TS NEWS

https://www.trialsitenews.com/a/gene-therapy-for-thalassemia-needs-to-be-safer-46e576ac

Gene Therapy For Thalassemia Needs To Be Safer

Tom Intrepid

Jan. 16, 2023, 6:30 p.m.

Opinion Article

Insertional oncogenesis is an inherent risk of lentiviral vectors because they are able to integrate into thousands of loci within the genome.

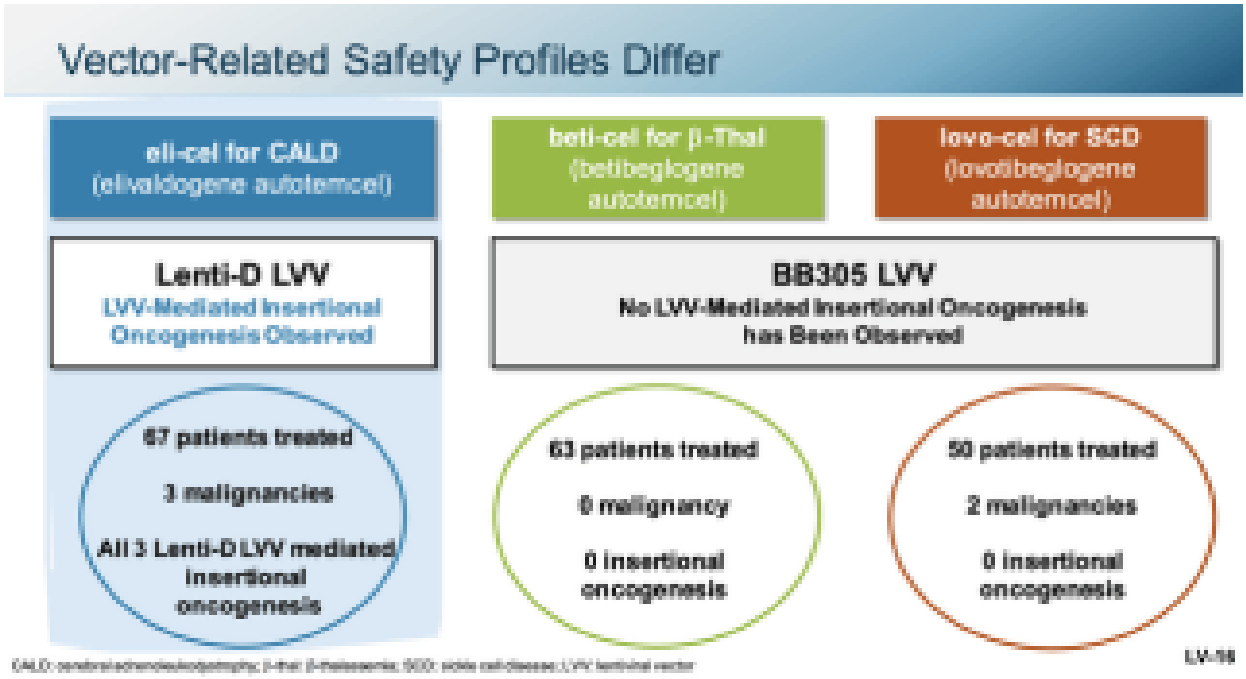
Bluebird's Lenti-D integrated into 1 or more oncogenes in nearly all 67 trial subjects with cerebral adrenoleukodystropy; 3 developed malignancy. The synthetic promoter that drives Lenti-D's ubiquitous expression is culpable.

BB305 is constructed with the natural, erythroid specific β-globin promoter, and has not caused malignancy or clonality in 63 with thalassemia or, on detailed analysis, in 50 with sickle cell.

Michel Sadelain developed β-globin vector TNS9 in 2000, and in 2021 still believed that a safe and effective genetic cure for thal and sickle cell disease "remains to be achieved".

Lentiviral vectors are antithetical to genomic safe harbors, a concept advanced by Sadelain to avoid insertional oncogenesis and which will be exploited with CRISPR to guide genes to designated sites.

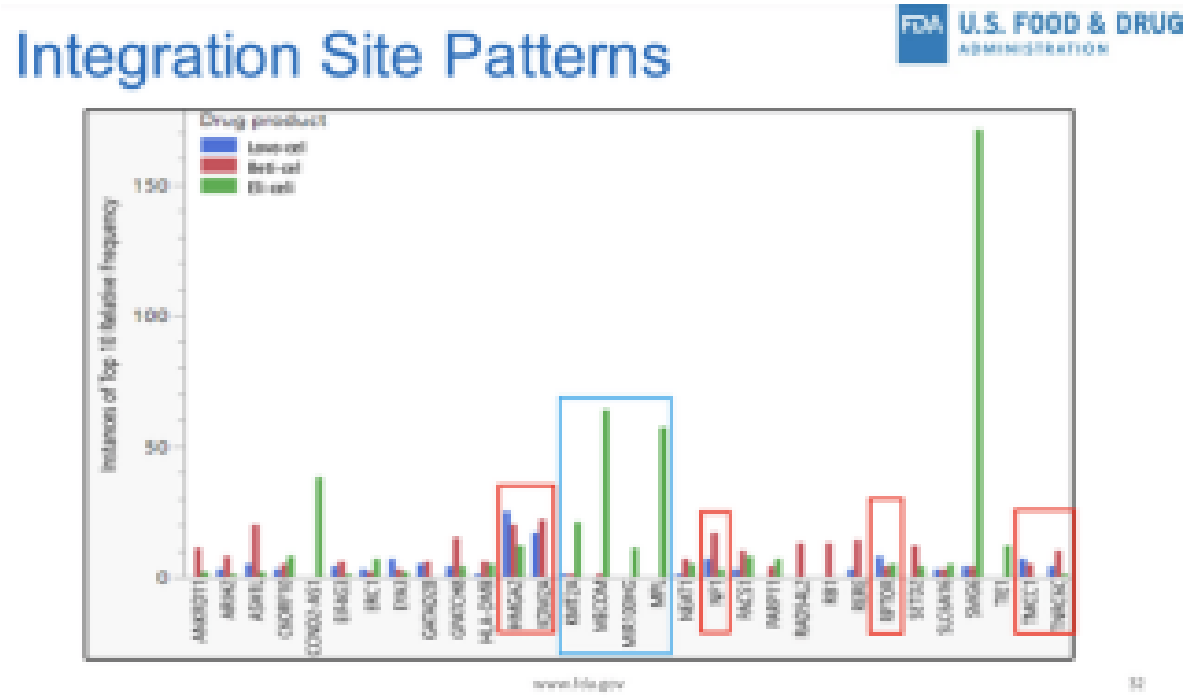
At the FDA Advisory Committee Meeting June 9, 2022 bluebird bio (BLUE) reported on malignancy risk and occurrence in patients treated with either of 2 Lentiviral vectors (LVV) used to manufacture 3 products (see slide LV-16 below). These cell products are derived from the recipient's own (autologous) mobilized (peripheral) bone marrow stem cells by ex-vivo transduction with the vectored functional gene. For BB305 that gene is beta-(β-)globin coded for a 1 amino acid substitution versus that of the normal adult hemoglobin HbA.



Source: https://www.fda.gov/media/159131/download

The bluebird presentation and the one by the FDA official that followed read like a primer on insertional oncogenesis. For example slide 32 of the latter presentation shows results of insertion site analysis for the 3 Bluebird products presented to the FDA. Insertions were found in many disease (including cancer) -related genes.

Eli-cel integration site patterns are represented by the green bars, lovo-cel the blue, beti-cel the red bars.



Source: https://www.fda.gov/media/159130/download

In addition to the 3 CALD trial subjects who developed myelodysplastic syndrome, 4 others had non-malignant bone marrow abnormalities. Lenti-D LVV integrated into the MECOM proto-oncogene in 53 of 54 subjects.

The modified sarcoma viral MNDU3 promoter that drives Lenti-D's ubiquitous (ie non-erythroid) expression is believed responsible for driving expression of nearby oncogenes.

Considering that CALD is devastating hereditary metabolic disease that destroys the central nervous system of young people led to accelerated FDA approval of the product despite ability of the MNDU3-armed vector to cause cancer.

Tissue and stage-specificity of promoters is critically important.

BB305 is constructed with the natural β-globin promoter which is erythroid specific, and BB305 has not yet caused malignancy or clonal predominance in 63 trial patients treated for thalassemia, 50 for sickle cell disease. However, HMGA2 integrations occur (see red bar inside 1st red box of slide 32 above), and that gene has been linked to benign clonal dominance after treatment with HPV569, a predecessor of BB305 (Cavazzana-Calvo, 2010).

More recently, benign clonal expansion was reported years after treating a thalassemic with a comparable globin vector TNS9.3.55 which had integrated into another cancer-related gene UBR2 (Boulad et al., 2022).

Based on experiments performed in his lab, Michel Sadelain, who leads the team that developed β-globin TNS9 vector (May et al., 2000), believes that enhancer elements (ie DNA sequences) within the locus control region of the globin vector, in particular one known as HS2 (see uspto.report) may cause

problems if integrated near (within 300k nucleotide bases) to a cancer-related gene. In his words:

"Due to the non-erythroid activity of HS2, HS2-containing globin vectors may pose a risk for their safe use in clinical treatment"

Some of this data was recently published (Cabrioulu et al. 2022).

All current LVV including BB305 require that a combination of variants of these so-called Hypersensitive Sites be incorporated into the LCR so that therapeutic levels of β-globin are expressed. The LCR is thought to regulate the expression of the β-globin-like genes by direct interaction with their promoters.

The number and exact boundaries of individual LCR elements (HS's) affects the safety and efficacy of a globin vector, and these are highly proprietary (see eg 1st page of the Cabrioulu paper).

Insulators. The search for a safer globin LVV has led Sadelain's team to the study of "insulators." Insulators are naturally occurring DNA elements that partition genes to prevent cross-talk so that one naturally activated gene does not unintentionally activate a silenced, neighboring gene which might be, for example, related to cell growth, ie a proto-oncogene. Another function of an insulator is to prevent a gene intended to be active from being silenced by its neighbor. Sadelain now has possession of a "small sized" insulator called A1 which was used in the laboratory and found to block non-erythroid

expression of β-globin vector TNS9.3.55 without compromising β-globin expression (Cabrioulu et al. 2022). In his 2021 lecture entitled "Gene & Cell Therapy Through the Lens of the β-Globin Gene" Sadelain states that the A1-insulated TNS9 vectors are "poised" to enter clinical trial. However there is a claim against the MKSCC patent by San Rocco Therapeutics (previously named Errant Gene Therapeutics or EGT) that can be read here. That may cause a delay.

For now, it is fair to conclude:

Insertional oncogenesis is a risk inherent to gene therapy with LVV

because LVV are integrated into thousands of genomic sites. In the case of β globin LVV TNS9.3.55 tested in human thalassemia stem cell lines, the vector was integrated into 5,840 unique sites (Papapetrou et al., 2011). It seems logical to conclude that it is only a matter of time and number of patients treated until a current version of β-globin LVV causes cancer.

Benefit weighed against the risk of cancer. In the phase 3 thalassemia trial, BB305 cell product beti-cel boosted total hemoglobin by only about 2 grams% from 9.6g at baseline to an average of 11.7g% during transfusion independence in the 20/22 who achieved that goal (Locatelli et al. 2022).

BB305 probably has more value for sickle cell because of the anti-sickling effect of the T87Q substitution in the encoded β-globin gene, and the resolution of severe vaso-occlusive events in 25 of 25 trial patients (Kanter et al., 2022). In that trial, lovo-cel BB305 boosted total hemoglobin by about 2.5 grams% from 8.5g at baseline to 11g%.

It may be worth the risk until there is a better solution. In my opinion, BB305 will retain a place in history as the 1st approved gene therapy for β thalassemia, but must be revamped to eliminate the risk of insertional oncogenesis.

Guided globin vector insertion into genomic safe harbors

The safe harbor concept for gene addition by retrovirus was developed in Sadelain's lab (see Papapetrou et al., 2011) and is further described section III of the MKSCC September 17, 2020 uspto.report (link to which is above). The proposal is to embody an "expression cassette comprised in the CRISPR-Cas system [between] two insulators." The RNA guide is to give site specificity (eg a unique DNA sequence on chromosome 1) where the therapeutic gene can be inserted into a double strand break created by the Cas enzyme, and is far enough (50k bases) away from any other gene to not disturb it, and even further (300k bases) from one that is known to be oncogenic.

This is a lofty goal that to my knowledge has not yet even been performed in mice, and is years away from clinical trials.

The near future. Since the Aug. 17, 2022 FDA approval of beti-cel for transfusion-dependent beta-thalassemia, bluebird reports that "approximately 40 patients have initiated benefits verification" for the $2.8 million treatment, and that 1 has begun apheresis to harvest stem cells. "Bluebird estimates that there are approximately 1,300-1,500 individuals with transfusion dependent beta-thalassemia in the U.S."

Not all will achieve great clinical benefit. Among 9 patients in the phase 1/2 trial (Thompson et al., 2018) with the most severe genotype (β0/β0) only 3 became transfusion independent.

I suspect that those who benefit most will be those who need only about 2 grams extra hemoglobin to get them over the 9 gram hurdle to achieve transfusion independence.

For the more intermediate term, I would look for insulated modifications of BB305 or more likely TNS9.3.55 - which has already been insulated with A1 - to enter clinical trials as soon as some data in mice can be presented. If gene expression data can show that insertions by chance into, or near, known oncogenes like HMGA2 do not activate them, practitioners and patients/families could be more confident they will not be trading transfusion dependence for a lethal MDS or even a somewhat worrisome clonal expansion.

Long-term, I would expect researchers of lentivirus or gamma retrovirus, with which it is apparently easier and less expensive to produce cell product in bulk, to develop vectors guided by CRISPR to accomplish gene addition much more precisely.