comparison.

Figure 2. miR-124 and miR-142 additive reprogramming effects do not impair reprogrammed cell function.

- 20 (A) Reprogramming efficiency measured by the percentage of Clec9a-tdT⁺ cells expressing antigen-presenting-cell markers CD45 and MHC-II. (n=2, mean ± SD) (B) Reprogramming fidelity measured by the percentage of Clec9a-tdT⁺ cells expressing the cDC1-restricted marker XCR1. (n=4, mean ± SD). (C) Flow cytometry plots showing expression of XCR1 in Clec9a-tdT⁺ cells. (D) Expression of the Clec9a-tdT
- 25 reporter and induced cDC1 markers CD45, MHC-II and XCR1 in response to increasing lentiviral titers of PIB transcription factors alone (top row) or increasing titers of miR-124, miR-142 or the combination. (E) Kinetics of Clec9a-tdT reporter activation (left) and expression of iDC markers CD45, MHC-II and XCR1 gated in Clec9a-tdT⁺ iDCs (right). (F) Cytometric bead array quantification of the pro-inflammatory cytokines
- 30 IL-12, CXCL10 and IFNα secreted by iDCs generated with and without miRNAs upon stimulation with polyinosinic:polycytidylic acid (pIC, TLR3 agonist), lipopolysaccharide (LPS, TLR4 agonist) and resiquimod (R848, TLR7/8 agonist). (n=3, mean ± SD). *P < 0.05, ***P < 0.001 one-way ANOVA with Dunnett's multiple comparison.

Figure 3. miR-124 and miR-142 enhance cDC1 reprogramming efficiency in human fibroblasts.

(A) Reprogramming efficiency measured by the percentage of cells expressing the markers CD45 and HLA-DR in reprogrammed human dermal fibroblasts (HDFs) at day 9 of reprogramming, with and without human miR-124, miR-142 or the combination. (B) Reprogramming fidelity measured by the expression of induced cDC1 marker CD226. (n=2, mean $\pm$ SD). (C) Flow cytometry plots of induced cDC1 markers CD45, HLA-DR and CD226, with or without human miR-124 and miR-142. *P < 0.05, **P < 0.01, ***P < 0.001 one-way ANOVA with Dunnett's multiple comparison.

Figure 4. miR-124 and miR-142 suppress fibroblast identity and promote cDC1 reprogramming.

(A) miR-124, miR-142 or a scrambled (SCR) pri-miRNA were co-transduced with PIB TFs into HDFs. Reprogrammed populations were sorted for total RNA-seq and ATAC-seq at day 3 (CD45+), day 6 (CD45+HLA-DR+ and CD45+HLA-DR-) and day 9 (CD45+HLA-DR+ and CD45+HLA-DR-). Partially reprogrammed CD45+ HLA-DR- (+) and fully reprogrammed CD45+ HLA-DR+ (++) populations were sorted at days 6 and 9. HDFs and DC1s (HLA-DR+ CD11c+ CD141+), DC2s (HLA-DR+ CD11c+ CD1c+ CD141-) and pDCs (HLA-DR+ CD11c- CD123+) from peripheral blood of 3 donors were sorted as controls. (B) Activation of a cDC1 gene expression signature measured by the percentage of genes upregulated in cDC1s when compared to HDFs. (C) Normalized read counts of the cDC1 genes CD141 and XCR1 in HDFs, DC controls and reprogrammed cells. (n=3, mean + SD). (D) Genes downregulated by miR-124 and miR-142 and their cell type enrichment on days 3, 6 and 9. (E) Genes upregulated by miR-124 and miR-142 and their cell type enrichment on days 3, 6 and 9. (F) Downregulated genes at day 3 in miR-124 and miR-142 expressing cells compared to scrambled control. The percentage of miRNA targets combining experimental evidence (mirTarBase) and seed-filtered CLIP-seq data (starBase) is shown in colored circles for each enriched mature miRNA strand.

Figure 5. miR-124 induces a permissive chromatin landscape.

miR-124, miR-142 or a scrambled (SCR) pri-miRNA were co-transduced with PIB TFs into HDFs. Reprogrammed populations were sorted for total RNA-seq and ATAC-seq at day 3 (CD45+), day 6 (CD45+HLA-DR+ and CD45+HLA-DR-) and day 9 (CD45+HLA-DR+ and CD45+HLA-DR-). Partially reprogrammed CD45+ HLA-DR- (+)

and fully reprogrammed CD45+ HLA-DR+ (++) populations were sorted at days 6 and 9. HDFs and DC1s (HLA-DR+ CD11c+ CD141+), DC2s (HLA-DR+ CD11c+ CD1c+ CD141-) and pDCs (HLA-DR+ CD11c- CD123+) from peripheral blood of 3 donors were sorted as controls. (A) Percent of accessible cDC1 genes compared to HDF gene accessibility. Activation of a cDC1 chromatin accessibility signature measured by the percentage of genes with increased accessibility in cDC1 when compared to HDFs. (B) Number of differentially accessible peaks compared to scrambled (SCR) control at days 3, 6 and 9. (C) TFs and chromatin-binding proteins enriched in more and less accessible genes with miR-124 or miR-124 compared to scrambled control on days 3, 6 and 9. (D) De novo TF motif enrichment analysis using more and less accessible peaks compared to scrambled control at day 3. (E) cDC1 reprogramming efficiency in the presence of EZH2 inhibitor GSK-126 or DMSO (control) was quantified 9 days after transduction. Small molecules or DMSO were added on days -2, 0, 3, 6, or on days 0, 3 and 6. (F) Flow cytometry quantification of cDC1 reprogramming efficiency measured by the frequency of cells expressing CD45 and/or HLA-DR in HDF cultures 9 days after transduction with PIB-encoding lentiviral vectors. Reprogramming was performed in the presence of GSK-126 or DMSO.

Figure 6. miR-124-3p mimics increase cDC1 reprogramming efficiency in human cells.

(A) miRNA mimics were transfected in HDFs on days 0, 3 and 6, after transduction with PIB TFs. (B) Proportion of human dermal fibroblasts (HDFs) expressing antigen-presenting-cell markers CD45 and HLA-DR upon transfection with canonical miR-124 and miR-142 mimics. (C) Exemplificative flow cytometry plots of CD45 and HLA-DR expression. (D) miRNA mimics were transfected in HDFs on days -2, 0, 3 or 6, before or after transduction with PIB TFs. (E) Fold change of reprogramming efficiency to CD45+ HLA-DR+ iDCs upon miR-124-3p transfection compared to scrambled control. *P < 0.05, one-way ANOVA with Dunnett's multiple comparison. (F) Flow cytometry analysis and quantification of proportion of HDFs expressing the reprogramming markers CD45 and HLA-DR upon transfection with canonical mimics and 5' isomiRs of miR-142. Exemplificative flow cytometry plots of CD45 and HLA-DR expression are shown on the top panel. (G) Flow cytometry analysis and quantification of proportion of HDFs expressing the cDC1 marker CD226 upon transfection with canonical mimics and 5' isomiRs of miR-142. (H) qPCR quantification of the cDC1 marker XCR1 expression in human dermal fibroblasts (HDFs) reprogrammed to CD45+ HLA-DR+

iDCs upon transfection with miR-142 canonical or isomiR mimics. (I) Proportion of reprogrammed human embryonic fibroblasts (HEFs) expressing antigen-presenting-cell markers CD45 and HLA-DR upon transfection with canonical miR-124 and miR-142 mimics and 5' isomiRs of miR-142. Exemplificative flow cytometry plots of CD45 and HLA-DR expression are shown on the top panel. (J) Proportion of HEFs reprogrammed to CD45+ HLA-DR+ iDCs expressing DC1 marker XCR1 upon transfection with canonical miR-124 and miR-142 mimics and 5' isomiRs of miR-142. Exemplificative flow cytometry plots of XCR1 expression are shown on the top panel. (K) Proportion of cells expressing antigen-presenting-cell markers CD45 and HLA-DR upon transfection with selected miR-124 and miR-142 mimics and combinations thereof. Exemplificative flow cytometry plots of CD45 and HLA-DR expression are shown on the top panel. (L) Proportion of CD45+ HLA-DR+ iDCs expressing DC1 marker XCR1 upon transfection with selected miR-124 and miR-142 mimics and combinations thereof. Exemplificative flow cytometry plots of XCR1 expression are shown on the top panel. (M) Fold change of reprogramming efficiency measured as frequency of cells expressing CD45 and HLA-DR upon transduction with PIB-encoding lentiviral vectors at day 0, and transfection with miR-142-3p, miR-142-3p-1, miR-142-3p-3 or miR-142-5p-1 at days -2, 0, 3, 6 or 0, 3 and 6, compared to scrambled control. SCR: scrambled control.

Figure 7. Pathway analysis of miR-124 and miR-142 overexpression in iDC reprogramming.

(A) Schematic representation of the approach to determine the mechanisms of miR-124 and miR-142 in DC reprogramming. Briefly, GRaNIE and GRaNPA packages were used to predict transcription factors (TFs) explaining gene expression changes based on transcriptome and genome accessibility data. Then, we combined target prediction for previously validated miRNA strands, and centrality within the differentially expressed (DE) protein network to infer first-order miRNA targets responsible for the effect on iDC reprogramming. (B) GRaNPA predictions for effector regulators behind miR-124 (left) or miR-142 overexpression. Strands of the respective miRNA targeting GRaNPA transcripts are listed to the right of each plot. KLF5-miR-124 interaction was not computationally predicted. (C) Gene expression fold-change for the predicted miRNA targets among GRaNPA predictions from panel B. Significantly up- and down-regulated transcripts are marked with an up- or down-wards facing triangle, respectively. (D) Top 10 proteins by STRING network degree downregulated on day 3 upon miR-124 overexpression, which are also predicted targets of miR-124-3p or miR-

124-5p, or upon miR-142 overexpression, which are also predicted targets of miR-142-3p, miR-142-3p-1, miR-142-3p-3 or miR-142-5p-1.

Figure 8. Delivery of PU.1, IRF8 and BATF3 using modified linear mRNA induces cDC1 reprogramming.

5 (A) PU.1, IRF8, BATF3 and GFP m1-Ψ-modified mRNAs were transfected in HDFs on days 0, 2, 4 and 6. (B) Reprogramming efficiency to CD45⁺ cells in GFP-positive cells on day 9 of reprogramming, with or without transfection with the scrambled, miR-124-3p, or miR-142-5/3p mimics. (C) Human dermal fibroblasts (HDFs) were plated a day
10 before and transduced on day 0 with PIB-encoding lentiviral vectors (Lenti-PIB) or transfected with PIB-encoding mRNAs, miRNAs or control mimics. Medium was supplemented with 10 μM of JAK1/2 inhibitor ruxolitinib and cells were analyzed on day 3 for the expression of CD45 and HLA-DR. (D) Reprogramming efficiency measured by the percentage of cells expressing markers CD45 and HLA-DR in HDFs (n=6, two
15 separate experiments, mean ± SD). (E) Quantification of cDC1 reprogramming yield as number of CD45⁺ cells 3 days after transfection or transduction. (F) Flow cytometry quantification of cDC1 reprogramming efficiency measured by the frequency of cells expressing CD45 and/or HLA-DR 60 hours after transfection of human cancer cell lines of melanoma (A2058) and sarcoma (SK-LMS-1). Exemplificative flow cytometry plots of
20 CD45 and HLA-DR expression are shown on the top panel for A2058 cell line. SCR: scrambled control. (G) Flow cytometry quantification of cDC1 reprogramming yield in human dermal fibroblasts 9 days after transduction with lentiviral vectors encoding PU.1, IRF8 and BATF3. Cells were incubated with different JAK inhibitors, including Ruxolitinib (rux), Baricitinib (Bari, JAK1/JAK2 inhibitor), Fedratinib (Fedra, JAK2
25 inhibitor), Upadacitinib (Upa, JAK1 inhibitor) and Filgotinib (Filgo, JAK 1 inhibitor), at 3 different concentrations (5, 10 or 15 uM) from day 0 to day 9. (H) Flow cytometry analysis and quantification of cDC1 reprogramming efficiency 6 days after transfection with linear mRNA encoding PIB, with or without transfection with the scrambled (SCR), miR-124-3p, or miR-142-5/3p mimics individually or mixed.

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Figure 9. Delivery of PU.1, IRF8 and BATF3 using self-amplifying RNA induces cDC1 reprogramming.

(A) Flow cytometry quantification of cDC1 reprogramming efficiency measured by the frequency of Clec9a-tdTomato⁺ and (B) CD45⁺ and MHC-II⁺ cells at day 9 of

reprogramming. Exemplificative plots showing expression of CD45 and MHC-II within Clec9a-tdTomato + cells is shown in (C).

Figure 10. Linear, circular and self-amplifying RNA platforms exhibit different payload expression levels and kinetics.

5 (A) Workflow for characterizing payload expression from three different RNA platforms: linear, circular, and self-amplifying RNA. SK-LMS-1 cells are transfected with an EGFP-encoding RNA on day 0 and monitored by flow cytometry from day 1 to day 8 to determine the transfection efficiency, dose-dependence, and kinetics of EGFP
10 expression. (B-D) Representative contour plots for EGFP expression after transfection with (B) linear, (C) circular, and (D) self-amplifying RNA, respectively. EGFP-positive cells are gated in live cells, and their frequencies are indicated. (E) Flow cytometry quantification of frequency of GFP+ transfected cells and (F) mean fluorescence intensity (MFI) overtime. (G) Workflow for characterizing payload expression from three
15 different RNA platforms: linear, circular, and self-amplifying RNA, after 1 transfection at day 0 (-) or 3 transfections at day 0, 3 and 6 (+). (H) Flow cytometry quantification of frequency and (I) GFP MFI of eGFP+ cells.

Figure 11. Delivery of PU.1, IRF8 and BATF3 using linear, circular and self-amplifying RNA induces cDC1 reprogramming in cancer cells.

20 (A) SK-MS-1 human cancer cells were transfected with linear, circular and self-amplifying RNA encoding PU.1, IRF8 and BATF3, and cDC1 reprogramming efficiency was profiled at day 3. (B) Flow cytometry quantification of cDC1 reprogramming efficiency measured as frequency of CD45+ HLA-DR+ cells gate in live cells (linear and
25 circular RNA) or eGFP+ cells (saRNA). Doses of RNA used for transfection are shown.

Figure 12. Multiple transfections can enhance cDC1 reprogramming efficiency mediated by RNA.

(A) SK-LMS-1 human cancer cells were treated with one (day 0), two (day 0 and 3) or
30 three (day 0, 3 and 6) with linear, circular and self-amplifying RNA encoding PU.1, IRF8 and BATF3, and reprogramming efficiency was profiled at day 3, 6 and 9. (B-D) Flow cytometry analysis of cDC1 reprogramming efficiency measured as frequency of cells expressing CD45 and HLA-DR after transfection with (B) linear, (C) circular, and (D) saRNA. Quantification is shown in (E). (F, G) Flow cytometry quantification of (F)
35 frequency of CD40+ cells and (G) mean fluorescence intensity (MFI) of HLA-ABC in

cancer cells after transfection with the 3 RNA platforms. (H) Flow cytometry quantification of cDC1 reprogramming efficiency measured as frequency of eGFP+ or eGFP- cells expressing CD45 and/or HLA-DR. (I) Flow cytometry quantification of number of CD45+ and/or HLA-DR+ reprogrammed cells 3, 6 and 9 days after
5 transfection with the 3 RNA platforms.

Detailed description

Definitions

10 As used herein an "RNA construct" refers to an arrangement or sequence of one or more ribonucleic acid molecule(s). The term RNA construct includes for example RNA sequences designed or engineered for applications such as gene expression and/or therapeutic purposes, and can for example form part of cell delivery systems, such as, but not limited to vectors or plasmids, or non-viral cell delivery systems, such as, but
15 not limited to, lipid-based delivery systems, polymer-based delivery systems or electroporation-mediated delivery systems.

"miRNA", or "mi-RNA", "miR", "mi-R", microRNA and micro-RNA are used interchangeably herein.

20 "pri-miRNA" or "primary miRNA" as used herein refers single strand RNA which transcribed from DNA and has a hairpin loop structure containing miRNA and its complementary strand. The "pre-miRNA" is produced from pri-miRNA partially by cleavage by an intranuclear enzyme called Drosha. "miRNA, or pri-miRNA thereof" as
25 used herein therefore relates to both mature the microRNA and its precursor form, encompassing the mature miRNA as well as the primary transcript.

"isomiRs" refer to heterogeneous variants of individual miRNAs that can differ in length or sequence from the archetype miRNA, also known as canonical miRNA. isomiRs
30 include 5' isomiRs (displaying sequence changes at 5' end of the archetype form), 3' isomiRs (displaying changes at the 3' end), polymorphic isomiRs (displaying changes within the sequence), and mixed type isomiRs (wherein at least two of the above listed changes occur), as defined in Wagner *et al.* 2024.

“miRNA mimic” as used herein refer to a class of molecules that can be used to imitate the gene silencing ability of one or more miRNAs. The term “miRNA mimic” refers to synthetic non-coding RNAs that are capable of entering the RNAi pathway and regulating gene expression. miRNA mimics can be designed as mature molecules (e.g. single stranded) or mimic precursors (e.g., pri- or pre-miRNAs). “miRNA mimic” as used herein cover both mimics of canonical miRNA sequences, and isomiRs mimics.

As used herein “encapsulation” refers to the process of enclosing or packaging RNA molecules within a protective carrier or delivery system. Encapsulation may be performed to enhance stability, delivery efficiency, and targeted delivery of RNA molecules and RNA-based therapeutics to the cells. Encapsulation methods include, but not limited to, liposomes, lipid nanoparticles (LNPs), polymers and polymer nanoparticles, cationic polymers, and exosomes.

As used herein, “contacting a cell with a composition” is defined as bringing said cell and said composition in physical contact with one another, for example under conditions appropriate for internalization. The term includes for example introduction of compositions of nucleic acids intracellularly, such as, but not limited to, transfection (non-viral delivery), transduction (viral vector delivery), electroporation, lipofection, sonoporation, and microinjection. The term also includes allowing effectors, such as drugs or small molecules, to perform their effects on said cells.

“Mesenchymal stem cells” or “Mesenchymal stromal cells” (both referred to as “MSCs”) are used herein interchangeably, and are referred to herein as being multipotent stromal cells that can differentiate into a variety of cell types, including but not limited to: osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells).

“Pluripotent stem cell” or “PSC” as used herein refers to a stem cell capable of producing all cell types of the organism and can produce cells of the germ layers, e.g. endoderm, mesoderm, and ectoderm, of a mammal and encompasses at least pluripotent embryonic stem cells and induced pluripotent stem cells.

As used herein, “multipotent” refers to cells that can give rise to several other cell types, but those cell types are limited in number. An example of multipotent cells is

hematopoietic cells - blood stem cells that can develop into several types of blood cells but cannot develop into brain cells for example.

5 As used herein, the term "somatic cell" refers to any cell other than a germ cell, such as an egg or a sperm, which does not directly transfer its DNA to the next generation. Typically, somatic cells have limited or no pluripotency.

10 "Reprogramming" refers herein to the process of converting of differentiating cells from one cell type into another. "Reprogramming" thus refers to reprogramming cells to a different type or lineage. In particular, in some embodiments of the present invention, reprogramming refers to converting or transdifferentiating any type of cell into a type 1 conventional dendritic cell or an antigen-presenting cell.

15 "replicon" as used herein a replicon refers to an RNA molecule capable of autonomous replication within a host cell.

20 "m1-Ψ-modified mRNA" as used herein refers to a RNA modified to contain the synthetic pyrimidine nucleoside N1-Methylpseudouridine (abbreviated m1-Ψ). As used herein "miR-142" and "miR-142a" can be used interchangeably.

"EGFP" or "eGFP" as used herein are used as synonyms and refer to a modified version of green fluorescent protein (GFP) exhibiting enhanced fluorescence compared to the naturally occurring variant.

25 *Compositions*

The present invention provides RNA-based cell reprogramming strategies, in particular RNA-based reprogramming of any cell type into a dendritic cell or and antigen presenting cell.

30 In one aspect, the present invention relates to a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of: PU.1, IRF8, and BATF3, wherein the RNA construct is selected from the group consisting of: self-amplifying RNA (saRNA),

circular RNA (circRNA), non-modified RNA, modified RNA (modRNA) and trans-amplifying RNA (taRNA).

5 The skilled person will appreciate that other RNA types suitable for encoding and expressing the transcription factors, or combinations thereof in cells may be used in the compositions or methods and uses as described herein.

As showed herein however (e.g. in Example 11) different RNA types exhibit different payload expression levels and kinetics and the results disclosed herein demonstrate that achieving efficient gene expression and reprogramming is not straightforward. The
10 experimental data provided herein, such as in the working Examples of the present application, underscore the advantages of the specific compositions and non-obvious advantages of the specific RNAs used in the methods described.

15 In some embodiments, the combination of at least two transcription factors encoded by the composition comprising one or more RNA construct(s) is:

- a. PU.1 and IRF8;
- b. PU.1 and BATF3; or
- c. IRF8 and BATF3.

20 In one embodiment, the combination of at least two transcription factors encoded by the composition comprising one or more RNA construct(s) is PU.1 and IRF8, or PU.1 and BATF3.

25 In another embodiment, the combination of at least two transcription factors is PU.1, IRF8, and BATF3.

In some embodiments, the combination of at least two transcription factors in the 5' to 3' order is:

- a. PU.1, IRF8, BATF3;
- 30 b. PU.1, BATF3, IRF8;
- c. IRF8, BATF3, PU.1;
- d. IRF8, PU.1, BATF3;
- e. BATF3, IRF8, PU.1; or
- f. BATF3, PU.1, IRF8.

35

In preferred embodiments, the composition comprises 2, such as 3 RNA constructs encoding the combination of at least two transcription factors

5 In other embodiments, each of the at least two transcription factors is encoded by a single RNA construct, optionally further encoding other genes such as reporter genes, such as fluorescent reporter genes, for example GFP, BFP or RFP. Thus in some embodiments, the composition comprises 3 RNA constructs, wherein the combination of at least two transcription factors is PU.1, IRF8, and BATF3, and wherein each of the RNA constructs encodes one of PU.1, IRF8, and BATF3.

10 In yet other embodiments, the RNA construct(s) are identical or different. By way of example the RNA constructs may be all modified RNAs.

In some embodiments of the composition of the present invention, PU.1 is encoded by a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO:13 or

15 SEQ ID NO:14, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:13 or SEQ ID NO:14.

In some embodiments of the composition of the present invention, IRF8 is

20 encoded by a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO:11 or SEQ ID NO:12, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO: SEQ ID NO:11 or SEQ ID NO:12.

25 In some embodiments of the composition of the present invention, BATF3 is encoded by a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO:15 or SEQ ID NO:16, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:15 or SEQ ID NO:16.

30 In other embodiments, PU.1 is of a polypeptide sequence with at least 90% sequence identity to SEQ ID NO:19 or SEQ ID NO:20, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:19 or SEQ ID NO:20.

35

In other embodiments, IRF8 is of a polypeptide sequence with at least 90% sequence identity to SEQ ID NO:17 or SEQ ID NO:18, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:17 or SEQ ID NO:18.

5

In other embodiments, BATF3 is of a polypeptide sequence with at least 90% sequence identity to SEQ ID NO:21 or SEQ ID NO:22, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:21 or SEQ ID NO:22.

10

Direct reprogramming of cells by expression of the combinations of transcription factors as described herein can be significantly improved by expression of miRNAs or miRNA mimics. In particular, the present invention provides compositions of isomiRs for use in cell reprogramming. Further, RNA-based co-expression of the transcription factors as described herein allows direct reprogramming of cells and reprogramming efficiency can be significantly improved by co-transfection with miRNA mimics.

15

One aspect of the present invention relates to a composition comprising one or more miRNA mimics, wherein said one or more miRNA mimics are isomiRs, for use in cell reprogramming.

20

In preferred embodiments, the one or more miRNA mimics are isomiRs of miRNA 124a-1, miRNA-124-1, miRNA-126a, miRNA-126, miRNA-142, and/or miRNA-150.

25

Another aspect of the invention relates to a composition comprising one or more miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics, selected from the group consisting of: miR-124, miR-124a-1, miR-124-1, miR-124-3p, miR-124-5p, miR-142a, miR-142, miR-126, miR-126a, miR-150, miR-142-3p-1, miR-142-3p-3, and miR-142-5p-1.

30

In some embodiments, said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics:

35

- a. downregulate the gene expression of SP1, KLF5, and/or RELA, preferably wherein said miRNAs, or primary miRNAs (pri-

- miRNAs) thereof, and/or miRNA mimics are of the miR-124 family, even more preferably wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are miR-124-3p or isomiRs thereof; and/or
- 5 b. downregulate the gene expression of KLF10, PATZ1, and/or ELK3 preferably wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are of the miR-142 family.
- 10 In some embodiments, said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics:
- 15 a. downregulate the gene expression of SP1, KLF5, and/or RELA, preferably wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are of the miR-124 family, even more preferably wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are miR-124-3p or isomiRs thereof; and/or
- 20 b. downregulate the gene expression of KLF10, PATZ1, and/or ELK3 preferably wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are of the miR-142 family.
- 25 In other embodiments wherein the miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are of the miR-124 family, preferably miR-124-3p or miR-124-5p, said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics downregulate the protein expression of STAT3, MAPK14, and/or RELA, preferably wherein STAT3, MAPK14, and RELA represent the nodes with the highest connectivity to differentially expressed proteins in the miR-124 network, as identified through protein
- 30 interaction analysis using STRING as described in Example 8 (Szklarczyk et al., 2023).
- 35 In other embodiments wherein the miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are of the miR-142 family, said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics downregulate the protein expression of CCND1, TGFBR1, and/or RAB2, preferably wherein CCND1, TGFBR1, and/or RAB2

represent the nodes with the highest connectivity to differentially expressed proteins in the miR-142 network, as identified through protein interaction analysis using STRING as described in Example 8 (Szkłarczyk et al., 2023).

5 In further embodiments, miR-142-3p-1 downregulates the protein expression of CCND1, preferably wherein CCND1 represents the node with the highest connectivity to differentially expressed proteins in the miR-142 network, as identified through protein interaction analysis using STRING as described in Example 8 (Szkłarczyk et al., 2023). In other embodiments, miR-142-3p-1 and miR-142-3p downregulate the protein expression of TGFBR1, and/or RAB2, preferably wherein TGFBR1, and/or RAB2
10 represent the node with the highest connectivity to differentially expressed proteins in the miR-142 network, as identified through protein interaction analysis using STRING as described in Example 8 (Szkłarczyk et al., 2023). In further embodiments, miR-142-5p-1 downregulates the protein expression of TGFBR1, preferably wherein TGFBR1 represents the node with the highest
15 connectivity to differentially expressed proteins in the miR-142 network, as identified through protein interaction analysis using STRING as described in Example 8 (Szkłarczyk et al., 2023).

20 It may be beneficial, as shown in the present invention that the composition comprises more than one miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics. This may be the case for example in order to improve reprogramming efficiency and/or fidelity in the methods described herein, such as improving cDC1 cell reprogramming.

Thus, in other embodiments, the composition comprises at least 2, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8 miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics, selected from the group consisting of: miR-124, miR-124a-1, miR-124-1, miR-124-3p, miR-124-5p, miR-142a, miR-142, miR-126, miR-126a, miR-150, miR-142-3p-12, miR-142-3p-3, and miR-142-5p-1.

30 In some embodiments, the one or more miRNAs are the two miRNAs miR-124 and miR-142.

In other embodiments, the one or more miRNAs are the two miRNAs miR-124 and
35 miR-142a.

In further embodiments, the composition comprises at least one or more miRNA mimic(s).

5 As described herein miRNA mimics include canonical miRNA mimics and isomiRs mimics.

In some embodiments, the composition comprises at least one, such as at least two, such as at least three, such as at least four, such as at least five miRNA mimic(s).

10 In other embodiments, said at least one, such as at least two, such as at least three, such as at least four, such as at least five miRNA mimic(s) are isomiRs. In some embodiments, the isomiRs are 5' isomiRs, 3' isomiRs, polymorphic isomiRs, and/or mixed type isomiRs. In preferred embodiments, the isomiRs are 5' isomiRs.

15 In preferred embodiments, the miRNA mimic(s) are the isomiRs miR-142-3p-1, miR-142-3p-3 and/or miR-142-5p-1.

In some embodiments, the composition comprises or consists of miR-142-3p, miR-142-3p-1 and miR-142-5p-1.

20

In other embodiments, the composition comprises or consists of miR-124-3p and miR-142-5p-1.

25 In other embodiments, the at least one or more miRNAs is a primary miRNA (pri-miRNA).

In preferred embodiments, at least one of the miRNA(s), or primary miRNA thereof, and/or miRNA mimics, is encoded by a sequence selected from the group consisting of:

30

- a. miRNA 124a-1 of SEQ ID NO: 1;
- b. miRNA-124-1 of SEQ ID NO: 2;
- c. miRNA-126a of SEQ ID NO: 3;
- d. miRNA-126 of SEQ ID NO: 4
- e. miRNA-142 of SEQ ID NO: 5 or SEQ ID NO: 6
- 35 f. miRNA-150 of SEQ ID NO: 7 or SEQ ID NO: 8

- g. miRNA-124-3p mimic of SEQ ID NO: 9;
- h. miRNA-124-5p mimic of SEQ ID NO: 10;
- i. miR-142-3p-1 of SEQ ID NO: 23;
- j. miR-142-3p-3 of SEQ ID NO: 24; and
- k. miR-142-5p-1 of SEQ ID NO: 25.

5

or a polynucleotide sequence with at least 90% sequence identity, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity thereto.

10 In some embodiments, at least one of the one or more RNA construct(s), or at least one of the one or more miRNA(s), or pri-miRNAs thereof, and/or miRNA mimics, is encapsulated.

15 In other embodiments, the encapsulation is selected from the group consisting of: nanoparticle encapsulation, polymeric encapsulation, viral encapsulation, exosome encapsulation, and lipid nanoparticle encapsulation.

20 In further embodiments, the one or more RNA construct(s) are naked RNA or RNA not encapsulated or incorporated into a viral particle, for example a lentiviral particle, such as naked non-modified or modified RNA, or non-modified or modified RNA not encapsulated or incorporated into a viral particle, for example a lentiviral particle.

25 In some applications, it may be beneficial that compositions of RNA construct(s) encoding the reprogramming transcription factors as described herein and the compositions of miRNAs, or pri-miRNAs thereof, and/or miRNA mimics as described herein are provided as a single composition

Therefore, one aspect of the present invention relates to a composition comprising:

- a. the composition comprising one or more RNA construct(s) as described herein; and
- 30 b. the composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein.

35 The inventors have validated herein that Polycomb Repressive Complex 2 (PRC2) inhibition can enhance cDC1 reprogramming efficiency. Thus in further embodiments, of the present invention, the compositions of RNA construct(s) encoding the

reprogramming transcription factors as described herein and/or the compositions of miRNAs, or pri-miRNAs thereof, and/or miRNA mimics as described herein further comprise compounds inhibiting Polycomb Repressive Complex 2 (PRC2), such as SUZ12 or EZH2 inhibitors, preferably GSK-126.

5

The one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics as described herein may be beneficially encoded by one or more construct(s) or vector(s), for example, but not limited to, construct(s) or vector(s) facilitating their entry and/or expression in cells.

10

Thus, another aspect of the present invention relates to a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics as described herein.

15

In some embodiments of the compositions of the present invention, at least one of the one or more miRNA(s) is encoded as a pri-miRNA.

The one or more vector(s) may be a viral, or a non-viral vector.

20

The viral vector(s) may be selected from the group consisting of: adenovirus, lentivirus, retrovirus, alphaviruses, flavivirus, rhabdovirus, Sendai virus, pox virus, adeno-associated virus (AAV), and herpes simplex virus (HSV).

The non-viral vector(s) may be selected from the group consisting of: liposomes, polymeric nanoparticles, dendrimers, cationic polymers, nanogels, inorganic

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nanoparticles, carbon nanotubes, hydrogels, electroporation, and ultrasound-mediated vectors

30

In some embodiments of the compositions and methods described herein, the at least two transcription factors, such as the at least two transcription factors selected from the group consisting of: PU.1, IRF8, and BATF3, are encoded as polycistronic constructs, optionally separated by 2A peptides. In other embodiments, the at least two transcription factors, such as the at least two transcription factors selected from the group consisting of: PU.1, IRF8, and BATF3, are encoded as monocistronic constructs.

In preferred embodiments, the compositions of the present invention further comprise one or more inhibitor(s) of interferons (IFN inhibitors), preferably wherein the IFN inhibitor(s) are selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors and virus proteins neutralizing type I interferons, even more preferably
5 wherein the IFN inhibitor(s) are selected from the group consisting of B18R and ruxolitinib, yet even more preferably wherein the IFN inhibitor is ruxolitinib.

The skilled person will appreciate that other IFN inhibitors include, but not limited to, Tofacitinib, Baricitinib, Ritlecitinib, Abrocitinib, Upadacitinib and Delgocitinib, and viral
10 proteins known as innate inhibiting proteins (IIPs), including but not restricted to HSV-2 Us1, HSV-1 Us1, HSV-1Us11, Orf OV20.0L, BVDV Npro, PIV-5 V, MERS-CoV M, MERS-CoV ORF4a, Langat NS5, Influenza NS1, vaccinia virus immune evasion protein E3, vaccinia virus immune evasion protein K3 and vaccinia virus immune
15 evasion protein B18.

In some embodiments, the composition is a pharmaceutical composition.

Method for reprogramming or inducing a cell

20 The present invention provides methods for reprogramming cells, said methods making use of miRNA mimics, in particular isomiRs.

In one aspect, the invention relates to a method for reprogramming or inducing a cell, said method comprising the steps of:

- 25 a. Contacting a cell with a composition comprising one or more constructs or vectors encoding a combination of at least two transcription factors;
 b. Expressing the at least two transcription factors;
 c. Contacting the cells with a composition comprising one or more
 miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a
30 composition comprising one or more construct(s) or vector(s) encoding
 one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;
whereby the cell is reprogrammed or induced.

In some embodiments, said reprogramming is direct cell reprogramming, or direct cell
35 fate reprogramming.

In preferred embodiments, the one or more miRNA mimic(s) are isomiRs.

In some embodiments, the one or more isomiRs are 5' isomiRs.

5

The isomiRs of the composition or methods of the present invention may preferably be isomiRs of the miRNAs of the compositions and methods of the present invention.

Thus, in other embodiments, the one or more miRNA mimics are isomiRs of miRNA 124a-1, miRNA-124-1, miRNA-126a, miRNA-126, miRNA-142, and/or miRNA-150.

10

In preferred embodiments, the one or more isomiRs are isomiRs of miR-142.

In yet other embodiments, the one or more isomiRs are the isomiRs miR-142-3p-1, miR-142-3p-3 and/or miR-142-5p-1.

15

Methods for reprogramming or inducing cells into a dendritic or antigen-presenting cell

One aspect of the present invention relates to a method for reprogramming or inducing a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of:

20

- a. Contacting a cell with a composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
- b. Expressing the at least two transcription factors;
- c. Contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;

25

30

whereby a dendritic cell or antigen-presenting cell is obtained.

In some embodiments, said reprogramming is direct cell reprogramming, or direct cell fate reprogramming.

35

In some embodiments, the one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8 and BATF3 comprise both RNA constructs as described herein, wherein the RNA constructs encode one or more of PU.1, IRF8 and BATF3, and one or more viral
5 vectors such as lentiviruses, adenoviruses or adeno associated viruses encoding one or more of PU.1, IRF8 and BATF3.

Another aspect of the present invention relates to a method for reprogramming or inducing a cell into a dendritic cell or antigen-presenting cell, said method comprising
10 the steps of:

- a. Contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
 - b. Expressing the at least two transcription factors;
- whereby a dendritic cell or antigen-presenting cell is obtained.
- 15

In some embodiments, the method comprises the steps of:

- a. Contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
 - b. Expressing the at least two transcription factors;
 - c. Contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;
- 20
- 25

whereby a dendritic cell or antigen-presenting cell is obtained.

30

In some embodiments, said reprogramming is direct cell reprogramming, or direct cell fate reprogramming.

The inventors have shown herein that different RNA types exhibit different payload
35 expression levels and kinetics, as in Example 11. The present invention shows that

repeating step a. of contacting and b. of expressing of the methods herein, also called “redosing”, has the advantage of rescuing the fading of the transgene expression levels over time and prolonging transgene expression of linear (m1-Ψ-modified mRNA), circular and self-amplifying RNA-encoded transgenes. Thus, in some preferred
5 embodiments of the methods of the present invention, the steps a. of contacting a cell with a composition comprising one or more RNA construct(s) and step b. of expressing are repeated.

10 In further embodiments, the step a. of contacting a cell with a composition comprising one or more RNA construct(s) and step b. of expressing are repeated twice, such as repeated three times, such as repeated 4 times, such as repeated 5 times, such as repeated 6 times, such as 7 times, for example at least 8 times.

15 In other embodiments, the step a. of contacting a cell with a composition comprising one or more RNA construct(s) and step b. of expressing are performed on day 0, day 3 and day 6, wherein day 0 is defined as the first-time step a. of contacting a cell with a composition comprising one or more RNA construct(s) is performed. In further
20 embodiments, the step a. of contacting a cell with a composition comprising one or more RNA construct(s) and step b. of expressing are performed on day 0, day 3 and day 7, wherein day 0 is defined as the first-time step a. of contacting a cell with a composition comprising one or more RNA construct(s) is performed.

In some embodiments, the methods comprise the step a. of contacting the cells once with one or more saRNA construct(s) .

25 In other embodiments, the methods comprise performing the step a. of contacting the cells and step b. of expressing twice at days 0 and 3 with one or more circular RNA construct(s) wherein day 0 is defined as the first-time step a. of contacting a cell with a composition comprising one or more circular RNA construct(s) is performed.

30 In yet other embodiments, the methods comprise performing the step a. of contacting the cells and step b. of expressing three times at days 0, 3 and 7 with one or more linear RNA construct(s), wherein day 0 is defined as the first-time step a. of contacting a cell with a composition comprising one or more linear RNA construct(s) is performed

In yet preferred embodiments, the step c. of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics is not performed prior to the
5 step a. of contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3 and/or the step b. of expressing the at least two transcription factors.

10 In some embodiments, the step c. of contacting the cells with a composition comprising miRNA mimics comprises miR-142 mimics, such as miR-142-3p, miR-142-3p-1, miR-142-3p-3 and/or miR-142-5p-1, and is performed on day 0, day 3 and day 6 following the step a. of contacting a cell with a composition comprising one or more RNA
15 construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3.

In other embodiments of the methods of the present invention, step c. comprises contacting the cells with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, and
20 the method further comprises a step of expressing the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics.

In preferred embodiments, the composition comprising one or more construct(s) or vector(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3 is as described herein in the section
25 *Compositions*.

In some embodiments of the methods of the present invention, the composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is as
30 described herein in the section *Compositions*.

In other preferred embodiments, the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is as described herein in the section *Compositions*.
35

In some embodiments, the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed prior to, simultaneously, and/or after, the step of contacting a cell with a composition comprising one or more constructs or vectors, or with a composition comprising one or more RNA construct(s). In preferred embodiments, the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed simultaneously to the step of contacting a cell with a composition comprising one or more constructs or vectors, or with a composition comprising one or more RNA construct(s).

In preferred embodiments of the methods of the present invention, the step of expressing the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed prior to, simultaneously, and/or after, the step of expressing the at least two transcription factors. In even more preferred embodiments, the step of expressing the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed simultaneously to the step of expressing the at least two transcription factors.

In yet other preferred embodiments of the methods of the present invention, the step c. of the methods comprises contacting the cells with miRNA-124 and/or miRNA-142 simultaneously to the step of expressing the at least two transcription factors.

In some preferred embodiments, the step c. of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics is repeated.

In more preferred embodiments, the step c. of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics is repeated after the step a. of contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group

consisting of PU.1, IRF8, and BATF3 and/or the step b. of expressing the at least two transcription factors, for example repeated twice, such as repeated three times, such as repeated 4 times, such as repeated 5 times, such as repeated 6 times, such as 7 times, for example at least 8 times.

5 In other embodiments, the step c. of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics is performed on day 0, day 3 and day 6 following the step a. of contacting a cell with a composition comprising one
10 or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3 and/or the step b. of expressing the at least two transcription factors.

15 In yet preferred embodiments, the step c. of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics is not performed prior to the step a. of contacting a cell with a composition comprising one or more RNA
20 construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3 and/or the step b. of expressing the at least two transcription factors.

In some embodiments, the step c. of contacting the cells with a composition comprising
25 miRNA mimics comprises miR-142 mimics, such as miR-142-3p, miR-142-3p-1, miR-142-3p-3 and/or miR-142-5p-1, and is performed on day 0, day 3 and day 6 following the step a. of contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3.

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RNA types

It may be beneficial that the RNA construct(s) of the present invention comprise of
35 consist of self-amplifying RNA (saRNA). saRNA typically include, but not limited to, replication elements of positive-strand RNA viruses, to be able to replicate and

increase the production of the antigens they encode, such as for example the transcription factors as described herein. The skilled person will appreciate that saRNAs can comprise, polyadenylation signals at the 3' end to enhance their stability and promote translation, as well as modified cap structures on their 5' end.

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In some embodiments of the compositions or methods of the present invention, the one or more RNA construct(s) comprise or consist of self-amplifying RNA.

Without wishing to be bound by theory, preferably the self-amplifying RNA is selected from the group consisting of: Venezuelan equine encephalitis (VEE) virus, Semliki forest virus, Sindbis virus, classical swine fever virus, tick-borne encephalitis virus, alphavirus chimera based on the Venezuelan equine encephalitis virus, and Sindbis virus replicons.

It may also be beneficial that the RNA constructs of the present invention comprise or consist of circular RNA, forming a closed-loop structure created by a back-splicing event. The skilled person will appreciate that circular RNAs have been engineered for example for optimal protein expression, including N6-methyladenosine (m6A) incorporation, vector topology, number of stop codons, 5' and 3' untranslated regions (UTRs), IRES sequences and synthetic aptamers. The skilled person will also appreciate that circular RNA are typically more stable than linear RNAs due to, for example, their resistance to exonucleases.

In some embodiments of the present invention, the one or more RNA construct(s) comprise or consist of circular RNA (circRNA).

25

In other embodiments, the one or more RNA construct(s) comprise or consist of linear RNA.

In some embodiments, the linear RNA molecule further comprises a 3' poly(A) tail, a Kozak sequence, an open reading frame, a 3' untranslated region, a 5' untranslated region, a 5' cap or any combination thereof.

In some embodiments, the skilled person will appreciate that linear RNAs have been modified for enhanced translatability or stability. In one embodiment, the modified RNA can comprise at least one modified nucleoside selected from the group consisting of, but not restricted to, 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-

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dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I).

Therefore in some embodiments, the one or more RNA construct(s) comprise or consist of non-modified RNA. In other embodiments, the one or more RNA construct(s) comprise or consist of modified RNA. In further embodiments, the one or more RNA construct(s) comprise or consist of linear modified RNA, such as linear m1-Ψ-modified mRNA.

Trans-amplifying RNAs are split-vector derivatives of self-amplifying RNAs (saRNAs), as they combine a non-replicating mRNA encoding an alphaviral replicase and a transreplicon RNA coding for the transgene.

In some embodiments of the present invention, the one or more RNA construct(s) comprise or consist of trans-amplifying RNA (taRNA). In preferred embodiments, the taRNA can be engineered for faster replication, greater potency and improved immunogenicity.

The skilled person will appreciate that RNA molecules may be modified to integrate, but not limited to, synthetic nucleosides, such as N1-Methylpseudouridine (m1-Ψ) in order to enhance translation of the proteins encoded by said RNAs.

In some embodiments, the one or more RNA construct(s) comprise or consist of modified RNA. In preferred embodiments, the modified RNA is a m1-Ψ-modified mRNA. In further embodiments, the modified RNA is linear modified RNA, such as linear m1-Ψ-modified mRNA.

30 *IFN inhibition*

In some embodiments of the methods of the present invention, the methods further comprise a step of inhibiting the IFN pathway of the cell.

In some embodiments, the step of inhibiting the IFN pathway of the cell is performed prior to, during, and/or after the step of contacting a cell with a composition comprising one or more constructs or vectors, or with a composition comprising one or more RNA construct(s).

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In other embodiments, the step of inhibiting the IFN pathway of the cell is performed prior to, during, and/or after the step of expressing the at least two transcription factors.

10 In some embodiments, the step of inhibiting the IFN pathway is performed using small molecules, viral proteins including, but not limited to, innate inhibiting proteins, delivery of shRNAs, siRNAs, miRNAs and miRNA mimetics targeting downstream mediators and/or effectors of IFN signaling.

15 In further embodiments, the step of inhibiting the IFN pathway is performed by contacting the cells with one or more IFN inhibitors.

In preferred embodiments, the one or more IFN inhibitors are selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors and virus proteins neutralizing type I interferons.

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In even more preferred embodiments, the one or more IFN inhibitors are selected from the group consisting of: B18R and ruxolitinib. In yet more preferred embodiments, the one or more IFN inhibitor is ruxolitinib. This may be the case in particular for methods described herein making use of linear RNA, circular RNA or saRNA, for example
25 methods of cDC1 reprogramming using linear RNA, circular RNA or saRNA, preferably wherein reprogramming efficiency is measured as induction of CD45 and MHC-II surface marker expression.

B18R is a Vaccinia virus protein that neutralizes type I interferons (IFNa, IFNb, IFNe,k,t,d,z,w,v) by inhibiting activation of interferon-mediated signal transduction.
30 Ruxolitinib belongs to the Janus kinase (JAK) inhibitors family, with selectivity for JAK1 and JAK2 subtypes.

The skilled person will appreciate that other IFN inhibitors include Tofacitinib, Baricitinib, Ritlecitinib, Abrocitinib, Upadacitinib and Delgocitinib, and viral proteins know as innate inhibiting proteins (IIPs), including but not restricted to HSV-2 Us1,
35 HSV-1 Us1, HSV-1Us11, Orf OV20.0L, BVDV Npro, PIV-5 V, MERS-CoV M, MERS-

CoV ORF4a, Langat NS5, Influenza NS1, vaccinia virus immune evasion protein E3, vaccinia virus immune evasion protein K3 and vaccinia virus immune evasion protein B18.

5 In some embodiments, the IFN inhibitors are Upadacitinib, Filgotinib, or Baricitinib. The inventors have showed herein that said inhibitors enhance cell reprogramming efficiency in some applications. This may be the case for example in methods of reprogramming cells to cDC1 cells, as measured by CD45 and/or HLA-DR positivity of reprogrammed cells, such as reprogrammed cDC1 cells from human dermal fibroblasts
10 (HDFs).

In preferred embodiments of the composition or the method described herein, the one or more IFN inhibitor is present at a concentration between 0 and 100 μ M, preferably between 10nM and 50 μ M, even more preferably between 100nM and 25 μ M, yet even
15 more preferably between 100nM and 10 μ M.

Cells and cell culture

In some embodiments of the methods of the present invention, the cell is a human or
20 non-human cell. In preferred embodiments, the cell is a human cell.

In yet other embodiments, the cell is a human fibroblast, such as a human dermal fibroblast (HDF), or a human embryonic fibroblast (HEF).

25 In some embodiments of the methods of the present invention the cell is a cancer cell, such as a cancer cell line, for example a primary cancer cell line. In other embodiments, the cell is a melanoma cancer cell, such as the A2058 cancer cell line, or a sarcoma cancer cell, for example Leiomyosarcoma, such as the SK-LMS-1 cancer cell line.

30 In some other embodiments, the cell is a mouse cell. In further embodiments, the cell is a mouse embryonic fibroblast (MEF).

In some embodiments of the methods of the present invention, the reprogrammed or induced cell is an induced dendritic cell (iDC). In preferred embodiments, the reprogrammed or induced cell is a human or non-human iDC, such as a mouse iDC.

- 5 In preferred embodiments, the reprogrammed or induced cell is a conventional type 1 dendritic cell (cDC1 cell).

In some embodiments, the reprogrammed or induced cell expresses CD45, XCR1 and/or MHC-II. This is for example the case when the cell is a mouse cell, for example
10 a mouse dendritic cell, such as a mouse cDC1 cell.

In preferred embodiments, the reprogrammed or induced cell expresses CD45, XCR1 and MHC-II.

In other embodiments, the reprogrammed or induced cell expresses CD45, CD226
15 and/or HLA-DR. This is for example the case when the cell is a human cell, for example a human dendritic cell, such as a human cDC1 cell.

In further embodiments, the reprogrammed or induced cell expresses CD45, CD226 and HLA-DR. In other embodiments, the reprogrammed or induced cell expresses
20 CD226. In yet other embodiments, the reprogrammed cell further expresses CD141.

In some embodiments of the methods of the present invention, the reprogramming or induction is *in vivo*, *in vitro*, or *ex vivo*.

- 25 In some embodiments, the method further comprises a step of culturing the cell in a cell media, wherein the culturing step is conducted before, during, and/or after expressing the at least two transcription factors.

In preferred embodiments, the cell is cultured during at least 2 days, such as at least 4
30 days, such as at least 5 days, such as at least 6 days, such as at least 8 days, such as at least 9 days, such as at least 10 days, such as at least 12 days.

In some embodiments, it may be beneficial that the cell culture media comprises one or more IFN inhibitor(s) selected from the group consisting of: kinase inhibitors,
35 JAK/STAT inhibitors, and virus proteins neutralizing type I interferons. In preferred

embodiments, the one or more IFN inhibitor(s) are selected from the group consisting of B18R and ruxolitinib. In even more preferred embodiments, the one or more IFN inhibitor is ruxolitinib.

5 In yet other embodiments of the methods of the present invention, the method further comprises culturing the cell in a cell media comprising one or more cytokines selected from the group consisting of: IFN β , IFN γ , TNF α , IFN α , IL-1 β , IL-6, CD40l, Flt3l, GM-CSF, IFN- λ 1, IFN- ω , IL-2, IL-4, IL-15, prostaglandin 2, SCF and oncostatin M (OM).

10 In other embodiments, the method further comprises culturing the cell in a cell media comprising one or more epigenetic modifiers, such as histone deacetylase inhibitors. In preferred embodiments, the histone deacetylase inhibitor is valproic acid.

15 In some embodiments of the methods of the present invention, the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed prior to, simultaneously, and/or after the step of expressing the at least two transcription factors.

20 In preferred embodiments, the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed no more
25 than 6h, such as no more than 12h, such as no more than 24 day, such as no more than 36h, such as no more than 48h, such as no more than 72h, such as no more than 96h, such as no more than 120h, such as no more than 144h after the step of expressing the at least two transcription factors.

30 *Markers expression and Methods of improving reprogramming efficiency and/or fidelity.*

In some embodiments of the methods of the present invention, the methods further comprise the steps of:

- 5 a. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II in the cell, after the step of expressing the at least two transcription factors;
- b. assessing the expression of CD45, XCR1, CD226, CD141, HLA-DR, HLA-ABC, CD40, and/or MHC-II in the cell after the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;
- 10 c. comparing the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II assessed in steps a. and b.,
- wherein a higher level of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II, preferably a higher level of expression of XCR1, even more preferably a higher level of expression of CD226 after contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics,
- 15 indicates increased cell reprogramming fidelity.
- 20

In other embodiments of the methods of the present invention, the methods further comprising the steps of:

- 25 a. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II in the cell, after the step of the step of expressing the at least two transcription factors;
- b. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II in the cell after the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;
- 30

- c. comparing the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II assessed in steps a. and b.,

wherein a higher level of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II, preferably a higher level of expression of CD45 and MHC-II, even more preferably a higher level of expression of CD45 and HLA-DR after contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, indicates increased cell reprogramming efficiency.

In some other embodiments of the methods comprising a step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, the dendritic cell or antigen-presenting cell displays increased cell reprogramming efficiency and/or fidelity.

The increased cell reprogramming efficiency and/or fidelity may be assessed as described herein.

In some embodiments of the methods of the present invention, the reprogramming efficiency and/or fidelity increases with increasing concentration of the miRNA(s), or pri-miRNAs thereof, and/or miRNA mimics, and/or with increasing concentration of the one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics.

In preferred embodiments, the assessment of the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II in the cell is performed using flow cytometry and/or single-cell RNA sequencing (scRNAseq).

In some embodiments of the methods of the present invention, the step c. of comparing the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II is performed by comparing the percentage of cells expressing CD45, XCR1, CD 141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II of step a. of the

method, with the percentage of cells expressing CD45, XCR1, CD141, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II of step b of the method.

5 Another aspect of the present invention relates to a method of improving cell reprogramming fidelity of a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of the methods as described herein.

10 A further aspect of the present invention relates to a method of improving cell reprogramming efficiency of a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of the methods as described herein.

15 In some embodiments, the cell reprogramming efficiency is improved by at least 25%, such as at least 50%, such as at least 75%, such as at least 100%, such as at least 200%, such as at least 300%, such as at least 500%.

20 In some embodiments, the cell reprogramming efficiency is improved by at least 0.25-fold, such as at least 0.5-fold, such as at least 1-fold, such as at least 1.5-fold, such as at least 1.6-fold, such as at least 1.7-fold, such as at least 1.8-fold, such as at least 1.9-fold, such as at least 2-fold, such as at least 2.1-fold, such as at least 2.2-fold, such as at least 2.3-fold, such as at least 2.4-fold, such as at least 2.5-fold, such as at least 2.6-fold, such as at least 2.7-fold, such as at least 2.8-fold, such as at least 2.9-fold, such as at least 3-fold, such as at least 5-fold, such as at least 10-fold.

25 In preferred embodiments, the cell reprogramming efficiency is improved by at least 1.5-fold, such as at least 1.6-fold, such as at least 1.7-fold, such as at least 1.8-fold, such as at least 1.9-fold, such as at least 2-fold, such as at least 2.1-fold, such as at least 2.2-fold, such as at least 2.3-fold, such as at least 2.4-fold, such as at least 2.5-fold, such as at least 2.6-fold, such as at least 2.7-fold, such as at least 2.8-fold, such as at least 2.9-fold, such as at least 3-fold, such as at least 5-fold, such as at least 10-fold.

30 In some embodiments, the cell reprogramming fidelity is improved by at least 25%, such as at least 50%, such as at least 75%, such as at least 100%, such as at least 200%, such as at least 300%, such as at least 500%.

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In some embodiments, the cell reprogramming fidelity is improved by at least 0.25-fold, such as at least 0.5-fold, such as at least 1-fold, such as at least 1.5-fold, such as at least 1.6-fold, such as at least 1.7-fold, such as at least 1.8-fold, such as at least 1.9-fold, such as at least 2-fold, such as at least 2.1-fold, such as at least 2.2-fold, such as at least 2.3-fold, such as at least 2.4-fold, such as at least 2.5-fold, such as at least 2.6-fold, such as at least 2.7-fold, such as at least 2.8-fold, such as at least 2.9-fold, such as at least 3-fold, such as at least 5-fold, such as at least 10-fold.

In other embodiments, the cell reprogramming fidelity is improved by at least 1.5-fold, such as at least 1.6-fold, such as at least 1.7-fold, such as at least 1.8-fold, such as at least 1.9-fold, such as at least 2-fold, such as at least 2.1-fold, such as at least 2.2-fold, such as at least 2.3-fold, such as at least 2.4-fold, such as at least 2.5-fold, such as at least 2.6-fold, such as at least 2.7-fold, such as at least 2.8-fold, such as at least 2.9-fold, such as at least 3-fold, such as at least 5-fold, such as at least 10-fold.

Another aspect of the present invention relates to a cell comprising the one or more RNA construct(s), or the one or more construct(s) or vector(s) as described herein.

In some embodiments, the cell is a mammalian cell, such as a human or murine cell.

In other embodiments, the cell is selected from the group consisting of: a stem cell, a differentiated cell, and a cancer cell, wherein:

a. the stem cell is selected from the group consisting of: a pluripotent stem cell and a multipotent stem cell, such as a mesenchymal stem cell or a hematopoietic stem cell;

b. the differentiated cell is any somatic cell, such as a fibroblast, for example a dermal fibroblast or an embryonic fibroblast, or a hematopoietic cell, such as a monocyte.

In some embodiments, the cell is a cancer cell, such as a cancer cell line, for example a primary cancer cell line. In other embodiments, the cell is a melanoma cancer cell, or a sarcoma cancer cell.

In preferred embodiments, the cell is a reprogrammed human dendritic cell or human antigen-presenting cell, such as a human type 1 conventional dendritic cell.

In even preferred embodiments, the cell expresses one or more surface marker(s) selected from the group consisting of: CLEC9A, CD45, CD141, XCR1, MHC-II, HLA-DR, HLA-ABC, CD40, and CD226.

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A further aspect of the present invention relates to a reprogrammed or induced cell obtained by the method as described herein.

10 In some embodiments, the reprogrammed or induced cell is an induced dendritic cell (iDC), preferably wherein the reprogrammed or induced cell is a human or non-human iDC, such as a mouse iDC.

In other embodiments, the reprogrammed or induced cell is a human or non-human iDC, such as a mouse iDC.

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In further embodiments, the reprogrammed or induced cell is a conventional type 1 dendritic cell (cDC1 cell).

20 In preferred embodiments, the reprogrammed or induced cell expresses CLEC9A, CD45, CD141, XCR1, CD226, HLA-DR, HLA-ABC, CD40, and/or MHC-II.

In some embodiments, for example wherein the cell is a mouse cell, the reprogrammed or induced cell expresses CD45, XCR1 and/or MHC-II.

In preferred embodiments, the reprogrammed or induced cell expresses HLA-ABC, and CD40.

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In other embodiments, for example wherein the cell is a human cell, the reprogrammed or induce cell expresses CLEC9A, CD141, HLA-DR and/or CD226.

Kit of parts

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Another aspect of the present invention relates to a kit-of-parts comprising:

a. The composition comprising one or more RNA construct(s) as described herein.

35 b. The composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics as described herein, or

the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein.

- 5 A further aspect of the present invention relates to a kit-of-parts comprising:
- a. The composition comprising one or more RNA construct(s) as described herein.
 - b. One or more IFN inhibitors selected from the group consisting of:
10 kinase inhibitors, JAK/STAT inhibitors such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected from the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more
15 preferably wherein the one or more IFN inhibitor is ruxolitinib.

Another aspect of the present invention relates to a kit-of-parts comprising:

- a. The composition comprising one or more RNA construct(s) as described herein.
- b. The composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics as described herein.
- c. One or more IFN inhibitors selected from the group consisting of:
20 kinase inhibitors, JAK/STAT inhibitors, such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected from the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more
25 preferably wherein the one or more IFN inhibitor is ruxolitinib.
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Another aspect of the present invention relates to a kit-of-parts comprising:

- 5
- a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors.
 - b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein.

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A further aspect of the present invention relates to a kit-of-parts comprising:

- a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3
- b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein.

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Another aspect of the present invention relates to a kit-of-parts comprising:

- a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors
- b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-

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miRNAs thereof, and/or miRNA mimics, as described herein

- 5 c. One or more IFN inhibitors selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected from the group consisting of B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is ruxolitinib.

10

A further aspect of the present invention relates to a kit-of-parts comprising:

15

- a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3

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- b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, as described herein.

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- c. One or more IFN inhibitors selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected from the group consisting of B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is ruxolitinib.

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Medical uses and method of treatment

Provided herein is the composition, the cell, the reprogrammed or induced cell, or the kit-of-parts as described herein, for use in medicine.

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Also provided herein is the composition, the cell, the reprogrammed or induced cell, or the kit-of-parts as described herein, for use in the treatment of cancer or infectious diseases.

In one embodiment, the cancer is selected from the group consisting of: colorectal cancer, head and neck cancer, basal cell carcinoma, cervical dysplasia, sarcoma, germ cell tumor, retinoblastoma, glioblastoma, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, blood cancer, prostate cancer, ovarian cancer, cervix cancer, oesophageal cancer, uterus cancer, vaginal cancer, breast cancer, gastric cancer, oral cavity cancer, naso-pharynx cancer, trachea cancer, larynx cancer, bronchi cancer, bronchioles cancer, lung cancer, pleural cancer, bladder and urothelial cancer, hollow organs cancer, esophagus cancer, stomach cancer, bile duct cancer, intestine cancer, colon cancer, rectum cancer, bladder cancer, ureter cancer, kidney cancer, liver cancer, gall bladder cancer, spleen cancer, brain cancer, lymphatic system cancer, bone cancer, pancreatic cancer, leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, skin cancer, melanoma and myeloma,

In preferred embodiments, the cancer is selected from the group consisting of: head and neck cancer, colorectal cancer, melanoma, breast cancer, lung cancer, liver cancer, lymphoma, bladder and urothelial cancer, pancreatic cancer and glioblastoma.

In even more preferred embodiments, the cancer is selected from the group consisting of: melanoma and sarcoma.

Provided herein is a method of treating cancer or infectious diseases, the method comprising administering to an individual in need thereof the composition, the cell, the reprogrammed or induced cell, or the kit-of-parts as described herein.

Also provided herein is the use of the composition, the cell, the reprogrammed or induced cell, or the kit-of-parts as described herein, for the manufacture of a medicament for the treatment of cancer or infectious diseases.

35

Examples

Example 1. General methods and materials

5 *Molecular cloning and plasmid preparation*

Selected mouse and human pri-miRNA sequences, together with approximately 300 bp upstream and downstream genomic sequence of the hairpin or hairpin cluster, were amplified from mouse or human genomic DNA using PCR. Resulting amplicons were size-selected with gel electrophoresis, purified and cloned into a constitutive lentiviral vector under control of the SFFV promoter using the Takara In-Fusion kit, according to the manufacturer's protocol. After cloning, plasmids were purified from bacterial cultures with the GeneJET Plasmid Miniprep Kit. Large scale plasmid production for lentivirus generation was performed using GenElute HP Plasmid DNA Maxiprep Kit. Insert regions of all plasmids used in this study were validated using Sanger sequencing.

Cell culture

All mouse experiments were performed by following the Swedish guidelines and with approval from a local ethics committee. Mouse embryonic fibroblasts were isolated from E13.5 Clec9a-tdTomato reporter mice as previously described (Rosa et al. 2018). MEFs were expanded until passage 2-4 before reprogramming. Human dermal fibroblasts were reprogrammed at passage 7-12. All cells were cultured in complete DMEM – Dulbecco's modified eagle medium with high glucose, 2 mM GlutaMAX Supplement, 1 mM sodium pyruvate, 10 µg/ml Penicillin/Streptomycin and 10 % fetal bovine serum (FBS) – at 5 % CO₂ and 37 °C. Fibroblasts were cultured in completed DMEM, on cell culture dishes pre-coated in 0.1 % gelatin for 15-30 minutes at 37 °C.

Lentiviral production

293Ts were grown in 15 cm tissue culture plates to 70-90 % confluency and transfected in serum-free DMEM with a mixture of 7.5 µg of psPAX2.G plasmid, 2.5 µg of pMD2 plasmid and 10 µg of the transfer plasmid coding for PIB TFs or pri-miRNA, together with 60 µL of PEI (1 mg/ml) in 2 mL of OptiMEM Reduced Serum Medium (Thermo Fisher Scientific). After 5 hours, transfection medium was replaced with complete DMEM. Next day it was changed again to 12 mL complete DMEM for collection. Virus-containing medium was collected every 8-16 hours to a total of three

times, after which the virus was filtered using a 0.45 µm low protein binding filter, concentrated 50-100X using Lenti-X Concentrator (Takara) and stored at -80 °C.

Mouse and human iDC reprogramming

5 On day -2 of reprogramming, fibroblasts were plated on 12- or 6-well plates, or 10 cm dishes at 250.000 cells per plate/dish. On day -1, cells were transduced with lentivirus containing PIB TFs and pri-miR sequences with polybrene at a final concentration of 8 µg/mL overnight. On day 0 of reprogramming, medium was changed back to complete DMEM. Medium was changed every 3 days. In the dose-dependency experiment, 10 either PIB or miRNA lentivirus titers were varied. When miRNA-titers were varied, PIB virus volume was kept constant at 10 µL. When miR-124 and miR-142 were co-transduced together, half the volume of each was used, for an equivalent total titer of the mixture to the individual miRNA vectors.

15 *Flow cytometry*

To assess reprogramming efficiency, cells were washed with PBS, raised with TrypLE Express, and resuspended in 200 µL PBS + 2.5 % FBS, with surface marker antibodies and 1:100 rat or mouse serum depending on the staining antibody species of origin. Cells were stained for 45 minutes at 4 °C, washed with PBS + 2.5 % FBS and kept on 20 ice until flow cytometry analysis. DAPI was used as a viability dye, unless otherwise specified. Flow cytometry analysis was performed on BD LSRII, BD LSRFortessa, BD LSRFortessa X-20, or BD FACSymphony A1. Flow cytometry data was analyzed using FlowJo (BD) software.

25 *Cell sorting*

For fluorescence-activated cell sorting, cells were raised and stained as described above for flow cytometry. MEFs for the cytokine expression assay were sorted on BD FACSMelody and HDFs for RNA-seq and ATAC-seq were sorted on BD 30 FACSymphony S6.

30 *Statistical analysis*

Graphing and statistical analysis were performed using GraphPad Prism 9 software. Data is shown as mean ± SD, unless otherwise specified. Statistical tests are reported in respective figure legends.

35

Example 2. miR-124 and miR-142 increase efficiency of cDC1 reprogramming mediated by PU.1, IRF8 and BATF3 in mouse cells.

Material and methods

5 Material and methods are as described herein above in Example 1.

Results

To screen the capacity of miRNAs to promote cDC1 reprogramming, the inventors used mouse embryonic fibroblast (MEF) harboring a DC-specific Clec9a-TdTomato (Clec9a-Cre X R26-stop-tdT mouse, abbreviated as Clec9a-tdT) reporter system. In
10 Clec9a-tdT MEFs, expression of tdTomato marks the acquisition of a DC fate during DC reprogramming (Rosa et al. 2018). 15 miRNA candidates were selected, cloned the respective pri-miRNA genomic sequences in constitutive lentiviral vectors, co-transduced Clec9a-tdT MEFs with pri-miRNA-expressing and PIB-expressing lentiviral
15 vectors and profiled reprogramming efficiency after 6 days (Fig. 1A). Beside quantifying activation of Clec9a-tdT reporter, the inventors also profiled surface markers associated with high cDC1 fidelity of reprogrammed cells, including CD45 (pan-hematopoietic marker) and MHC-II (antigen-presenting-cell marker) which co-expression marks successfully reprogrammed cells(Rosa et al. 2018), and XCR1
20 (cDC1-restricted marker) marking high cDC1 identity of reprogrammed cells. Although none of the miRNA candidates increased Clec9a-tdT reporter activation compared PIB alone (Fig. 1B), miR-124 increased the percentage of CD45⁺ MHC-II⁺ cells to 44.1 ± 6.6 % compared to 25.3 ± 4.7 % with PIB alone (Fig. 1C), suggesting that miR-124 increases reprogramming efficiency by promoting successful cDC1 reprogramming.
25 Several miRNA candidates decreased reprogramming efficiency (%CD45⁺ MHC-II⁺ cells) when combined with PIB, confirming that pri-miRNA overexpression by itself does not increase reprogramming efficiency. Interestingly, several candidates increased XCR1 expression, including miR-124, miR-126a, miR-142a and miR-150, suggesting that these miRNAs allow the generation of reprogrammed DCs with
30 statistically higher cDC1 fidelity when compared to PIB overexpression alone. In particular, miR-142a induced the highest increase (3.5-fold) in XCR1⁺ cells (23.7 ± 1.1 %) compared to PIB alone (6.8 ± 1.0 %) (Fig. 1D, E).

Conclusion

Together, these data suggest that miR-124 and miR-142a increase cDC1 reprogramming efficiency and fidelity of reprogrammed DCs when co-expressed with PIB.

5

Example 3. miR-124 and miR-142 show additive effect in DC reprogramming.*Material and methods*

Material and methods are as described herein above in Example 1.

10

Results

To assess whether miR-124 and miR-142a synergize to promote iDC reprogramming, the inventors co-transduced both pri-miRNA vectors in combination with PIB and assessed reprogramming efficiency. While there was no significant increase in the CD45+ MHC-II+ population (Fig. 2A), the inventors observed an additive increase in XCR1 expression (Fig. 2B, C). Increasing amounts of PU.1, IRF8 and BATF3 TFs increased Clec9a-tdT reporter activation, but not cDC1 fidelity of reprogrammed cells marked by the expression of CD45, MHC-II and XCR1 (Fig. 2D, top). In contrast, miR-124 and miR-142a promoted a dose dependent increase in the proportion of successfully reprogrammed cells based on the expression of CD45, MHCII and XCR1. Moreover, although Clec9a-tdT reporter activation was not affected by miR-124, miR-142 or the combination at day 2, 4, 6 or 9 of reprogramming (Fig. 2E, left), the percentage of CD45+ MHC-II+ XCR1+ reprogrammed DCs increased with the miRNAs on days 6 and 9 (Fig. 2E, right), suggesting that miRNAs increase the velocity and fidelity of cDC1 reprogramming. Interestingly, reprogrammed cells generated with PIB together with miR-124 and/or miR-142 were functional and responded to inflammatory stimulation by secreting proinflammatory cytokines including IL-12, CXCL10 and TNF α at reprogramming day 9 (Fig. 2F).

30 *Conclusion*

Together, these data suggests that miR-124 and miR-142a have an additive effect in DC reprogramming mediated by overexpression of PIB by enhancing the velocity of reprogramming and the fidelity of reprogrammed DCs. Moreover, these data also suggests that reprogrammed cells generated with PIB together with miR-124 and/or miR-142a are functional cDC1-like cells.

35

Example 4. miR-124 and miR-142 increase efficiency of cDC1 reprogramming mediated by PU.1, IRF8 and BATF3 in human cells.

5 *Material and methods*

Material and methods are as described herein above in Example 1.

Results

Next, the inventors wanted to evaluate if the activity of miR-124 and miR-142 was
10 conserved across mouse and human species. To this end, human dermal fibroblasts (HDFs) were transduced with human pri-miR sequences together with PIB factors and quantified reprogramming efficiency by analysing the surface expression of CD45 and HLA-DR (human equivalent to MHC-II) 9 days after transduction. Interestingly, the inventors observed that miR-124 and miR-142 increased reprogramming efficiency 2.8
15 and 1.9- fold (50.4 ± 16.1 % and 35.1 ± 5.8 %, respectively) compared to PIB alone (18.3 ± 5.5 %) (Fig. 3A, C). The inventors also measured the expression of CD226 as a surrogate marker for high cDC1 fidelity of reprogrammed DCs. The inventors observed that miR-142 increased the fidelity of reprogramming by increasing CD226 expression 2.3-fold (29.1 ± 9.6 % compared to 12.9 ± 6.5 %) compared to PIB alone (Fig. 3B, C).

20

Conclusion

Taken together, these data suggest that activity of miR-124 and miR-142 is conserved in mouse and human DC reprogramming. While miR-124 increases efficiency of the cell fate conversion by increasing the surface expression of the successful DC
25 reprogramming markers CD45 and MHC-II/HLA-DR, miR-142 enhances cDC1 fidelity of reprogrammed DCs measured by surface expression of the cDC1 restricted markers XCR1 (in the mouse) and CD226 (in the human).

30 **Example 5. miR-124 and miR-142 enhances cDC1 reprogramming efficiency by repressing the original cell fate and promoting cDC1 gene expression**

Material and methods

RNA-seq experiment library preparation and sequencing

On day 3, 6 and 9 of reprogramming, HDFs or iDCs were sorted for reprogramming
35 markers CD45 and HLA-DR. Empty vector-transduced control HDFs were sorted on

day 3 using the parent live cell gate. Human DC controls were isolated from buffy coats with the Pan-DC Enrichment Kit (Miltenyi Biotec) and sorted for cDC1s (HLA-DR+ CD11c+ CD141+), cDC2s (HLA-DR+ CD11c+ CD1c+ CD141-) and pDCs (HLA-DR+ CD11c- CD123+). 1000-2000 cells were sorted and their RNA extracted using

5 TRIZol (Thermo Fisher Scientific) extraction. RNA-seq libraries were prepared using SMARTer Stranded Total RNA-Seq Kit v3 (Takara) according to manufacturer's instructions. Two technical replicate RNA-seq library pools of 79-samples each were sequenced in a single run with the S2 reagent kit, split with a lane divider, on a NovaSeq 6000 (Illumina) sequencer.

10 *RNA-seq analysis*

To pre-process FASTQ files, the nf-core RNA-seq pipeline version 3.9 with default parameters was used, except for UMI extraction: `--with_umi --umitools_extract_method "regex" --umitools_bc_pattern2 "(?P<umi_1>.{8})(?P<discard_1>.{6}).*"`. In brief, the

15 inventors mapped the paired-end reads to the human genome (Ensembl, release 110) using STAR (Dobin et al. 2013) and performed downstream quantification with Salmon (Patro et al. 2017). (REF). Resulting gene counts were further processed with R package DESeq2 (Love, Huber, and Anders 2014) and normalized using RLE method. DESeq2 package and used for performing differential expression analysis based on

20 Wald test. The inventors defined upregulated genes by a fold change (FC) > 0.5 and Benjamini Hochberg (BH) corrected p-value < 0.05 and downregulated genes by FC < -0.5 and BH-corrected p-value < 0.05. To order conditions by reprogramming efficiency, the inventors calculated for each gene the average difference between the corresponding condition and empty vector-transduced HDFs and normalized it to the

25 difference between cDC1s and empty vector-transduced HDFs for individual conditions. CLEC9A and XCR1 expression and miRNA target downregulation were quantified with CPM normalization. Downregulated gene threshold in miRNA target prediction analysis was a mean fold change > 2 across three biological replicates. Cell type enrichment was performed against ARCHS4 using EnrichR.

30 *Results*

To explore the transcription changes induced by miR-124 and miR-142 in human DC reprogramming, the inventors performed total RNA sequencing (RNA-seq) at reprogramming days 3, 6 and 9 on HDFs co-transduced with PIB and miR-124, miR-

35 142 or scrambled (non-targeting) pri-miRNA sequence (Fig. 4A). Reprogrammed cells

were sorted for CD45⁺ on day 3, and for partially (CD45⁺ HLA-DR⁻) and successfully reprogrammed (CD45⁺ HLA-DR⁺) populations at days 6 and 9. As controls, empty vector-transduced HDFs as well as human cDC1s, cDC2s and pDCs isolated from peripheral blood of healthy donors were sorted. First, the inventors compared the expression of a cDC1 gene signature in reprogrammed cells and observed that miR-142-transduced CD45+HLA-DR+ iDCs at days 6 and 9 were more enriched in cDC1 genes compared to reprogrammed cells transduced with PIB and the scrambled control, indicating that miR-142 enhances cDC1 fidelity of reprogrammed DCs (Fig. 4B). The inventors further validated this finding by detecting expression of the cDC1-specific genes CLEC9A, THBD (encoding CD141) and XCR1 (Fig. 4C). Interestingly, miR-124 also showed an increase in cDC1 gene expression at day 3 compared to scrambled control (Fig. 4B) and allowed expression of XCR1, which was not detected at any point in reprogrammed DCs transduced with PIB and scrambled miRNA (Fig. 4C). Next, the inventors investigated the genes downregulated in reprogrammed DCs by miRNA overtime. Genes downregulated at least 2-fold by miR-124 at day 3 were strongly enriched for fibroblast identity, while genes related to pDC and macrophage identity were downregulated at days 6 and 9 (Fig. 4D). It was also observed that genes downregulated by miR-142 were associated with fibroblast identity throughout the time course of reprogramming (Fig. 4E). To explore possible targets of miR-124 and miR-142 underlying enhanced DC reprogramming, the inventors compared experimentally validated miRNA interactions (Huang et al. 2019) and miRNA seed sequence-filtered Argonaute CLIP-seq data (J.-H. Li et al. 2014) with downregulated genes on day 3 as an estimate of degree of direct targeting versus indirect facilitation of cell state transition by PIB TFs. miR-124 was predicted to directly target 57 % (224/392) of downregulated genes compared to 38 % by miR-142 (113/300) (Fig. 4F), suggesting that miR-124 directly represses a higher percentage of genes downregulated at early stages of DC reprogramming.

Conclusion

Taken together, miR-124 and miR-142 repress the original cell fate and promote cDC1 gene expression, with miR-124 acting primarily early in reprogramming and miR-142 driving increasing iDC fidelity over time.

Example 6. miR-124 induces a permissive chromatin state in the context of cDC1 reprogramming

Material and methods

5 *ATAC-seq experiment library preparation and sequencing*

On day 3, 6 and 9 of reprogramming, HDFs or iDCs were sorted for reprogramming markers CD45 and HLA-DR. Empty vector-transduced control HDFs were sorted on day 3 using the parent live cell gate. Human DC controls were isolated from buffy coats with the Pan-DC Enrichment Kit (Miltenyi Biotec) and sorted for cDC1s (HLA-DR+ CD11c+ CD141+), cDC2s (HLA-DR+ CD11c+ CD1c+ CD141-) and pDCs (HLA-DR+ CD11c- CD123+). The inventors sorted 5000-10000 cells per sample and followed a previously published protocol (Buenrostro et al. 2015) adapted for 96-plex indexing and lower cell inputs, with the transposition reaction scaled down to 10 μ L/10.000 cell sample (with 0.5 μ L of TDE1 transposase). The two technical replicate
10 ATAC-seq library pools of 79-samples were sequenced in a single run with an S4 reagent kit split with a lane divider, on a NovaSeq 6000 (Illumina) sequencer.
15

ATAC-seq analysis

In order to pre-process the FASTQ files, the nf-core (Ewels et al. 2020) ATAC-seq pipeline version 1.2.2 was used with default parameters. Reads were mapped to the GRCh38 reference genome using BWA (H. Li and Durbin 2009). Peak calling was performed with MACS2 separately for each sample. A combined peak list for all samples was obtained by using BEDTools (Quinlan and Hall 2010). Finally, read counts on a combined peak list were calculated using featureCounts. The resulting
20 read counts were processed with R package DESeq2 (Quinlan and Hall 2010) (REF) and normalized using the RLE method. For peak annotation ChIPseeker R library (Yu, Wang, and He 2015)) was used. To map common chromatin changes, a modified procedure for ATAC-seq data was used, as described above. Briefly, for each peak associated with individual genes from the cDC1 signature the inventors calculated an
25 average difference between each condition and empty vector-transduced HDFs and normalized it to the difference between cDC1s and empty vector-transduced HDFs. For motif discovery, findMotifsGenome.pl procedure from HOMER (Heinz et al. 2010) was used on differential ATAC-seq peaks with default parameters. Pathway enrichment analysis for genes associated with differential ATAC-seq peaks was performed with
30 EnrichR (Kuleshov et al. 2016).
35

Results

The inventors sought to characterize the effects of the miRNAs on PIB-driven chromatin remodeling. To do this, an assay for transposase-accessible chromatin with sequencing (ATAC-Seq) was performed at reprogramming days 3, 6 and 9 using HDFs co-transduced with PIB and miR-124, miR-142 or scrambled (non-targeting) pri-miRNA sequence (Fig. 4A). The inventors analyzed chromatin accessibility in regions containing cDC1s genes and observed that the majority was accessible from as early as day 3 (Fig. 5A), which goes in line with our previous findings in DC reprogramming of cancer cells (Zimmermann et al. 2023). miR-124 increased accessibility of cDC1 genes compared to miR-142 or the scrambled control (Fig. 5A). Accordingly, miR-124 induced a gain of 3503 accessible peaks and a loss of 1299 accessible peaks compared to scrambled control on day 3, indicating an early induction of permissive chromatin landscape (Fig. 5B). miR-142 showed an opposite effect on chromatin accessibility, with 77.6 % less differentially accessible peaks when compared to cells treated with miR-124, with the majority (858 peaks; 79,7 %) being less accessible when compared to the scrambled control at day 3. Peaks with increased accessibility upon miR-124 overexpression were enriched in Polycomb Repressive Complex (PRC) members SUZ12 and EZH2, as well as in transcriptional repressor REST (Fig. 5C), indicating a possible role in H3K27me3 mark inhibition in increasing PU.1 binding site accessibility in early reprogramming. Interestingly, both miRNAs showed an enrichment for SPI1 (PU.1) motifs in regions with higher accessibility (Fig. 5D), indicating that both miRNAs may facilitate chromatin binding of PU.1 and consequently increase DC reprogramming efficiency and fidelity. Less accessible regions were enriched in IRF motifs, suggesting that increased DC reprogramming efficiency observed with miRNAs can be induced by restricting chromatin binding of IRF8 and IRF8-driven gene expression. To validate the effect of Polycomb Repressive Complex 2 (PRC2) inhibition on DC reprogramming, the inventors treated HDFs with the EZH2 inhibitor GSK-126 at various timepoints of reprogramming (Fig. 5E). Interestingly, pre-treatment with GSK-126 increased reprogramming efficiency compared to DMSO control (Fig. 5F).

Conclusion

These data suggest that miR-124 and miR-142 modify chromatin accessibility favoring PU.1 binding and restricting IRF8 binding, which can be important molecular

mechanisms underlying efficient cDC1 reprogramming. These data also suggests that PRC2 inhibition can enhance cDC1 reprogramming efficiency.

Example 7. miR-124-3p mimics promote iDC reprogramming in HDFs

5

Material and methods

Transfection of miRNA mimics

mirVana miRNA mimics were transfected using Lipofectamine RNAiMAX (Thermo Fisher Scientific) according to the manufacturer's protocol to the final miRNA
10 concentration of 1 or 10 nM.

RT-qPCR quantification

Day 9 CD45+ HLA-DR+ cells were sorted into 350 µL of RNeasy Micro (Qiagen) lysis buffer from which total RNA was later isolated according to the manufacturer's protocol.
15 DNA libraries were prepared using the SuperScript IV Reverse Transcriptase (Invitrogen) with random hexamer primers. XCR1 expression was quantified by qPCR using TaqMan probes (ThermoFisher Scientific), with PPIA used as the reference gene.

Results

Next, the inventors wanted to investigate whether the effects of miRNA overexpression mediated by lentiviral vectors could be replicated by co-transfection of miRNA mimics, which are chemically synthesized double-stranded RNA molecules imitating mature miRNA duplexes. The inventors started by generating miRNA mimics for miR-124 (Fig.
25 6A), and transfected miRNA mimics on days 0, 3 and 6 of reprogramming to simulate constitutive pri-miRNA expression. miR-124-3p replicated the increase in reprogramming efficiency in a dose-dependent manner (Fig. 6B, C), with expected strand preference since miR-124-3p is known to be the primary mature strand of miR-124 (Kozomara, Birgaoanu, and Griffiths-Jones 2019). To assess whether prior
30 transfection with miRNA mimics induces a cellular or chromatin environment which favors iDC reprogramming, miR-124-3p was added on days -2, 0, 3 or 6 (Fig. 6D). Surprisingly, pre-treatment or day 6 addition of miR-124-3p did not increase reprogramming efficiency (Fig. 6E), underscoring the importance of cooperation between the PIB transcription factors and miR-124 for enhanced cDC1 reprogramming
35 efficiency. Unexpectedly, none of the canonical miR-142 strands affected

reprogramming efficiency (Fig. 6B). Since miR-142 processing results in heterogeneous products (Manzano et al. 2015), the inventors selected the 3 most expressed 5' isomiRs of each strand based on the isomiRdb microRNA expression database (Aparicio-Puerta et al. 2023) and investigated their impact in cDC1 reprogramming efficiency. Upon co-transfection, miR-142-3p-1 and miR-142-5p-1 increased the frequency of CD45+ and CD45+ HLA-DR+ cells, respectively (Fig. 6F), while miR-142-3p-3 induced upregulation of the cDC1 marker CD226 (Fig. 6G). XCR1 was primarily upregulated by the canonical 3' strand of miR-142 by qPCR (Fig. 6H). miRNA mimics had comparable effect on HEF reprogramming, with miR-124-3p, miR-142-3p-1, miR-142-3p-1 and miR-142 combination increasing reprogramming efficiency compared to the scrambled control (Fig. 6I). The miR-142-3p, miR-142-3p,5p and miR-142-3p-1 increased XCR1 expression in HEFs (Fig. 6J). The inventors also detected a notable additive effect of co-transfection with all 8 miR-142 mimics. Taken together, the inventors selected the canonical miR-142-3p and the isomiRs miR-142-3p-1, miR-142-3p-3 and miR-142-5p-1 for follow-up analyses, alongside miR-124-3p. When added together, these mimics showed limited additive effects on reprogramming efficiency to CD45+ HLA-DR+ iDC, apart from the combination of miR-124-3p and miR-142-5p-1 (Fig. 6K). However, mimics had a synergistic effect on reprogramming fidelity quantified by XCR1 expression, with the combination of PIB and miR-142-3p, miR-142-3p-1 and miR-142-5p-1 producing 9.3 ± 6.0 % XCR1+ iDCs, compared to 0.6 ± 0.2 % with the scrambled control (Fig. 6L). In line with previous findings, repeated transfection with selected miR-142 mimics on days 0, 3 and 6, moderately increased iDC reprogramming efficiency, while pre-treatment significantly decreased it (Fig. 6M).

25 *Conclusion*

These data suggests that transfection of miRNA mimics and/or isomiRs mimics can be used to replace lentiviral-mediated overexpression of immature miRNAs in the context of cDC1 reprogramming and demonstrates that cooperation between PU.1, IRF8 and BATF3 transcription factors and miR-124 and/or isomiRs of miR-142 is critical for enhanced cDC1 reprogramming efficiency.

Example 8. Identification of targets of canonical strands and isomiRs in iDC reprogramming

The inventors integrated transcriptomic and genomic accessibility data using GRaNP
5 (Kamal *et al.* 2023) to identify miRNA targets which could mediate their effect in DC reprogramming (Fig. 7A). They identified SP1 and KLF5 as the most significant TFs targeted by miR-124-3p, and KLF10, PATZ1 and ELK3 by validated miR-142 strands (Fig. 7B). Considering the role of miRNAs in target downregulation, the inventors investigated whether GRaNP target predictions were downregulated upon hairpin
10 overexpression and confirmed that SP1, KLF5 and RELA were significantly downregulated on co-transduction with miR-124 (Fig. 7C).
To further explore the mechanisms downstream miRNA overexpression, the authors plotted STRING networks of differentially expressed proteins on day 3 together with GRaNP hits (Fig. 7D) (<https://string-db.org/cgi/about>, Szklarczyk *et al.* 2023). Within
15 the miR-124 network, STAT3, MAPK14 and RELA represented nodes with the highest connectivity to differentially expressed proteins, indicating that they could be mediators downstream of miR-124 hairpin overexpression in DC reprogramming. Within the miR-142 network, the CCND1 node had the highest degree and was a predicted target of the miR-142-3p-1 isomiR. Additionally, TGFBR1 and RAB2A were predicted targets of
20 at least 3 miR-142 mimics.

Conclusion

These data suggests that inhibition of MAPK14, STAT3, RELA, CCND1 and TGFRB1
25 can enhance DC reprogrammed and/or fidelity.

Example 9. Linear RNA encoding PU.1, IRF8 and BATF3 allows cDC1 reprogramming

30 *Material and methods*

Transfection of miRNA mimics and mRNAs

Linear m1-Ψ-modified monocistronic and bicistronic mRNAs coding for human PU.1, PU.1-T2A-mTagBFP2 (PU.1-BFP), IRF8, IRF8-T2A-miRFP670nano (IRF8-RFP), BATF3, BATF3-T2A-mNeonGreen (BATF3-GFP) and GFP were generated and in vitro
35 synthesized. Monocistronic linear mRNAs were co-transfected together with miRNA

mimics using Lipofectamine RNAiMAX with a total of 1 µg of mRNAs combined with a 1:1:1:1 mass ratio and the miRNA mimics for a final concentration of 1 nM. For transfection of human cancer cells, the three linear m1-Ψ-modified bicistronic mRNAs were co-transfected using Lipofectamine RNAiMAX with a total of 100ng of mRNAs in the presence or absence of ruxolitinib, Baricitinib, Fedratinib, Upadacitinib or Filgotinib, and reprogramming efficiency was profiled 60h after transfection.

Results

To test whether RNA-mediated delivery of transcription factors allows cDC1 reprogramming in human cells, the inventors transfected HDFs with modified linear mRNAs encoding human PU.1, IRF8, BATF3 or GFP with or without miRNA mimics on days 0, 2, 4 and 6 and profiled cDC1 reprogramming efficiency at day 9 (Fig. 8A). Interestingly, the inventors observed expression of the cDC1 reprogramming marker CD45 in transfected HDFs, suggestion that cDC1 reprogramming is possible using modRNA. Moreover, it was observed that co-delivery of miR-124 mimetics with the mRNA mix encoding PIB and GFP increased 2-fold cDC1 reprogramming efficiency measure by the surface expression of CD45 when compared to transfection with mRNA mix encoding PIB and GFP alone or combined with the scrambled mimic control (Fig. 8B). Next, the inventors transfected HDFs with modified bicistronic linear mRNAs encoding human PU.1-BFP2, human IRF8-RFP, and human BATF3-GFP with or without miRNA mimics and/or the JAK1/2 inhibitor ruxolitinib and profiled cDC1 reprogramming efficiency at day 3 after one single transfection at day 0 (Fig. 8C). Interestingly, the inventors observed similar cDC1 reprogramming efficiency at day 3 induced lentiviral vector-transduced GFP+ cells (Gated in GFP+) and mRNA+ cells (gated in BFP+RFP+GFP+) (Fig. 8D). Moreover, the inventors also observed that miR-124-3p increased reprogramming efficiency compared to either PIB mRNA alone or the scrambled control, and that PIB mRNA with no mimics, miR-142-3p-1, miR-142-3p-3 and the combination of all 5 miRNA mimics increased the yield of CD45+ iDCs when compared to reprogramming mediated by lentiviral vectors (Fig. 8E). Next, the inventors investigated whether modified linear mRNA allows reprogramming of cancer cells. Interestingly, the inventors observed that one single transfection was sufficient to induce upregulation of the cDC1 reprogramming markers CD45 and/or HLA-DR in the human cancer cell lines SK-LMS-1 (sarcoma) and A2058 (Melanoma) (Fig. 8F). To explore the impact of JAK signaling inhibition in cDC1 reprogramming efficiency, the

inventors transduced human dermal fibroblasts with lentiviral vectors encoding PU.1, IRF8 and BATF3 and profiled reprogramming yield in the presence of different IFN inhibitors, including Ruxolitinib (rux), Baricitinib (Bari, JAK1/JAK2 inhibitor), Fedratinib (Fedra, JAK2 inhibitor), Upadacitinib (Upa, JAK1 inhibitor) and Filgotinib (Filgo, JAK 1 inhibitor), at 3 different concentrations 9 days after transduction. First, the inventors detected reprogrammed cells in the presence of all inhibitors (Fig. 8G). While treatment with Febratinib and ruxolitinib reduced the total yield of reprogrammed cells, it was observed that treatment with Upadacitinib, Filgotinib and Baricitinib increase the total number of reprogrammed cells. The inventors also observed that transfection of HDFs with mRNA encoding PU.1, IRF8 and BATF3 together with miR-124-3p resulted in high cDC1 reprogramming efficiency 6 days after transfection, associated with the emergence of a clear population of HDFs co-expressing CD45 and HLA-DR at similar levels to lentiviral vector-mediated overexpression of PU.1, IRF8 and BATF3.

15 *Conclusion*

These data demonstrates that cDC1 reprogramming in human somatic healthy and cancer cells can be induced using linear RNA. The data also suggests that JAK inhibition can increase cDC1 reprogramming efficiency, and that miR-124-3p synergises with PU.1, IRF8 and BATF3 overexpression mediated by mRNA to induce high cDC1 reprogramming efficiency.

Example 10. saRNA encoding PU.1, IRF8 and BATF3 induces cDC1 reprogramming

25 *Material and methods*

Transfection of constructs

Synthetic polycistronic self-amplifying RNAs were generated based on the Venezuelan equine encephalitis (VEE) genome (Yoshioka et al. 2013; Yoshioka and Dowdy 2017) encoding the human sequences of PU.1, IRF8 and BATF3 separated by 2A self-cleaving peptides in a tricistronic cassette (saRNA-PIB). saRNA-PIB and GFP-encoding saRNA (saRNA-GFP) were synthesized in vitro and cells co-transfected with saRNA-PIB (0.5ug) and saRNA-GFP (0.5ug), using Lipofectamin Messenger Max. After transfection, the media was daily replaced by fresh culture media with or without B18R, ruxolitinib or both.

35

Results

To test whether saRNA-mediated delivery of PU.1, IRF8 and BATF3 allows cDC1 reprogramming, Clec9a-tdT MEFs were co-transfected at day 0 with saRNA-PIB and saRNA-GFP and cDC1 reprogramming efficiency was evaluated by flow cytometry quantification of tdTomato+ reprogrammed cDC1-like cells. Because saRNA replication was previously shown to elicit strong interferon (IFN) responses that can affect protein expression and reprogramming efficiency, the inventors also tested whether treatment of transfected cells with the IFN inhibitors B18R (Vaccinia virus protein that neutralizes type I interferons) and ruxolitinib (JAK1/JAK2 inhibitor) could allow higher reprogramming efficiency. First, the inventors observed that saRNA-PIB allowed tdTomato reporter activation and expression of CD45 and MHC-II, suggesting that saRNA can induce cDC1 reprogramming (Fig 9. A-C). Although no clear differences were observed in tdTomato reporter activation in cell cultures treated with B18R or ruxolitinib, the inventors observed that treatment with ruxolitinib was critical to induce high surface expression of the reprogramming markers CD45 and MHC-II.

Conclusion

These data demonstrates that cDC1 reprogramming can be induced using saRNA and shows that treatment with IFN inhibitors enhances cDC1 reprogramming efficiency mediated by saRNA-PIB.

Example 11. Linear, circular and self-amplifying RNA platforms exhibit different payload expression levels and kinetics.

Material and methods

Cell culture

Human leiomyosarcoma cell line SK-LMS-1 was cultured in complete DMEM – Dulbecco's modified eagle medium with high glucose, 2 mM GlutaMAX Supplement, 1 mM sodium pyruvate, 10 µg/ml Penicillin/Streptomycin and 10 % fetal bovine serum (FBS) – at 5 % CO₂ and 37 °C.

Transfection of RNA constructs

Linear m¹-Ψ-modified RNA, circular RNA, and self-amplifying RNA, coding for EGFP were in vitro synthesized and purified by Genescript. On day -1 of transfection, SK-

LMS-1 cells were plated in 48-well plates. On day 0 of transfection, cells reached 70-90 % confluency, and medium was changed to complete DMEM with ruxolitinib at a final concentration of 1 μ M. Then, cells were transfected with linear, circular, or self-amplifying RNA coding for EGFP, together with 0.2 μ L of Lipofectamine RNAiMAX in 20 μ L of OptiMEM Reduced Serum Medium. In the dose-dependency experiment, the total amount of RNA varied. On day 1 and 4 of transfection, cells were raised with TrypLE Express and resuspended in complete DMEM with ruxolitinib at a final concentration of 1 μ M. A fraction of the cells from each well were transferred to new 48-well plates to avoid over-confluency due to high growth rate of SK-LMS-1 cells. In the redosing experiment, cells were transfected on day 0, 3, and 6, respectively.

Flow cytometry

To assess transfection efficiency from day 1 to 8, cells were raised with TrypLE Express and resuspended in autoMACS running buffer (Miltenyi Biotec). Cells were washed once with autoMACS running buffer and stained with Fixable Viability Stain 620 (BD Biosciences). Cells were stained for 10 minutes at room temperature in the dark, washed once with autoMACS running buffer, and kept on ice until flow cytometry analysis on Cytex Aurora (4L V/B/YG/R). Flow cytometry data were analyzed using FlowJo (BD) software.

Statistical analysis

Graphing and statistical analysis were performed using GraphPad Prism 10 software. Data is shown as mean $\pm$ SD, unless otherwise specified.

Results

To benchmark linear, circular and self-amplifying RNA platforms, the inventors started by profiling eGFP expression dynamics after transfection of SK-LMS-1 human cancer cells using eGFP-encoding modified linear, circular and saRNAs (Fig. 10A-F). The inventors observed that frequency of eGFP+ cells peaked at day 2 post transfection for the 3 RNA platforms, with linear and circular RNA allowing higher frequency of eGFP+ cells overtime. While eGFP mean fluorescence intensity (MFI, readout of level of transgene expression) also peaked at day 2 post transfection for linear and circular RNAs, it remained unchanged from day 1 to day 8 for saRNA. After day 2 post transfection, frequency of eGFP+ cells decreased overtime for the 3 platforms, with saRNA and circular RNA showing fastest and slowest decline of frequency of eGFP+

cells overtime, respectively. Moreover, the inventors detected the presence of a small population of eGFP+ cells expressing high levels of eGFP from day 1 to day 8 in cultures specifically in saRNA-treated cultures, suggesting that saRNA can amplify within transfected cells allowing high eGFP expression even 8 days after transfection.

5 Interestingly, the inventors observed that circular RNA allowed the highest frequency of eGFP+ cells at day 8 post transfection (between 20-40% eGFP+ cells) when compared to linear RNA (<20% eGFP+ cells) and saRNA (<5% eGFP+ cells) (Fig. B-E). Next, the inventors asked whether redosing allows prolonged transgene expression mediated by the 3 RNA platforms (Fig. 10G). They demonstrated that redosing allows higher
10 frequency of eGFP+ cells overtime for the 3 platforms, associated with increased frequency of eGFP+ positive cells (Fig. 10H) and enhanced eGFP MFI (Fig. 10I).

Conclusion

These data show that linear RNA allows highest transgene expression at day 2 post
15 transfection (early timepoint), followed by circular RNA and saRNA, and that saRNA shows highest transgene expression at day 8 (late timepoint). The different RNA platforms show distinct kinetics of frequency of transfected cells, with circular RNA allowing higher frequency of eGFP+ transfected cells at day 8 post transfection when compared to linear and saRNA. Moreover, these data also suggest that repeated
20 transfections can enhance the frequency of transfected cells overtime.

Example 12. Linear, circular and self-amplifying RNA platforms allow cDC1 reprogramming in cancer cells.

25 *Material and methods*

Transfection of RNA constructs

Linear m1-Ψ-modified monocistronic RNAs, circular monocistronic RNAs, and self-amplifying polycistronic RNA, coding for human PU.1, IRF8, and BATF3 were in vitro synthesized and purified by Genescript.

30 On day -1 of reprogramming, SK-LMS-1 cells were plated in 48-well plates. On day 0 of reprogramming, cells reached 70-90 % confluency, and medium was changed to complete DMEM containing 1 μM ruxolitinib. Cells were then transfected with linear, circular, or self-amplifying RNA coding for PIB TFs, together with 0.2 μL of Lipofectamine RNAiMAX in 20 μL of OptiMEM Reduced Serum Medium. For linear and
35 circular RNAs encoding monocistronic PIB TFs, three PIB TFs were mixed at 1:1:1

molar ratio. In the dose-dependency experiment, the total amounts of RNAs were varied. On day 1 of reprogramming, cells were raised with TrypLE Express and resuspended in complete DMEM with ruxolitinib at a final concentration of 1 μ M. A fraction of the cells from each well were transferred to a new well in 48-well plates to avoid crowding due to the high growth rate of SK-LMS-1 cells.

Flow cytometry

To assess reprogramming efficiency, cells were raised with TrypLE Express on day 3 and resuspended in autoMACS running buffer. Cells were washed once with autoMACS running buffer and stained with surface marker antibodies and 1:50 mouse serum. Cells were stained for 20 minutes at 4 °C, washed twice with autoMACS running buffer and kept on ice until flow cytometry analysis. DAPI was used as a viability dye. Flow cytometry analysis was performed with Cytex Aurora (4L V/B/YG/R). Flow cytometry data were analyzed using FlowJo (BD) software.

Results

The inventors profiled cDC1 reprogramming efficiency after transfection with linear (monocistronic RNAs encoding PU.1, IRF8 and BATF3), circular (monocistronic RNAs encoding PU.1, IRF8 and BATF3) and self-amplifying RNA (tracistronic RNA encoding PU.1, IRF8, BATF3 and eGFP) constructs using SK-LMS-1 human cancer cells (Fig. 11A). First, the inventors observed that all RNA platforms could induce cDC1 reprogramming measured by the frequency of cancer cells expressing CD45 and/or HLA-DR in live (for linear and circular RNA) or eGFP+ (for saRNA) cells 3 days after transfection (Fig. 11B). Interestingly, they observed that circular RNA allowed the highest frequency of CD45+HLA-DR+ cells across the 3 RNA platforms.

Conclusion

These data demonstrate that linear RNA, circular RNA and saRNA allows cDC1 reprogramming in cancer cells and highlight circular RNA as the platform allowing highest reprogramming efficiency.

Example 13. Redosing of RNA improves reprogramming efficiency.*Material and methods**Transfection of constructs*

5 The same RNA constructs as those in example 12 were used, following the same transfection protocol. In the redosing experiment, cells were treated with 1 dose (day 0), two doses (day 0 and 3), and three doses (day 0, 3, and 7) of linear, circular, or self-amplifying RNA coding for PIB TFs. On day 3 and 6 of reprogramming, cells were subcultured to avoid crowding.

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Results

Next, the inventors investigated the kinetics of cDC1 reprogramming after 1 or multiple doses (transfections) of linear, circular and saRNA encoding PU.1, IRF8 and BATF3. SK-LMS-1 cells were transfected at day 0, 3 and/or 7 with the different RNA platforms and reprogramming efficiency was evaluated at day 3, 6 and 9 after the first dose by flow cytometry quantification of CD45, HLA-DR, CD40 and HLA-ABC (Figure 12A). As previously demonstrated in Example 12, one dose of linear, circular or saRNA allowed high cDC1 reprogramming efficiency detected at day 3 post-transfection (Figure 12B-E). The inventors also observed upregulation of CD40 and HLA-ABC expression induced by the 3 PIB-encoding RNA platforms, suggesting that reprogrammed cells generated by RNA platforms are functional antigen-presenting cells (Figure 12F, G). Reprogramming efficiency decreased over time to frequencies of CD45+ and/or HLA-DR+ cells below 5% independently of the reprogramming platform. Nevertheless, circular RNA allowed higher reprogramming efficiency at day 6 with one single dose at day 0 when compared to linear and saRNA. A small population of SK-LMS-1 cells co-expressing CD45 and HLA-DR was detected specifically in cultures transfected with PIB-encoding saRNA. To dissect this observation, the inventors profiled reprogramming efficiency within eGFP+ cells 3, 6 and 9 days after transfection with PIB-eGFP – encoding saRNA. In agreement with the previous observation, the frequency of CD45+ and/or HLA-DR+ cells within eGFP+ cells remained constant until day 9, suggesting that saRNA amplified within transfected cells allowing reprogrammed cells to persist over time (Figure 12H).

The inventors observed that the frequency of reprogrammed cells could be maintained over time or enhanced with multiple dosing at day 3 and/or day 7 for the different RNA platforms. Importantly, two doses of circular RNA (at day 0 and 3) allowed highest

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reprogramming efficiency at day 9 (approx. 40% CD45+ and/or HLA-DR+ cells) when compared to 2 doses of linear or saRNA (approx. 10% CD45+ and/or HLA-DR+ cells). Next, the inventors looked at the absolute numbers of reprogrammed cells expressing CD45 and/or HLA-DR after single or multiple doses of PIB-encoding linear, circular and saRNA (Figure 12I). With one dose at day 0, saRNA allowed highest number of reprogrammed cells at day 9. With two doses at days 0 and 3, circular RNA allowed the highest number of reprogrammed cells at day 9. With 3 doses at days 0, 3 and 7, linear RNA allowed the highest number of reprogrammed cells.

Conclusion

These data demonstrates that multiple dosing can allow higher reprogramming efficiency and yield for RNA-mediated reprogramming, particularly for linear and circular RNA. Moreover, these data suggests that reprogrammed cells generated by linear, circular and saRNA are functional antigen-presenting cells.

Example 14. Delivery of RNA encoding DC reprogramming transcription factors for cancer immunotherapy (Prophetic)

The inventors have previously demonstrated that in vivo delivery of a viral vector encoding the transcription factors PU.1, IRF8, and BATF3 enables in situ direct reprogramming of cancer cells into type 1 conventional dendritic cells (cDC1). This reprogramming remodels the tumor microenvironment, induces tumor regression, and establishes long-term systemic anti-tumor immunity in mouse syngeneic models (Asci *et al.* 20242024). It also exhibited strong synergistic effects when combined with immune checkpoint blockade (ICB) therapy.

The RNA modalities described herein provide an alternative non-viral system for direct in vivo tumor cell reprogramming.

In this prophetic example, in vivo delivery of RNA to a subject will induce efficient reprogramming of tumor cells into functional cDC1s. These reprogrammed cells will acquire enhanced antigen-presentation capacity, recruiting and activating cytotoxic T-cells within the tumor microenvironment so as to induce antitumor immune response that will result in in vivo efficacy, such as control of tumor growth, or such as complete tumor regression. Additionally, when combined with ICB therapy in a subject such as anti-PD-1 or anti-CTLA-4 antibodies, RNA-based reprogramming will further enhance T-cell infiltration and overall antitumor efficacy. In vivo delivery of RNA to a subject will

be achieved using multiple methods, such as electroporation, such as hydrodynamic injection or such as delivery mediated by nanocarriers. Nanocarriers include for example exosomes, liposomes, lipid-based, silicon-based, polymer-based and peptide-based nanoparticles. The feasibility of these delivery methods for RNA has been widely established in the literature.

The inventors have also previously demonstrated that adoptive transfer of in vitro reprogrammed or in vitro transduced cancer cells (generated with viral vectors encoding DC reprogramming factors) induces antitumor efficacy as monotherapy and in combination with ICB (Zimmermannova *et al.* 2023, Ascic *et al.* 2024). The RNA modalities described herein also provide an alternative non-viral system for tumor cell transfection and reprogramming in vitro. RNAs encoding PU.1, IRF8 and BATF3 have been shown herein to allow efficient DC reprogramming of cancer cells.

In this prophetic example, such reprogrammed cancer cells will be administered in vivo for cancer immunotherapy or will be used in vitro for other applications including neoantigen identification or expansion of lymphocytes from peripheral blood and tumors. These approaches will show the potential of RNA-based DC reprogramming for cancer immunotherapy, providing a safe, effective, and scalable alternative to viral vectors for DC reprogramming.

Sequence overview

SEQ. ID NO. 1: Mus musculus microRNA 124a-1 (Mir124a-1, NR_029813.1)
AGGCCTCTCTCTCCGTGTTACAGCGGACCTTGATTAAATGTCCATACAATTAAG
GCACGCGGTGAATGCCAAGAATGGGGCTG

SEQ. ID NO. 2: Homo sapiens microRNA 124-1 (Mir124-1, NR_029668.1), DNA
sequence
AGGCCTCTCTCTCCGTGTTACAGCGGACCTTGATTAAATGTCCATACAATTAAG
GCACGCGGTGAATGCCAAGAATGGGGCTG

SEQ. ID NO. 3: Mus musculus microRNA 126a (Mir126a, NR_029541.1), DNA sequence

TGACAGCACATTATTACTTTTGGTACGCGCTGTGACACTTCAAACCTCGTACCGTGA
GTAATAATGCGCGGTCA

5

SEQ. ID NO. 4: Homo sapiens microRNA 126 (Mir126, NR_029695.1), DNA sequence
CGCTGGCGACGGGACATTATTACTTTTGGTACGCGCTGTGACACTTCAAACCTCGT
ACCGTGAGTAATAATGCGCCGTCCACGGCA

10

SEQ. ID NO. 5: Mus musculus microRNA 142 (Mir142, NR_029555.1), DNA sequence
ACCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTGTAGTGTTCCTACTT
TATGGATG

15

SEQ. ID NO. 6: Homo sapiens microRNA 142 (Mir142, NR_029683.1), DNA sequence
GACAGTGCAGTCACCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTGT
GTGTTTCCTACTTTATGGATGAGTGTACTGTG

20

SEQ. ID NO. 7: Mus musculus microRNA 150 (Mir150, NR_029560.1), DNA sequence
CCCTGTCTCCCAACCCTTGTACCAGTGCTGTGCCTCAGACCCTGGTACAGGCCTG
GGGGATAGGG

25

SEQ. ID NO. 8: Homo sapiens microRNA 150 (Mir150, NR_029703.1), DNA sequence
CTCCCCATGGCCCTGTCTCCCAACCCTTGTACCAGTGCTGGGCTCAGACCCTGGT
ACAGGCCTGGGGGACAGGGACCTGGGGAC

SEQ. ID NO. 9: miR-124-3p mimic
UAAGGCACGCGGUGAAUGCCAA

30

SEQ. ID NO. 10: miR-124-5p mimic
CGUGUUCACAGCGGACCUUGAU

SEQ. ID NO. 11: Mus musculus interferon regulatory factor 8 (IRF8), transcript variant
1 (NM_001301811.1), DNA sequence

ATGTGTGACCGGAACGGCGGGCGGCGGCTGCGGCAGTGGCTGATCGAACAGAT
CGACAGCAGCATGTACCCGGGGCTGATCTGGGAAAATGATGAGAAGACCATGTT
CCGTATCCCCTGGAAGCATGCCGGCAAGCAGGATTACAATCAGGAGGTGGATGC
TTCCATCTTCAAGGCCTGGGCAGTTTTTAAAGGGAAGTTTAAAGAGGGAGACAAA
5 GCTGAACCAGCCACGTGGAAGACGAGGTTACGCTGTGCTCTGAACAAGAGCCCA
GATTTTGAAGAAGTGACTGACCGGTCCCAGCTGGACATTTCTGAGCCATATAAAG
TTTACCGAATTGTCCCCGAGGAAGAACAAAAATGCAAGCTGGGCGTGGCACCTGC
AGGCTGCATGAGCGAAGTTCCTGAGATGGAGTGTGGCCGCTCAGAGATTGAGGA
GCTGATCAAGGAACCTTCTGTGGATGAGTACATGGGTATGACCAAGAGGAGCCCA
10 TCCCCACCAGAGGCCTGCAGGAGCCAGATCCTCCCTGACTGGTGGGTCCAGCAG
CCCAGTGCAGGCCTGCCACTGGTGACCGGATATGCCGCCTATGACACACACCAT
TCAGCTTTCTCCAGATGGTCATCAGCTTCTACTACGGGGGCAAGCTGGTGGGCC
AGGCCACCACCACCTGCCTTGAAGGCTGCCGTCTCTCCCTGAGCCAGCCGGGGC
TGCCTAAGTTGTATGGGCCGGATGGCCTGGAACCCGTGTGCTTTCCGACGGCCG
15 ACACCATCCCCAGTGAGCGGCAGAGGCAGGTGACCCGGAAGCTGTTTGGGCACC
TGGAACGTGGCGTGCTACTGCACAGCAACCGCAAGGGCGTGTCGTGAAGCGGC
TGTGCCAGGGCCGCGTGTTCTGCAGCGGCAACGCGGTGGTGTGCAAGGGCAGG
CCCAACAAGCTGGAGCGGGACGAGGTGGTGCAGGTCTTTGACACCAACCAAGTTC
ATCCGAGAGCTGCAGCAATTCTACGCCACCCAGAGCCGCCTACCTGACAGCAGG
20 GTGGTCCTGTGCTTCGGGGAGGAGTTTCCGGACACTGTGCCCTTGCGCTCCAAA
CTCATTCTGGTGCAGGTAGAGCAGCTGTATGCCAGGCAGCTGGTGGAGGAAGCG
GGCAAGAGCTGCGGTGCTGGCTCCCTGATGCCAGCCCTGGAGGAGCCCCAGCC
GGACCAGGCTTTCCGCATGTTTCCGGATATCTGTACCTCACACCAGAGACCCTTT
TTTAGAGAAAATCAACAGATCACCGTCTAA

25

SEQ. ID NO. 12: Homo sapiens interferon regulatory factor 8 (IRF8), transcript variant 1 (NM_001363907.1), DNA sequence

ATGGCAGGTGTCCCGGAGTCCCTGAATCTGATGTGTGACCGGAATGGTGGTCGG
CGGCTTCGACAGTGGCTGATCGAGCAGATTGACAGTAGCATGTATCCAGGACTGA
30 TTTGGGAGAATGAGGAGAAGAGCATGTTCCGGATCCCTTGAAACACGCTGGCAA
GCAAGATTATAATCAGGAAGTGGATGCCTCCATTTTTTAAGGCCTGGGCAGTTTTTA
AAGGGAAGTTTAAAGAAGGGGACAAAGCTGAACCAGCCACTTGGAAGACGAGGT
TACGCTGTGCTTTGAATAAGAGCCCAGATTTTGAGGAAGTGACGGACCGGTCCCA
ACTGGACATTTCCGAGCCATACAAAGTTTACCGAATTGTTCCCTGAGGAAGAGCAAA
35 AATGCAAACTAGGCGTGGCAACTGCTGGCTGCGTGAATGAAGTTACAGAGATGGA

GTGCGGTCGCTCTGAAATCGACGAGCTGATCAAGGAGCCTTCTGTGGACGATTAC
ATGGGGATGATCAAAAGGAGCCCTTCCCCGCCGGAGGCCTGTCGGAGTCAGCTC
CTTCCAGACTGGTGGGCGCAGCAGCCCAGCACAGGCGTGCCGCTGGTGACGGG
GTACACCACCTACGACGCGCACCATTGAGCATTCTCCCAGATGGTGATCAGCTTC
5 TACTATGGGGGCAAGCTGGTGGGCCAGGCCACCACCACCTGCCCCGAGGGCTG
CCGCCTGTCCCTGAGCCAGCCTGGGCTGCCCGGCACCAAGCTGTATGGGCCCCG
AGGGCCTGGAGCTGGTGCGCTTCCCCGCCGGCCGACGCCATCCCCAGCGAGCGA
CAGAGGCAGGTGACGCGGAAGCTGTTTCGGGCACCTGGAGCGCGGGGTGCTGCT
GCACAGCAGCCGGCAGGGCGTGTTGTCGTCAGCGGCTGTGCCAGGGCCGCGTGT
10 TCTGCAGCGGCAACGCCGTGGTGTGCAAAGGCAGGCCCAACAAGCTGGAGCGT
GATGAGGTGGTCCAGGTCTTCGACACCAGCCAGTTCTTCCGAGAGCTGCAGCAG
TTCTATAACAGCCAGGGCCGGCTTCCCTGACGGCAGGGTGGTGTGCTTTGGG
GAAGAGTTTCCGGATATGGCCCCCTTGCCTCCAACTCATTCTCGTGCAGATTG
AGCAGCTGTATGTCCGGCAACTGGCAGAAGAGGCTGGGAAGAGCTGTGGAGCCG
15 GCTCTGTGATGCAGGCCCCCGAGGAGCCGCCGCGCCAGACCAGGTCTTCCGGATGT
TTCCAGATATTTGTGCCTCACACCAGAGATCATTTTTCAGAGAAAACCAACAGATC
ACCGTCTAA

SEQ. ID NO. 13: *Mus musculus* spleen focus forming virus (SFFV) proviral integration
20 oncogene (Spi1), transcript variant 2 (NM_011355.2), DNA sequence
ATGTTACAGGCGTGCAAAATGGAAGGGTTTTCCCTCACCGCCCCTCCATCGGATG
ACTTGGTTACTTACGATTCAGAGCTATACCAACGTCCAATGCATGACTACTACTCC
TTCGTGGGCAGCGATGGAGAAAGCCATAGCGATCACTACTGGGATTTCTCCGCAC
ACCATGTCCACAACAACGAGTTTGAGAACTTCCCTGAGAACCACTTCACAGAGCT
25 GCAGAGTGTGCAGCCCCCGCAGCTACAGCAGCTCTATCGCCACATGGAGCTGGA
ACAGATGCACGTCCTCGATACTCCCATGGTGCCACCCACACCGGCCTCAGTCAC
CAGGTTTCCTACATGCCCCGGATGTGCTTCCCTTATCAAACCTTGTCCCCAGCCC
ACCAGCAGAGCTCAGATGAGGAGGAGGGTGAGAGGCAGAGCCCTCCCCTGGAG
GTGTCTGATGGAGAAGCTGATGGCTTGGAGCCTGGGCCAGGTCTTCTGCACGGG
30 GAGACAGGCAGCAAGAAAAAGATTCGCCTGTACCAGTTCTGCTGGACCTGCTGC
GCAGCGGCGACATGAAGGACAGCATCTGGTGGGTGGACAAGGACAAAGGTACCT
TCCAGTTCTCGTCCAAGCACAAAGGAGGCGCTGGCGCACCGCTGGGGCATCCAGA
AGGGCAACCGCAAGAAGATGACCTACCAGAAGATGGCGCGCGCGCTGCGCAACT
ACGGCAAGACAGGCGAGGTGAAGAAAGTCAAGAAGAAGCTCACCTACCAGTTCA

GCGGCGAGGTGCTGGGCCGTGGGGGCCTGGCCGAGCGGCGCCTCCCGCCCCA
CTGA

SEQ. ID NO. 14: Homo sapiens spleen focus forming virus (SFFV) proviral integration
oncogene (Spi1), transcript variant 2 (NM_003120.3), DNA sequence

5 ATGTTACAGGCGTGCAAATGGAAGGGTTTCCCCTCGTCCCCCTCCATCAGAAG
ACCTGGTGCCCTATGACACGGATCTATACCAACGCCAAACGCACGAGTATTACCC
CTATCTCAGCAGTGATGGGGAGAGCCATAGCGACCATTACTGGGACTTCCACCCC
CACCACGTGCACAGCGAGTTCGAGAGCTTCGCCGAGAACAACCTTCACGGAGCTC
10 CAGAGCGTGACAGCCCCCGCAGCTGCAGCAGCTCTACCGCCACATGGAGCTGGA
GCAGATGCACGTCCTCGATACCCCCATGGTGCCACCCCATCCCAGTCTTGGCCA
CCAGGTCTCCTACCTGCCCCGGATGTGCCTCCAGTACCCATCCCTGTCCCCAGC
CCAGCCCAGCTCAGATGAGGAGGAGGGCGAGCGGCAGAGCCCCCACTGGAGG
TGTCTGACGGCGAGGCGGATGGCCTGGAGCCCGGGCCTGGGCTCCTGCCTGGG
15 GAGACAGGCAGCAAGAAGAAGATCCGCCTGTACCAGTTCCTGTTGGACCTGCTC
CGCAGCGGCGACATGAAGGACAGCATCTGGTGGGTGGACAAGGACAAGGGCAC
CTTCCAGTTCTCGTCCAAGCACAAGGAGGCGCTGGCGCACCGCTGGGGCATCCA
GAAGGGCAACCGCAAGAAGATGACCTACCAGAAGATGGCGCGCGCGCTGCGCAA
CTACGGCAAGACGGGCGAGGTCAAGAAGGTGAAGAAGAAGCTCACCTACCAGTT
20 CAGCGGCGAAGTGCTGGGCCGCGGGGGCCTGGCCGAGCGGCGCCACCCGCCC
CACTGA

SEQ. ID NO. 15: Mus musculus basic leucine zipper ATF-like transcription factor 3
(BATF3) (NM_030060.2), DNA sequence

25 ATGTCGCAAGGGCCCCCGCGGTCAGCGTGCTGCAGAGAAGCGTGATGCGCC
CGGGAACCAGCCGAGAGCCCCAAGGACGATGACAGGAAAGTTTGAAGGAGAG
AGAAAAACCGGGTTGCAGCTCAGAGGAGCCGGAAGAAGCAGACCCAGAAGGCTG
ACAAGCTCCACGAGGAGCACGAGAGCCTGGAGCAGGAGAACTCTGTGCTGCGCA
GGGAGATTTTGAAGCTGAAGGAGGAGCTGCGTCACCTGAGCGAGGTGCTGAAGG
30 AGCACGAGAAGATGTGCCCGCTGCTGCTGTGTCCTATGAACTTTGTGCAGCTTCG
GTCAGACCCCGTGGCCAGCTGTCTACCACGATGA

SEQ. ID NO. 16: Homo sapiens basic leucine zipper ATF-like transcription factor 3
(BATF3) (NM_018664.3), DNA sequence

ATGTCGCAAGGGCTCCCGGCCGCCGGCAGCGTCCTGCAGAGGAGCGTCGCGGC
GCCCCGGAACCAGCCGCAGCCGCAGCCGCAGCAGCAGAGCCCTGAGGATGATG
ACAGGAAGGTCCGAAGGAGAGAAAAAACCGAGTTGCTGCTCAGAGAAGTCGGA
AGAAGCAGACCCAGAAGGCTGACAAGCTCCATGAGGAATATGAGAGCCTGGAGC
5 AAGAAAACACCATGCTGCGGAGAGAGATCGGGAAGCTGACAGAGGAGCTGAAGC
ACCTGACAGAGGCACTGAAGGAGCACGAGAAGATGTGCCCGCTGCTGCTCTGCC
CTATGAACTTTGTGCCAGTGCCTCCCCGGCCGGACCCTGTGGCCGGCTGCTTGC
CCCGATGA

10 SEQ. ID NO. 17: Mus musculus interferon regulatory factor 8 (IRF8)
(NP_001288740.1), polypeptide sequence
MCDRNGGRRRLRQWLIEQIDSSMYPGLIWENDEKTMFRIPWKHAGKQDYNQEVDASIF
KAWAVFKGKFKEGDKAEPATWKTRLRCALNKSPDFEEVTDRSQLDISEPYKVYRIVP
EEEQKCKLGVAPAGCMSEVPEMECGRSEIEELIKEPSVDEYMGMTKRSPSPPEACRS
15 QILPDWWWQQPSAGLPLVTGYAAYDTHHSAFSQMVISFYGGKLVGQATTTCLEGCR
LSLSQPGLPKLYGPDGLEPVCFTADTIPSERQRQVTRKLFHGLERGVLLHSNRKGVF
VKRLCQGRVFCSGNAVVCCKGRPNKLERDEVVQVFDTNQFIRELQQFYATQSRLPDS
RVVLCFGEEFPDTVPLRSKLILVQVEQLYARQLVEEAGKSCGAGSLMPALEEPQPDQ
AFRMFPDICTSHQRPFFRENQQITV

20 SEQ. ID NO. 18: Homo sapiens interferon regulatory factor 8 (IRF8), isoform 1
(NP_001350836.1), polypeptide sequence
MAGVPESLNLMDRNGGRRRLRQWLIEQIDSSMYPGLIWENEEKSMFRIPWKHAGKQ
DYNQEVDASIFKAWAVFKGKFKEGDKAEPATWKTRLRCALNKSPDFEEVTDRSQLDI
25 SEPYKVYRIVPEEEQKCKLGVATAGCVNEVTEMECGRSEIDELIKEPSVDDYMGMIKR
SPSPPEACRSQLLPDWWAQQPSTGVPLVTGYTTYDAHHSQFSQMVISFYGGKLVG
QATTTCEGCRLSLSQPGLPGTKLYGPEGLELVRFPADAIPSERQRQVTRKLFHGLE
RGVLLHSSRQGVFVKRLCQGRVFCSGNAVVCCKGRPNKLERDEVVQVFDTSQFFREL
QQFYNSQGRLPDGRVVLCFGEEFPDAPLRSKLILVQIEQLYVRQLAEEAGKSCGAG
30 SVMQAPEEPPPDQVFRMFPDICASHQRSFFRENQQITV

SEQ. ID NO. 19: Mus musculus spleen focus forming virus (SFFV) proviral integration
oncogene (Spi1), isoform 2 (NP_035485.1), polypeptide sequence
MLQACKMEGFSLTAPPSDDLVTYDSELYQRPMHDYYSFVGSDGESHSDDHYWDFSA
35 HHVHNNEFENFPENHFTLQSVQPPQLQQLYRHMELEQMHVLDTPMVP PHTGLSHQ

VSYMPRMCFPYQTLSPAHHQQSSDEEEGERQSPPLEVSDGEADGLEPGPGLLHGETG
SKKKIRLYQFLDLLRSGDMKDSIWWWVDKDKGTFQFSSKHKEALAHRWGIQKGNRKK
MTYQKMARALRNYGKTGEVKKVKKLTYQFSGEVLGRGGLAERRLPPH

5 SEQ. ID NO. 20: Homo sapiens spleen focus forming virus (SFFV) proviral integration
oncogene (Spi1), isoform 2 (NP_003111.2), polypeptide sequence
MLQACKMEGFPLVPPPSDLVPYDLDLYQRQTHEYYPYLSSDGESHSDHYWDFHPPH
HVVHSEFESFAENNFTELQSVQPPQLQLYRHMELEQMHLVDTPMVPPHPSLGHQVS
YLPRMCLQYPSLSPAQPSSDEEEGERQSPPLEVSDGEADGLEPGPGLLPGETGSKK
10 KIRLYQFLDLLRSGDMKDSIWWWVDKDKGTFQFSSKHKEALAHRWGIQKGNRKKMTY
QKMARALRNYGKTGEVKKVKKLTYQFSGEVLGRGGLAERRHPPH

SEQ. ID NO. 21: Mus musculus basic leucine zipper transcriptional factor ATF-like 3
(NP_084336.1), polypeptide sequence
15 MSQGPPAVSVLQRSVDAPGNQPQSPKDDDRKVRRREKNRVAAQRSRKKQTQKADK
LHEEHESLEQENSVLRREISKLKEELRHLSEVLKEHEKMCPLLLCPMNFVQLRSDPVA
SCLPR

SEQ. ID NO. 22: Homo sapiens basic leucine zipper transcriptional factor ATF-like 3
(NP_061134.1), polypeptide sequence
20 MSQGLPAAGSVLQRSVAAPGNQPQPQPPQSPEDDDRKVRRREKNRVAAQRSRK
KQTQKADKLHEEYESLEQENTMLRREIGKLTEELKHLTEALKEHEKMCPLLLCPMNFV
PVPPRPDPVAGCLPR

25 SEQ ID NO: 23: isomiR miR-142-3p-1
GTAGTGTTCCTACTTTATGGA

SEQ ID NO: 24: isomiR miR-142-3p-3
AGTGTTCCTACTTTATGGATG

30 SEQ ID NO: 25: isomiR miR-142-5p-1
ATAAAGTAGAAAGCACTACT

References

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Claims

1. A composition comprising one or more RNA construct(s) encoding the combination
of at least two transcription factors selected from the group consisting of: PU.1,
5 IRF8, and BATF3, wherein the RNA construct is selected from the group consisting
of: circular RNA (circRNA), self-amplifying RNA (saRNA), non-modified RNA,
modified RNA (modRNA) and trans-amplifying RNA (taRNA).
2. A composition comprising one or more RNA construct(s) encoding the combinations
10 of at least two transcription factors selected from the group consisting of: PU.1,
IRF8, and BATF3, wherein the RNA construct is selected from the group consisting
of: circular RNA (circRNA), self-amplifying RNA (saRNA), naked non-modified RNA,
non-modified RNA not incorporated into a viral particle, linear m1-Ψ-modified
mRNA, and trans-amplifying RNA (taRNA).
- 15 3. The composition according to any one of the preceding claims, wherein the
combination of at least two transcription factors encoded by the composition
comprising one or more RNA construct(s) is:
 - a. PU.1 and IRF8;
 - 20 b. PU.1 and BATF3; or
 - c. IRF8 and BATF3.
4. The composition according to any one of the preceding claims, wherein the
combination of at least two transcription factors is PU.1 and IRF8, or PU.1 and
25 BATF3.
5. The composition according to any one of the preceding claims, wherein the
combination of at least two transcription factors is PU.1, IRF8, and BATF3.
- 30 6. The composition according to any one of the preceding claims, wherein the
combination of at least two transcription factors in the 5' to 3' order is:
 - a. PU.1, IRF8, BATF3;
 - b. PU.1, BATF3, IRF8;
 - c. IRF8, BATF3, PU.1;
 - 35 d. IRF8, PU.1, BATF3;

- e. BATF3, IRF8, PU.1; or
- f. BATF3, PU.1, IRF8.

- 5 7. The composition according to any one of the preceding claims, comprising 2, such as 3 RNA constructs encoding the combination of at least two transcription factors
- 10 8. The composition according to any one of the preceding claims, comprising 3 RNA constructs, wherein the combination of at least two transcription factors is PU.1, IRF8, and BATF3, and wherein each of the RNA constructs encodes one of PU.1, IRF8, and BATF3.
- 15 9. The composition according to any one of the preceding claims, wherein PU.1 is encoded by a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO:13 or SEQ ID NO:14, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:13 or SEQ ID NO:14.
- 20 10. The composition according to any one of the preceding claims, wherein IRF8 is encoded by a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO:11 or SEQ ID NO:12, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO: SEQ ID NO:11 or SEQ ID NO:12.
- 25 11. The composition according to any one of the preceding claims, wherein BATF3 is encoded by a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO:15 or SEQ ID NO:16, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:15 or SEQ ID NO:16.
- 30 12. The composition according to any one of the preceding claims, wherein PU.1 is of a polypeptide sequence with at least 90% sequence identity to SEQ ID NO:19 or SEQ ID NO:20, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:19 or SEQ ID NO:20.

13. The composition according to any one of the preceding claims, wherein IRF8 is of a polypeptide sequence with at least 90% sequence identity to SEQ ID NO:17 or SEQ ID NO:18, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:17 or SEQ ID NO:18.
14. The composition according to any one of the preceding claims, wherein BATF3 is of a polypeptide sequence with at least 90% sequence identity to SEQ ID NO:21 or SEQ ID NO:22, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity to SEQ ID NO:21 or SEQ ID NO:22.
15. A composition comprising one or more miRNA mimics, wherein said one or more miRNA mimics are isomiRs, for use in cell reprogramming.
16. The composition according to claim 15, wherein the one or more miRNA mimics are isomiRs of miRNA 124a-1, miRNA-124-1, miRNA-126a, miRNA-126, miRNA-142, and/or miRNA-150.
17. A composition comprising one or more miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics, selected from the group consisting of: miR-124, miR-124a-1, miR-124-1, miR-124-3p, miR-124-5p, miR-142a, miR-142, miR-126, miR-126a, miR-150, miR-142-3p-1, miR-142-3p-3, and miR-142-5p-1.
18. The composition according to any one of claims 15 to 17, wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics:
- a. downregulate the gene expression of SP1KLF5, and/or RELA, preferably wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are of the miR-124 family, even more preferably wherein said miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics are miR-124-3p or isomiRs thereof; and/or
 - b. downregulate the gene expression of KLF10, PATZ1, and/or ELK3 preferably wherein said miRNAs, or primary miRNAs (pri-

miRNAs) thereof, and/or miRNA mimics are of the miR-142 family.

- 5 19. The composition according to any one of claims 15 to 18, wherein said composition comprises at least 2, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8 miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics, selected from the group consisting of: miR-124, miR-124a-1, miR-124-1, miR-124-3p, miR-124-5p, miR-142a, miR-142, miR-126, miR-126a, miR-150, miR-142-3p-1, miR-142-3p-3, and miR-142-5p-1.
- 10 20. The composition according to any one of claims 15 to 19, wherein the one or more miRNAs are the two miRNAs miR-124 and miR-142.
- 15 21. The composition according to any one of claims 15 to 19, wherein the one or more miRNAs are the two miRNAs miR-124 and miR-142a.
- 20 22. The composition according to any one of the preceding claims, comprising at least one or more miRNA mimic(s).
- 25 23. The composition according to any one of the preceding claims, comprising at least one, such as at least two, such as at least three, such as at least four, such as at least five miRNA mimic(s).
- 30 24. The composition according to any one of the preceding claims, wherein said at least one, such as at least two, such as at least three, such as at least four, such as at least five miRNA mimic(s) are isomiRs.
25. The composition according to any one of the preceding claims, wherein the miRNA mimic(s) are the isomiRs miR-142-3p-1, miR-142-3p-3 and/or miR-142-5p-1.
26. The composition according to any one of the preceding claims, comprising or consisting of miR-142-3p, miR-142-3p-1 and miR-142-5p-1.
- 35 27. The composition according to any one of the preceding claims, comprising or consisting of miR-124-3p and miR-142-5p-1.

28. The composition according to any one of the preceding claims, wherein the at least one or more miRNAs is a primary miRNA (pri-miRNA).

5 29. The composition according to any one of the preceding claims, wherein at least one of the miRNA(s), or primary miRNA thereof, and/or miRNA mimics, is encoded by a sequence selected from the group consisting of:

- a. miRNA 124a-1 of SEQ ID NO: 1;
- b. miRNA-124-1 of SEQ ID NO: 2;
- 10 c. miRNA-126a of SEQ ID NO: 3;
- d. miRNA-126 of SEQ ID NO: 4;
- e. miRNA-142 of SEQ ID NO: 5 or SEQ ID NO: 6;
- f. miRNA-150 of SEQ ID NO: 7 or SEQ ID NO: 8;
- g. miRNA-124-3p mimic of SEQ ID NO: 9;
- 15 h. miRNA-124-5p mimic of SEQ ID NO: 10;
- i. miR-142-3p-1 of SEQ ID NO: 23;
- j. miR-142-3p-3 of SEQ ID NO: 24; and
- k. miR-142-5p-1 of SEQ ID NO: 25;

or a polynucleotide sequence with at least 90% sequence identity, such as at least
20 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% sequence identity thereto.

30. The composition according to any one of the preceding claims, wherein at least one of the one or more RNA construct(s), or at least one of the one or more miRNA(s),
25 or pri-miRNAs thereof, and/or miRNA mimics, is encapsulated.

31. The composition according to claim 30, wherein the encapsulation is selected from the group consisting of: nanoparticle encapsulation, polymeric encapsulation, viral encapsulation, exosome encapsulation, and lipid nanoparticle encapsulation.

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32. A composition comprising:

- a. the composition comprising one or more RNA construct(s) according to any one of the preceding claims; and

- b. the composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims.
- 5 33. A composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics according to any one of claims 15 to 31.
- 10 34. The composition according to claim 33, wherein at least one of the one or more miRNA(s) is encoded as a pri-miRNA.
35. The composition according to any one of the preceding claims, wherein the vector is a viral, or a non-viral vector.
- 15 36. The composition according to claim 35, wherein the viral vector is selected from the group consisting of: adenovirus, lentivirus, retrovirus, adeno-associated virus (AAV), alphaviruses, flavivirus, rhabdovirus, Sendai virus, pox virus, and herpes simplex virus (HSV).
- 20 37. The composition according to claim 35, wherein the non-viral vector is selected from the group consisting of: liposomes, polymeric nanoparticles, dendrimers, cationic polymers, nanogels, inorganic nanoparticles, carbon nanotubes, hydrogels, electroporation, and ultrasound-mediated vectors
- 25 38. The composition according to any one of the preceding claims, further comprising one or more IFN inhibitor(s), preferably wherein the IFN inhibitor(s) are selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, even more preferably wherein the IFN inhibitor(s) are selected from the group consisting of:
- 30 B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, yet even more preferably wherein the IFN inhibitor is ruxolitinib.
- 35 39. The composition according to any one of the preceding claims, wherein the composition is a pharmaceutical composition.

40. A method for reprogramming or inducing a cell, said method comprising the steps of:

- 5
- a. Contacting a cell with a composition comprising one or more constructs or vectors encoding a combination of at least two transcription factors;
 - b. Expressing the at least two transcription factors;
 - c. Contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a
- 10
- composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;

whereby the cell is reprogrammed or induced.

15 41. A method for reprogramming or inducing a cell, said method comprising the steps of:

- a. Contacting a cell with a composition comprising one or more constructs or vectors encoding a combination of at least two transcription factors;
 - b. Expressing the at least two transcription factors;
 - c. Contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a
- 20
- composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or
- 25
- miRNA mimics selected from the group consisting of: miR-124, miR-124a-1, miR-124-1, miR-124-3p, miR-124-5p, miR-142a, miR-142, miR-126, miR-126a, miR-150, miR-142-3p-1, miR-142-3p-3, and miR-142-5p-1;

whereby the cell is reprogrammed or induced.

30 42. The method according to any one of claims 40 and 41, wherein the one or more miRNA mimic(s) are isomiRs.

43. The method according to claim 42, wherein the one or more isomiRs are 5' isomiRs.

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44. The method according to any one of claims 40 to 43, wherein the one or more miRNA mimics are isomiRs of miRNA 124a-1, miRNA-124-1, miRNA-126a, miRNA-126, miRNA-142, and/or miRNA-150.
- 5
45. The method according to any one of claims 40 to 44, wherein the one or more isomiRs are isomiRs of miR-142.
46. The method according to any one of claims 40 to 45, wherein the one or more isomiRs are the isomiRs miR-142-3p-1, miR-142-3p-3 and/or miR-142-5p-1.
- 10
47. A method for reprogramming or inducing a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of:
- a. Contacting a cell with a composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
- 15
- b. Expressing the at least two transcription factors;
- c. Contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;
- 20
- whereby a dendritic cell or antigen-presenting cell is obtained.
- 25
48. A method for reprogramming or inducing a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of:
- a. Contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
- 30
- b. Expressing the at least two transcription factors;
- whereby a dendritic cell or antigen-presenting cell is obtained.

49. The method according to any one of claims 40 to 48, said method comprising the steps of:

- 5
- a. Contacting a cell with a composition comprising one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3;
- b. Expressing the at least two transcription factors;
- 10 c. Contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;

whereby a dendritic cell or antigen-presenting cell is obtained.

- 15 50. The method according to any one of claims 40 to 47 and 49, wherein step c. comprises contacting the cells with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, and wherein the method further comprises a step of expressing the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics.

- 20 51. The method according to any one of claims 40 to 50, wherein the composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3 is as described in any one of the preceding claims.

- 25 52. The method according to any one of claims 40 to 51, wherein the composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is as described in any one of claims 15 to 39.

- 30 53. The method according to any one of claims 40 to 52, wherein the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is as described in any one of claims 33 to 39.

54. The method according to any one of claims 40 to 53, wherein the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed prior to, simultaneously, and/or after, the step of contacting a cell with a composition comprising one or more constructs or vectors, or with a composition comprising one or more RNA construct(s), preferably wherein the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed simultaneously to the step of contacting a cell with a composition comprising one or more constructs or vectors, or with a composition comprising one or more RNA construct(s).
55. The method according to any one of claims 50 to 54, wherein the step of expressing the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed prior to, simultaneously, and/or after, the step of expressing the at least two transcription factors, preferably wherein the step of expressing the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed simultaneously to the step of contacting or the step of expressing the at least two transcription factors.
56. The method according to any one of claims 40 to 55, wherein step c. comprises contacting the cells with miRNA-124 and/or miRNA-142 simultaneously to the step of contacting or the step of expressing the at least two transcription factors.
57. The composition or the method according to any one of the preceding claims, wherein the one or more RNA construct(s) comprise or consist of self-amplifying RNA, preferably wherein the self-amplifying RNA is selected from the group consisting of: Venezuelan equine encephalitis virus, Semliki forest virus, Sindbis virus, classical swine fever virus, tick-borne encephalitis virus, alphavirus chimera based on the Venezuelan equine encephalitis virus, and Sindbis virus replicons.
58. The composition or the method according to any one of the preceding claims, wherein the one or more RNA construct(s) comprise or consist of circular RNA.

59. The composition or the method according to any one of the preceding claims, wherein the one or more RNA construct(s) comprise or consist of non-modified RNA.
- 5
60. The composition or the method according to any one of the preceding claims, wherein the one or more RNA construct(s) comprise or consist of trans-amplifying RNA.
- 10
61. The composition or the method according to any one of the preceding claims, wherein the one or more RNA construct(s) comprise or consist of modified RNA, preferably wherein the modified RNA is a m1-Ψ-modified mRNA, even more preferably wherein the modified RNA is a linear m1-Ψ-modified mRNA.
- 15
62. The method according to any one of the claims 40 to 61, further comprising a step of inhibiting the IFN pathway of the cell.
63. The method according to claim 62, wherein the step of inhibiting the IFN pathway of the cell is performed prior to, during, and/or after the step of contacting a cell with a composition comprising one or more constructs or vectors, or with a composition comprising one or more RNA construct(s).
- 20
64. The method according to any one of claims 62 to 63, wherein the step of inhibiting the IFN pathway of the cell is performed prior to, during, and/or after the step of expressing the at least two transcription factors.
- 25
65. The method according to any one of claims 62 to 64 wherein the step of inhibiting the IFN pathway is performed using small molecules, viral proteins including, but not limited to, innate inhibiting proteins, delivery of shRNAs, siRNAs, miRNAs and miRNA mimetics targeting downstream mediators and/or effectors of IFN signaling.
- 30
66. The method according to any one of claims 62 to 65, wherein the step of inhibiting the IFN pathway is performed by contacting the cells with one or more IFN inhibitors.
- 35

67. The composition or the method according to any one of claims 38 to 66, wherein the one or more IFN inhibitors are selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons.
- 5
68. The composition or the method according to any one of the claims 38 to 67, wherein the one or more IFN inhibitors are selected from the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib.
- 10
69. The composition or the method according to any one of the claims 38 to 68, wherein the one or more IFN inhibitors are selected from the group consisting of: B18R and ruxolitinib.
- 15
70. The composition or the method according to any one of the claims 38 to 69, wherein the one or more IFN inhibitors is ruxolitinib.
71. The composition or the method according to any one of claims 38 to 70, wherein the one or more IFN inhibitor is present at a concentration between 0 and 100 μ M, preferably between 10nM and 50 μ M, even more preferably between 100nM and 25 μ M, yet even more preferably between 100nM and 10 μ M.
- 20
72. The method according to any one of claims 40 to 71, wherein the cell is a human or non-human cell.
- 25
73. The method according to any one of claims 40 to 72, wherein the cell is a human cell.
74. The method according to any one of claims 40 to 73, wherein the cell is a human fibroblast, such as a human dermal fibroblast (HDF), or a human embryonic fibroblast (HEF).
- 30
75. The method according to any one of claims 40 to 74, wherein the cell is a cancer cell, such as a cancer cell line, for example a primary cancer cell line.

76. The method according to claim 75, wherein the cell is a melanoma cancer cell, or a sarcoma cancer cell.
- 5 77. The method according to any one of claims 40 to 72 and 75 to 76, wherein the cell is a mouse cell.
78. The method according to claim 77, wherein the cell is a mouse embryonic fibroblast (MEF).
- 10 79. The method according to any one of claims 40 to 78, wherein the reprogrammed or induced cell is an induced dendritic cell (iDC), preferably wherein the reprogrammed or induced cell is a human or non-human iDC, such as a mouse iDC.
- 15 80. The method according to any one of claims 40 to 79, wherein the reprogrammed or induced cell is a human or non-human iDC, such as a mouse iDC.
81. The method according to any one of claims 40 to 80, wherein the reprogrammed or induced cell is a conventional type 1 dendritic cell (cDC1 cell).
- 20 82. The method according to any one of claims 40 to 81, wherein the reprogrammed or induced cell expresses CD45, XCR1 and/or MHC-II.
83. The method according to any one of claims 40 to 82, wherein the reprogrammed or induced cell expresses CD45, XCR1 and MHC-II.
- 25 84. The method according to any one of claims 40 to 83, wherein the reprogrammed or induced cell expresses CD45, CD226, CD40, and/or HLA-DR.
85. The method according to any one of claims 40 to 84, wherein the reprogrammed or induced cell expresses CD40, and HLA-DR.
- 30 86. The method according to any one of claims 40 to 85, wherein the reprogrammed or induced cell expresses CD45, CD226 and HLA-DR.

87. The method according to any one of claims 40 to 86, wherein the reprogrammed or induced cell expresses CD226.
88. The method according to any one of claims 40 to 87, wherein the reprogrammed cell
5 further expresses CD141.
89. The method according to any one of claims 40 to 88, wherein the reprogramming or induction is *in vivo*, *in vitro*, or *ex vivo*.
- 10 90. The method according to any one of claims 40 to 89, wherein the method further comprises a step of culturing the cell in a cell media, wherein the culturing step is conducted before, during, and/or after expressing the at least two transcription factors.
- 15 91. The method according to claim 90, wherein the cell is cultured during at least 2 days, such as at least 4 days, such as at least 5 days, such as at least 6 days, such as at least 8 days, such as at least 9 days, such as at least 10 days, such as at least 12 days.
- 20 92. The method according to any one of claims 90 to 91, wherein the cell media comprises one or more IFN inhibitor(s) selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK 1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitor(s) are selected from the group consisting of B18R, Ruxolitinib, Fedratinib,
25 Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is ruxolitinib.
- 30 93. The method according to any one of claims 40 to 92, wherein the method further comprises culturing the cell in a cell media comprising one or more cytokines selected from the group consisting of: IFN β , IFN γ , TNF α , IFN α , IL-1 β , IL-6, CD40L, Flt3l, GM-CSF, IFN- λ 1, IFN- ω , IL-2, IL-4, IL-15, prostaglandin 2, SCF and oncostatin M (OM).

94. The method according to any one of claims 40 to 93, wherein the method further comprises culturing the cell in a cell media comprising one or more epigenetic modifiers, such as histone deacetylase inhibitors.
- 5 95. The method according to claim 94, wherein the histone deacetylase inhibitor is valproic acid.
- 10 96. The method according to any one of claims to 40 to 95, wherein the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed prior to, simultaneously, and/or after the step of contacting the cells or the step of expressing the at least two transcription factors, preferably wherein the step of contacting the cells with a composition
- 15 comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, is performed no more than 6h, such as no more than 12h, such as no more than 24 day, such as no more than 36h, such as no more than 48h, such as no more than 72h, such as no more than 96h, such as no more than 120h, such as no more than 144h after the
- 20 step of contacting the cells or the step of expressing the at least two transcription factors.
- 25 97. The method according to any one of claims 40 to 96, further comprising the steps of:
- a. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II in the cell, after the step of expressing the at least two transcription factors;
- b. assessing the expression of CD45, XCR1, CD226, CD141, HLA-DR, CD40, and/or MHC-II in the cell after the step of
- 30 contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;

- c. comparing the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II assessed in steps a. and b.,

wherein a higher level of expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40 and/or MHC-II, preferably a higher level of expression of XCR1, even more preferably a higher level of expression of CD226 after contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, indicates increased cell reprogramming fidelity.

98. The method according to any one of claims 40 to 97, further comprising the steps of:

- a. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II in the cell, after the step of the step of expressing the at least two transcription factors;
- b. assessing the expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II in the cell after the step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics;
- c. comparing the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II assessed in steps a. and b.,

wherein a higher level of expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II, preferably a higher level of expression of CD45 and MHC-II, even more preferably a higher level of expression of CD45 and HLA-DR after contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, indicates increased cell reprogramming efficiency.

99. The method according to any one of claims 40 to 98, comprising a step of contacting the cells with a composition comprising one or more miRNAs, or pri-miRNAs

thereof, and/or miRNA mimics, or with a composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, and wherein the dendritic cell or antigen-presenting cell displays increased cell reprogramming efficiency and/or fidelity.

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100. The method according to any one of claims 40 to 99, wherein the reprogramming efficiency and/or fidelity increases with increasing concentration of the miRNA(s), or pri-miRNAs thereof, and/or miRNA mimics, and/or with increasing concentration of the one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics.

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101. The method according to any one of claims 40 to 100, wherein the assessment of the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR and/or MHC-II in the cell is performed using flow cytometry and/or single-cell RNA sequencing (scRNAseq).

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102. The method according to any one of claims 40 to 101, wherein the step c. of comparing the levels of expression of CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II is performed by comparing the percentage of cells expressing CD45, XCR1, CD 141, CD226, HLA-DR, CD40, and/or MHC-II of step a., with the percentage of cells expressing CD45, XCR1, CD141, CD226, HLA-DR, CD40, and/or MHC-II of step b.

20

103. A method of improving cell reprogramming fidelity of a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of any one of claims 40 to 102.

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104. A method of improving cell reprogramming efficiency of a cell into a dendritic cell or antigen-presenting cell, said method comprising the steps of any one of claims 40 to 102.

30

105. The method according to any one of claims 40 to 104, wherein the cell reprogramming efficiency is improved by at least 1.5-fold, such as at least 1.6-fold, such as at least 1.7-fold, such as at least 1.8-fold, such as at least 1.9-fold, such as at least 2-fold, such as at least 2.1-fold, such as at least 2.2-fold, such as at least

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2.3-fold, such as at least 2.4-fold, such as at least 2.5-fold, such as at least 2.6-fold, such as at least 2.7-fold, such as at least 2.8-fold, such as at least 2.9-fold, such as at least 3-fold, such as at least 5-fold, such as at least 10-fold.

5 106. The method according to any one of claims 40 to 105, wherein the cell reprogramming fidelity is improved by at least 1.5-fold, such as at least 1.6-fold, such as at least 1.7-fold, such as at least 1.8-fold, such as at least 1.9-fold, such as at least 2-fold, such as at least 2.1-fold, such as at least 2.2-fold, such as at least 2.3-fold, such as at least 2.4-fold, such as at least 2.5-fold, such as at least 2.6-fold, 10 such as at least 2.7-fold, such as at least 2.8-fold, such as at least 2.9-fold, such as at least 3-fold, such as at least 5-fold, such as at least 10-fold.

107. The method according to any one of the claims 40 to 106, wherein the one or more miRNAs, or primary miRNAs (pri-miRNAs) thereof, and/or miRNA mimics, are 15 selected from the group consisting of: miR-124, miR-124a-1, miR-124-1, miR-124-3p, miR-124-5p, miR-142a, miR-142, miR-126, miR-126a, miR-150, miR-142-3p-1, miR-142-3p-3, and miR-142-5p-1.

108. The method according to any one of claims 40 to 107, wherein at least 20 one of the miRNA(s), or pri-miRNAs thereof, and/or miRNA mimics is encoded by a sequence selected from the group consisting of:

- a. mi-RNA 124a-1 of SEQ ID NO: 1;
- b. miRNA-124-1 of SEQ ID NO: 2;
- c. miRNA-126a of SEQ ID NO: 3;
- 25 d. miRNA-126 of SEQ ID NO: 4
- e. miRNA-142 of SEQ ID NO: 5 or SEQ ID NO: 6
- f. miRNA-150 of SEQ ID NO: 7 or SEQ ID NO: 8
- g. miRNA-124-3p mimic of SEQ ID NO: 9;
- h. miRNA-124-5p mimic of SEQ ID NO: 10;
- 30 i. miR-142-3p-1 of SEQ ID NO: 23;
- j. miR-142-3p-3 of SEQ ID NO: 24; and
- k. miR-142-5p-1 of SEQ ID NO: 25,

or a polynucleotide sequence with at least 90% sequence identity, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, 35 such as at least 99%, such as 100% sequence identity thereto.

109. The method according to any one of the claims 40 to 108, further comprising a step of inhibiting the IFN pathway of the cell prior to, simultaneously, and/or after the step of contacting the cell with a composition comprising the one or more constructs or vectors, or the one or more RNA construct(s) encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3, preferably wherein the inhibition of the IFN pathway is performed using one or more IFN inhibitor(s) selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK 1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, even more preferably wherein the one or more IFN inhibitor(s) are selected from the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is Ruxolitinib.
110. A cell comprising the one or more RNA construct(s), or the one or more construct(s) or vector(s) according to any one of the preceding claims.
111. The cell according to claim 110, wherein the cell is a mammalian cell, such as a human or murine cell.
112. The cell according to any one of claims 110 to 111, wherein the cell is selected from the group consisting of: a stem cell, a differentiated cell, and a cancer cell, wherein:
- a. the stem cell is selected from the group consisting of: a pluripotent stem cell and a multipotent stem cell, such as a mesenchymal stem cell or a hematopoietic stem cell;
 - b. the differentiated cell is any somatic cell, such as a fibroblast, for example a dermal fibroblast or an embryonic fibroblast, or a hematopoietic cell, such as a monocyte.
113. The cell according to any one of claims 110 to 112, wherein the cell is a cancer cell, such as a cancer cell line, for example a primary cancer cell line.

114. The cell according to claim 113, wherein the cell is a melanoma cancer cell, or a sarcoma cancer cell.
115. The cell according to any one of claims 110 to 112, wherein the cell is a reprogrammed human dendritic cell or human antigen-presenting cell, such as a human type 1 conventional dendritic cell.
116. The cell according to any one of claims 110 to 115, wherein the cell expresses one or more surface marker(s) selected from the group consisting of: CLEC9A, CD45, CD141, XCR1, MHC-II, HLA-DR, CD40, and CD226.
117. A reprogrammed or induced cell obtained by the method defined in any one of claims 40 to 116.
118. The reprogrammed or induced cell according to claim 117, wherein the reprogrammed or induced cell is an induced dendritic cell (iDC), preferably wherein the reprogrammed or induced cell is a human or non-human iDC, such as a mouse iDC.
119. The reprogrammed or induced cell according to any one of claims 117 to 118 wherein the reprogrammed or induced cell is a human or non-human iDC, such as a mouse iDC.
120. The reprogrammed or induced cell according to any one of claims 117 to 119 wherein the reprogrammed or induced cell is a conventional type 1 dendritic cell (cDC1 cell).
121. The reprogrammed or induced cell according to any one of claims 117 to 120 wherein the reprogrammed or induced cell expresses CLEC9A, CD45, CD141, XCR1, CD226, HLA-DR, CD40, and/or MHC-II.
122. The reprogrammed or induced cell according to any one of claims 117 to 121 wherein the reprogrammed or induced cell expresses CD45, XCR1 and/or MHC-II.

123. The reprogrammed or induced cell according to any one of claims 117 to 122 wherein the reprogrammed or induced cell expresses CD40 and HLA-DR.
124. The reprogrammed or induced cell according to any one of claims 117 to 123,
5 wherein the reprogrammed or induce cell expresses CLEC9A, CD141, HLA-DR and/or CD226.
125. A kit-of-parts comprising:
- 10 a. The composition comprising one or more RNA construct(s) according to any one of the preceding claims.
- b. The composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof,
15 and/or miRNA mimics, according to any one of the preceding claims.
126. A kit-of-parts comprising:
- a. The composition comprising one or more RNA construct(s) according to any one of the preceding claims.
- 20 b. One or more IFN inhibitors selected form the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected form the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib,
25 and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is ruxolitinib.
127. A kit-of-parts comprising:
- 30 a. The composition comprising one or more RNA construct(s) according to any one of the preceding claims.
- b. The composition comprising one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims, or the composition comprising one or more construct(s) or

vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics according to any one of the preceding claims.

- 5 c. One or more IFN inhibitors selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected from the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is
- 10 ruxolitinib.

128. A kit-of-parts comprising:

- a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors.
- 15 b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to
- 20 any one of the preceding claims.

129. A kit-of-parts comprising:

- a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3
- 25 b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or
- 30 miRNA mimics, according to any one of the preceding claims.

130. A kit-of-parts comprising:

- a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors
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- 5 b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims
- 10 c. One or more IFN inhibitors selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected from the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is ruxolitinib.
- 15
131. A kit-of-parts comprising:
- 20 a. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of PU.1, IRF8, and BATF3
- 25 b. The composition comprising one or more constructs or vectors encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims, or the composition comprising one or more construct(s) or vector(s) encoding the one or more miRNAs, or pri-miRNAs thereof, and/or miRNA mimics, according to any one of the preceding claims
- 30 c. One or more IFN inhibitors selected from the group consisting of: kinase inhibitors, JAK/STAT inhibitors, such as JAK1 and/or JAK2 inhibitors, and virus proteins neutralizing type I interferons, preferably wherein the one or more IFN inhibitors are selected from the group consisting of: B18R, Ruxolitinib, Fedratinib, Upadacitinib, Filgotinib, and Baricitinib, more preferably selected from the group consisting of: B18R and ruxolitinib, even more preferably wherein the one or more IFN inhibitor is ruxolitinib.

132. The composition, the cell, the reprogrammed or induced cell, or the kit-of-parts according to any one of the preceding claims, for use in medicine.
133. The composition, the cell, the reprogrammed or induced cell, or the kit-of-parts according to any one of the preceding claims, for use in the treatment of cancer or infectious diseases.
134. The composition, the cell, the reprogrammed cell, or the kit-of-parts according to claim 133, wherein the cancer is selected from the group consisting of: basal cell carcinoma, cervical dysplasia, sarcoma, germ cell tumor, retinoblastoma, glioblastoma, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, blood cancer, prostate cancer, ovarian cancer, cervix cancer, oesophageal cancer, uterus cancer, vaginal cancer, breast cancer, head and neck cancer, gastric cancer, oral cavity cancer, naso-pharynx cancer, trachea cancer, larynx cancer, bronchi cancer, bronchioles cancer, lung cancer, pleural cancer, bladder and urothelial cancer, hollow organs cancer, esophagus cancer, stomach cancer, bile duct cancer, intestine cancer, colon cancer, colorectal cancer, rectum cancer, bladder cancer, ureter cancer, kidney cancer, liver cancer, gall bladder cancer, spleen cancer, brain cancer, lymphatic system cancer, bone cancer, pancreatic cancer, leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, skin cancer, melanoma and myeloma, preferably wherein the cancer is selected from the group consisting of: melanoma, head and neck cancer, breast cancer, colorectal cancer, liver cancer, lymphoma, bladder and urothelial cancer, pancreatic cancer and glioblastoma, even more preferably wherein the cancer is selected from the group consisting of: melanoma and sarcoma.
135. A method of treating cancer or infectious diseases, the method comprising administering to an individual in need thereof the composition, the cell, the reprogrammed or induced cell, or the kit-of-parts according to any one of the preceding claims.
136. Use of the composition, the cell, the reprogrammed or induced cell, or the kit-of-parts according to any one of the preceding claims, for the manufacture of a medicament for the treatment of cancer or infectious diseases.

137. A composition comprising one or more constructs or vectors encoding the combination of at least two transcription factors selected from the group consisting of: PU.1, IRF8, and BATF3,
wherein at least one of PU.1, IRF8, and BATF3 is encoded by a RNA construct,
5 wherein the RNA construct is selected from the group consisting of: self-amplifying RNA (saRNA), circular RNA (circRNA), non-modified RNA, modified RNA (modRNA) and trans-amplifying RNA (taRNA), and
wherein at least one of PU.1, IRF8, and BATF3 is encoded by a viral vector selected from the group consisting of: adenoviral, lentiviral, retroviral, adeno-
10 associated viral (AAV), alphaviral, flaviviral, rhabdoviral, Sendai viral, pox viral, and herpes simplex viral (HSV) vector.
138. The composition, the method, the cell, the reprogrammed or induced cell, and the
15 kit of parts according to any one of claims 1 to 136, wherein the composition comprising one or more RNA construct(s) of any one of claims 1 to 136 is the composition defined in claim 137.