Abstract ID:- 69
Abstract Topic:- Rare disease therapeutics
Abstract Title:- Lentiviral Vectors in Gene Therapy of β-Globinopathies;A Pursuit for a Strong Backbone
Presenting author name :- Karthik C
Presenting author institute:- CMC-CSCR
Co-authors name:- Abhirup Bagchi, Dr. Alok Srivastava, Dr. R.V.Shaji

Co-authors institute:-CMC-CSCR, Vellore & CMC Dept. of Hematology

Aims:-Assessing and optimizing lentiviral backbones for gene therapy of β globinopathies.

Methods:- Our lab-made H23B-Ery-Lin-shRNA vector (8.8 Kb) expresses an erythroid-specific transgene payload (3.3 kB) called HSBZmiRE. It consists of erythroid enhancer regions (HS2 and HS3), a minimal β-globin promoter, a ZSgreen GFP reporter, and a mir30 scaffold for the expression of shRNAs. This payload is used across various LV backbones known to exhibit high titers despite carrying larger payloads allowing for comparative analysis of transgene expression and titers. Vectors selected for this study are pLentiCRISPR v25 (14.9 Kb), STEMCCA (12.5 Kb), and pCL45 (7.5 Kb)] are selected. Different transgenes present in these vectors are replaced with HSBZmiRE. Lentiviral vectors(LV) are titrated using different volumes in the HUDEP-2 immortalized erythroid cell line. LVs demonstrating comparative GFP expression are selected for analysis of mean fluorescence intensity (MFI), indicating the efficiency of transgene expression. Viral titers of selected vectors are also analyzed. Transduced HUDEP cells are analyzed for fetal globin induction. The selected vector will be later transduced into human CD34+ HSPCs and transplanted into an immunocompromised mice model called NBSGW. After 4 months of transplantation, mice models will be sacrificed to assess their engraftment, differentiation, gene expression, and safety of LV transduced cells.

Results:- Among the vectors tested, FOLCV2BZmiRE showed notably elevated GFP expression (approximately 4.9 times higher than the control vector). It was followed by FO MSSV BZmiREW (2.52 times) and FO pCL45BZmiRE(1.9 times). However, the STEMCCA BZmiRE vector displayed a 15% reduction in GFP% during terminal differentiation, hinting at potential silencing. Titer analysis of virus concentrate of FO pCL45 BZmiRE, FO LCV2BZmiRE, and FO MSSV mL23 BZmiRE shows 4.2*109 TU/mL, 8.46*109 TU/mL, and 4.02*108 TU/mL respectively. Cell lysate from HUDEP-2 cells transduced with FO pCL45 BZshBCL11A and FO LCV2 BZshBCL11A is used to perform western to analyze BCL11A knockdown. Control samples are untransduced HUDEP cells and negative sorted cells from the cell population transduced with FO pCL45 BZshBCL11A and FO LCV2 BZshBCL11A.CD34+ HSPCs transduced with vectors are cultured in erythroid media and sorted. The transduction efficiency of the virus is 61.8% for FOpCL45 BZmiRE, 63.8% for FOLCV2 BZmiRE, and 50.7% for FO MSSV mL23 BZmiRE. Flow cytometry analysis of fetal hemoglobin (HbF) has shown that knockdown of BCL11A and fetal globin induction happened. Untransduced cells show a background HbF of 16.5%. The enhanced HbF% for FO pCL45BZmiRE is 48.46%, FO LCV2BZmiRE is 46.74% and FO MSSV mL23 BZmiRE is 30.21%.

Conclusions:- FO LCV2 BZmiRE is the most effective vector in terms of transgene expression. The knockdown of BCL11A in CD34+ HSPCs using specific vectors leads to an increased percentage of fetal hemoglobin (HbF) which is significant for potential therapeutic applications related to conditions like sickle cell disease and β thalassemia. Further validation of this study is to be performed after transplantation. After the analysis of engrafted CD34+ HSPCs derived from transplanted mice, differentiation potential, gene expression, and safety of the vectors were to be validated.

Keywords:- Lentiviral vectors, Gene therapy, Beta globinopathies, HbF induction