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A review of exploration of RNA interference of BCL11A gene approach for induction of fetal hemoglobin in transgenic sickle cell mouse model

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Abstract
Sickle-cell Disease (SCD) is a neglected chronic disorder of increasing global health importance. Worldwide estimation of about 20-25 million sickle cell disease individuals and out of which 5-10 million are living in India[1]. Still now, there is no complete treatment for SCD. Hematopoietic stem cell transplantation (HSCT) which is only hopeful treatment for SCD but this area is in beginning stag. Blood transfusion temporary treatment but has limitations, as most patients do not get a matched donor and unrelated donors have higher risks of complications, owing to immune reactions between the donor and the recipient. In this review we detail described about BCL11A silencing and how it will increases the fetal hemoglobin that is now emerging therapy for Sickle Cell Disease.

Keywords: RNA interference, BCL11A gene, Sickle-cell Disease, fetal hemoglobin

Introduction
According to Urade (2013) the frequency of sickle cell gene mostly occurred in south and central Indian tribal population which ranges from 5 to 35 percent. Census of 2011, exhibits around 1.80 crore sickle cell trait and 14 lakhs SCD out of a large number of 18 crore tribal community. As per the ICMR survey mortality rate of SCD is 30% before the age of 14 years whereas 70% by the age of 50 years [2]. First India-specific-model-based map about SCD newborns frequency in 2020 reported that shows the highest predicted frequencies ~12% spanned in Jammu and Kashmir and ~10% in central India [3]. In India ~5,200 live babies are born with inherited SCD every year [2]. Around 30% of babies developed serious complications within the first 2 to 2.6 years of life [4]. These comprehensive data help us to understand the importance of public health concern but notably the SCD is taking into account the non curable disease in India. The DBT took the initiatives from 1990s onwards and now working with the MoTAs, DHR, MoH & FW for scaling-up of existing programs for management of sickle cell disease (SCD).

History of Sickle cell disease and its research findings

S. No	Findings	Year
1	Globin difference in sickle cell Disease	1949
2	Glutamic acid-valine substitution	1957
3	Different Haplotypes of β-globin gene by RFLP	1984
4	Hyderoxyurea enhance the fetal hemoglobin	1984
5	Correction of SCD in Adult Mice by Interference with Fetal Hemoglobin Silencing	2011
6	Correction of sickle β-globin gene by CRISPR/cas9	2016

Sickle cell disease comprises highly complex pathological and molecular biological mechanism. It is known that *Silencing of BCL11A* will ameliorate the clinical complications of sickle cell disease [7].

Importance of BCL11A gene:
At of birth, fetal hemoglobin (γ-globin) expression is silenced, and the adult hemoglobin (β-globin) gene is predominantly expressed; this process is referred *as γ to β-hemoglobin switching*. A locus control region (LCR) located 40–60 kb upstream of the β-globin genes.

LCR contains DNA enhancer elements called DNase hypersensitive sites (HSs), which are necessary for high-level globin gene expression. The LCR loops to the β -like globin promoters in a developmentally dynamic manner and is juxtaposed to the fetal γ-globin and adult β -globin promoters in fetal and adult erythroblasts, respectively. Here, B cell CLL/lymphoma 11A (BCL11A) is a transcription factor and regulator of *as γ to β-hemoglobin switching* that has emerged as a promising therapeutic target for sickle cell disease. Because, In SCD homologues patients exhibit sickling and other pathological consequences only after the switching of γ-globins become β-globins. Therefore, reducing the BCL11A level will reduce γ to β-hemoglobin switching.

Findings	***Gene***	***Model***	***Year***
Correction of sickle cell by BCL11A silencing[6]	Bcl11a	*Mouse*	2011
miRNA-embedded shRNAs for Lineage-specific BCL11A Knockdown and Hemoglobin F Induction[7]	Bcl11a	*Mouse*	2015
Bcl11a-deficiency leads to hematopoietic stem cell defects with an aging-like phenotype[8]	Bcl11a, Cdk6	*Mouse*	2016
BCL11A control the fetal hemoglobin switch[9]	BCL11A	*Adult*	2018

BCL11A gene silencing by RNA interference

The double-stranded RNA (dsRNA) has been applied to induce sequence specific post-transcription gene silencing, a phenomenon known as RNA interference (RNAi). This technique to achieve sequence-specific knockdown of target mRNA and for reducing expression of endogenously expressed proteins[10]. Ability to obtain potent and stable RNAi silencing is critical for number of applications especially for gene knockdown studies for target gene validation. To achieve silencing of BCL11A gene by using RNAi which reverse the fetal hemoglobin silencing (γ to β-hemoglobin switching) [12]. Many researchers reported that they achieved silencing BCL11A by using different viral vectors. Here, we formulated our own designed ds-siRNA for BCL11A gene silencing and we using Non viral vectors. Which may be promise safety and simple, and also easily applicable in clinical trials [14, 15].

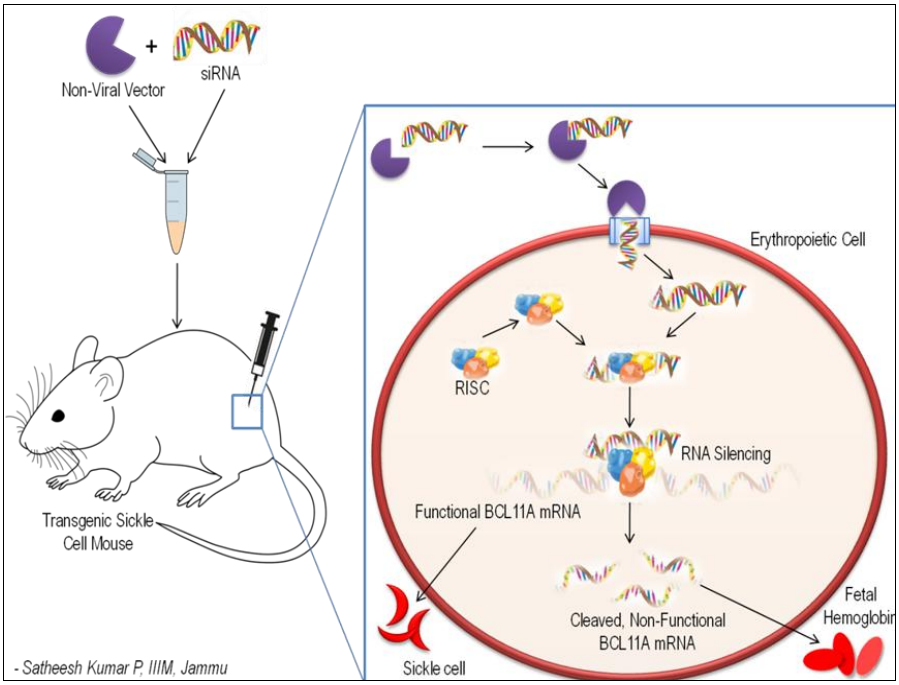

Fig 1: BCL11A silencing results induction of fetal hemoglobin

Table 1: Ongoing active gene therapy clinical trials for sickle-cell disease

Country	Sponsor	Gene	Vector	Conditioning (TD)	Enrolment	Identifier	Status
France	Bluebird Bio	$\beta^{A\text{-}T87Q}$ globin	BB305	Busulfan: 12.8 mg/kg, pk-adjusted	Age 5–37 years, 7 patients	NCT02151526	Active: 3 patients treated; not recruiting
United States	Bluebird Bio	$\beta^{A\text{-}T87Q}$ globin	BB305	Busulfan: 12.8 mg/kg	Adults, 29 patients	NCT02140554	Active: 9 patients treated
United States	UCLA	βAS3 globin	βAS3-FB	Busulfan: 12.8 mg/kg, pk-adjusted	Adults, 6 patients	NCT02247843	Active: 1 patient treated; recruiting
United States, Jamaica	Cincinnati Children's Hospital	γ-globin	mLARβΔγV5	Melphalan: 140 mg/m^2	Age 18–35 years, 10 patients	NCT02186418	Active: recruiting
United States	Boston Children's Hospital	BCL11A shRNA^{mir}	LCR-shRNA^{mir}	Busulfan: 12.8 mg/kg, pk-adjusted	Age 3–40 years, 7 patients	NCT03282656	Active, 1 patient treated; recruiting

Most recent and FDA approved clinical trial is BCL11A silencing [14, 15, 16].

Conclusion

We concluded that findings of "BCL11A silencing through viral vectors is increases the fetal hemoglobin" that is now emerging therapy for Sickle Cell Disease. But using viral vectors causes immunogenic and oncogenecity risks and also shows highest Bcl11a-silecncing which are leads to hematopoietic stem cell defects. Need a better understanding this and their related fields of research study on BCL11A gene silencing by using siRNA/shRNA with non-viral vectors in the transgenic sickle cell mouse.

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