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Pharmacovigilance Risk Assessment Committee (PRAC)

PRAC assessment report

Procedure under Article 20 of Regulation (EC) No 726/2004 resulting from pharmacovigilance data

Invented name: Zynteglo

Active substance: betibeglogene autotemcel

Procedure number: EMEA/H/A-20/1504/C/003691/0023

Note:

Assessment report as adopted by the PRAC and considered by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AE	adverse event
AIDS	acquired immunodeficiency syndrome
allo-HSCT	allogeneic hematopoietic stem cell transplantation
ANX	Annex IID
ATC	Anatomical Therapeutic Chemical
ATMP	Advanced Therapeutic Medicinal Product
AUC	area under the curve
BLA	Biologics License Application
BSC	biosafety cabinet
CAT	Committee for Advanced Therapies
CI	confidence interval
CMA	Conditional Marketing Authorisation
COMP	Committee for Orphan Medicinal Products
CSR	clinical study report
DIN	Donation Identification Number
DMSO	dimethyl sulfoxide
DP VCN	drug product vector copy number
DPI	drug product infusion
EC	European Commission
eCTD	electronic common technical document
EEA	European Economic Area
EMA	European Medicines Agency
EP	European Pharmacopoeia
EU	European Union
EUNetHTA	European Network for Health Technology Assessment
EUPI	European Union Product Information
FAL	Final Advice Letter
FISH	Fluorescent in situ-hybridization
G-CSF	Granulocyte colony-stimulating factor
GVHD	Graft-versus-host disease
Hb	hemoglobin
HbA^{T87Q}	hemoglobin containing $\beta^{A\text{-}T87Q}$-globin
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPLC	high performance liquid chromatography
HRQoL	health-related quality of life
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation

IARC	International Agency for Research on Cancer
ICF	informed consent form
INN	international non-proprietary name
ISA	integration site analysis/analyses
ITT	Intent-to-Treat
LIC	liver iron content
LVV	lentiviral vector
MAH	Marketing Authorisation Holder
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
NA	not applicable / not available
NE	neutrophil engraftment
NGS	Next Generation Sequencing
ODD	Orphan Drug Designation
PAES	post-approval efficacy study
PAM	Post-Approval Measure
PASS	post-approval safety study
PB VCN	peripheral blood vector copy number
PD	pharmacodynamic(s)
PDCO	Paediatrics Committee
PE	platelet engraftment
PIP	Paediatric Investigation Plan
pRBC	packed red blood cell
PSUR	Periodic Safety Update Report
PT	Preferred Term
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
RCL	replication competent lentivirus
REC	Recommendation
RMP	Risk Management Plan
SAE	serious adverse event
SAP	Statistical Analysis Plan
SCD	Sickle cell Disease
SD	standard deviation
SmPC	Summary of Product Characteristics
SOB	specific obligation
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TDT	transfusion-dependent β-thalassemia
TI	transfusion independence

TP	Transplant Population
TR	transfusion reduction
US FDA	United States Food and Drug Administration
VCN	vector copy number
VOD	Veno-occlusive liver disease
WBC	white blood cell
WHO	World Health Organization

1. Information on the procedure

In February 2021, the EMA received information about a case of acute myeloid leukaemia (AML), reported in a patient with sickle cell disorder (SCD). The patient had been treated, 5.5 years earlier, in a clinical study, with an investigational medicinal product (bb1111) for the treatment of SCD. The treatment used the same lentiviral vector as Zynteglo, to transduce CD34+ cells.

Some of the characteristics of the patient's adverse event (time to onset, presence of lentiviral integration in AML blast cells), occurring after exposure to bb1111, raise concerns regarding a possible causal association between the lentiviral vector or other aspects related to the therapy and the event. In addition, two cases reported as myelodysplastic syndrome (MDS) after use of bb1111 also occurred in the same study.

On 18 February 2021 the EC therefore triggered a procedure under Article 20 of Regulation (EC) No 726/2004 resulting from pharmacovigilance data, and requested the PRAC to assess the impact of the above concerns on the benefit-risk balance of Zynteglo and to issue a recommendation on whether the relevant marketing authorisations should be maintained, varied, suspended or revoked.

2. Scientific discussion

2.1. Introduction

Zynteglo (betibeglogene autotemcel) is a gene therapy medicinal product, classified as an Advanced Therapeutic Medicinal Product (ATMP) consisting of autologous CD34+ cell enriched population transduced with a lentiviral vector (LentiGlobin BB305-Vector) encoding the βA-T87Q-globin gene.

Zynteglo received an EU conditional marketing authorisation in May 2019 and is currently not authorised in any other part of the world. It is indicated for the treatment of patients 12 years and older with transfusion-dependent β-thalassemia (TDT) with non-β0/β0genotype and no (HLA)-matched related HSC donor is available. It is given as a one-time therapy with expected life-long effect.

ß-Thalassaemia is a severe congenital haemoglobinopathy, which in its most severe, transfusion dependent form impairs function of heart, liver and kidneys in the long-term, with high impact on living-conditions and lifespan of the affected individuals. It is caused by mutations in the HBB gene that encodes β-globin, resulting in reduced or absent production of functional adult haemoglobin (HbA) that normally accounts for > 95% of the total haemoglobin (Hb) in the blood of adults. β-Thalassemia is caused by mutations resulting in a single nucleotide substitution. More than 350 β-thalassemia mutations have been described, and they are commonly assigned a severity index, with β+ denoting mild mutations that cause a relative reduction of β-globin chain synthesis and β0 referring to severe mutations that can lead to a complete absence of β-globin chain product.

Standard of care is transfusion and iron-chelation therapy and for a minority of patients, HLA-matched HSCT from a not affected sibling donor. Patients who have been inadequately transfused and/or chelated may develop serious complications including cardiomyopathy; liver disease; bone disease; and endocrine disorders (Angelucci et al. 2002[1]; Rund and Rachmilewitz 2005[2]; Cappellini et al.

[1] Angelucci E, Muretto P, Nicolucci A, et al (2002) Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 100:17–21
[2] Rund D, Rachmilewitz E (2005) Beta-thalassemia. N Engl J Med 353:1135–46. doi: 10.1056/NEJMra050436

2014[3]; Tubman et al. 2015[4]; Farmakis et al. 2020[5]). Even with the correct application of blood transfusions and chelation treatments, patients with transfusion-dependent β-thalassaemia continue to have many complications (Bonifazi et al. 2017[6]; Betts et al. 2019[7]).

Beyond HSC transplantation, Zynteglo is the only available curative therapeutic approach. Zynteglo is prepared individually for each patient out of HSC collected from the patient and will only be given to the patient for whom it is made. It is administered as an intravenous infusion and the dose depends on patient's bodyweight. Zynteglo contains CD34+cell enriched erythropoietic stem cells (HSCs), transduced with a lentiviral vector (LVV) encoding ßA-TA7Q, so that these transduced autologous HSCs produce functional ß-globin (ßA-TA7Q-globin), providing the patient with a sufficient amount of functional haemoglobin. Zynteglo is a once-only treatment and its therapeutic effect is expected to be life-long.

The treatment with Zynteglo starts with a collection/apheresis and a myeloablative pre-conditioning phase. During the collection phase the patient receives plerixafor as a single dose 1 to 3 times in combination with G-CSF to enhance mobilisation. Then apheresis is performed to collect CD34+ stem cells (target number of cells for collection is 12×10^6 CD34+ cells/kg). Myelopablative pre-conditioning is performed with busulfan, which is only started when the full set of bags has been received at the administration (treatment center) site.

A total of 63 patients have been included in 4 clinical trials for Zynteglo (HGB-204, HGB-205, HGB-207 and HGB-212), two of which (HGB-207 and HGB-212) are still ongoing. 23 subjects are also currently included in the long-term follow-up study (LTF-303), which is a condition of the marketing authorisation (category 2 study).

Zynteglo was first launched for marketing in Germany on 13 November 2019 and was at the time of the start of this procedure in the EU only available from two centres in Germany, where one patient has received the medicine outside the context of clinical studies.

On 15 February 2021, the EMA received information, from the marketing authorisation holder of ZYNTEGLO, about an adverse event in a sickle cell disorder (SCD) patient treated in a clinical study with an investigational medicinal product (bb1111) for treatment of SCD, which uses the same lentiviral vector to transduce CD34+ cells. The patient was diagnosed with acute myeloid leukaemia (AML) about 5.5 years after the treatment with bb1111.

Cumulatively, 47 subjects have been exposed to the LVV/bb1111 in the sickle cell disease (SCD) clinical development programme. No post-marketing exposure occurred since the product is not approved in any country of the world.

The EC requested to assess the impact of the above concerns on the benefit-risk balance of Zynteglo and issue a recommendation on whether the relevant marketing authorisations should be maintained, varied, suspended or revoked. Therefore, the PRAC, in close collaboration with the experts from the Committee for Advanced Therapies, reviewed all data available regarding the development of acute myeloid leukaemia (AML) in sickle cell disease patients that occurred with the drug product bb1111 containing the same lentiviral vector also included in Zynteglo (betiblogene autotemcel, or beti-cel),

[3] Cappellini M, Cohen A, Porter J, et al (2014) Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT) (3rd Edition). Thalassaemia International Federation (TIF)
[4] Tubman VN, Fung EB, Vogiatzi M, et al (2015) Guidelines for the Standard Monitoring of Patients With Thalassemia: Report of the Thalassemia Longitudinal Cohort. J Pediatr Hematol Oncol 37:e162-9. doi: 10.1097/MPH.0000000000000307
[5] Farmakis D, Giakoumis A, Angastiniotis M, Eleftheriou A (2020) The changing epidemiology of the ageing thalassaemia populations: A position statement of the Thalassaemia International Federation. Eur J Haematol 105:16–23. doi: 10.1111/ejh.13410
[6] Bonifazi F, Conte R, Baiardi P, et al (2017) Pattern of complications and burden of disease in patients affected by beta thalassemia major. Curr Med Res Opin 33:1525–1533. doi: 10.1080/03007995.2017.1326890
[7] Betts M, Flight PA, Paramore LC, et al (2019) Systematic literature review of the burden of disease and treatment for transfusion-dependent beta-thalassemia. Clin Ther. doi: 10.1016/j.clinthera.2019.12.003

the quality of the bb1111 product administered to the AML/MDS cases, as well as quality, non-clinical and clinical / post-marketing data on Zynteglo.

2.2. Data on efficacy

The efficacy of Zynteglo in its authorised indication has been previously established and is not questioned in the present procedure.

2.3. Data on safety

In terms of safety data, the PRAC reviewed available clinical and post marketing data from Zynteglo, as well as the available information on the MDS/AML cases in clinical trials with bb1111. The PRAC further reviewed scientific literature on predisposing risk factors for haematological malignancies both in the SCD and TDT population

Clinical trial data on TDT subjects treated with betiblogene autotemcel/beti-cel

By the cut-off date 3 March 2020 used by the MAH in their responses, the safety of Zynteglo was evaluated in 50 subjects ≥ 12 years of age with TDT (35 subjects with a non-β0/β0 genotype and 15 subjects with a β0/β0 genotype) in the Intent-to- Treat (ITT) population. Forty-seven of these subjects were included in the study populations treated with beti-cel (33 subjects with a non-β0/β0 genotype and 14 subjects with a β0/β0 genotype). Subjects treated with beti-cel in bluebird bio clinical studies are to be followed for up to 15 years post drug product infusion.

The range (min, max) of duration of follow-up post-drug product infusion for patients from study HGB-204 was 47.0 to 71.3 months and for and HGB-205 it was 52.8 to 71.8. representing approximately 4 to 6 years of follow-up.

For the subjects in the ongoing Phase 3 Studies HGB-207 (N=15) and HGB-212 (n=4) the range (min, max) duration of follow-up post-drug product infusion was 15.9 to 36.2 months and 7.8 to 17.8 months respectively.

During clinical trials, a total of 61 adverse events (AEs) were reported in 48 subjects. Most commonly reported AEs were reported in the system organ class (SOC) infections and infestations (n=17), followed by blood and lymphatic system disorders (n=9) and hepatobiliary disorders (n=7). Most commonly reported AEs (≥2) were veno-occlusive liver disease (n=5), pyrexia (n=4), neutropenia (n=3), thrombocytopenia (n=3), febrile neutropenia (n=2) and stomatitis (n=2).

Post-marketing safety data for Zynteglo

At this stage, only one patient received Zynteglo in the post-marketing setting, i.e. outside clinical trial setting before the start of this Article 20 referral. Available safety data from this patient appears to be in line with the known safety profile. Cytogenetic investigations prior to treatment have not been performed. This is in line with the *"Guidelines for the management of Transfusion dependent Thalassaemia" 3rd edition* (2014) which do not require such testing for treatment of ß-thalassaemia patients scheduled for HSC treatment andd of Zynteglo.

Serious Adverse Events (SAEs) referring to MDS/AML in clinical trials with bb1111/ betiblogene autotemcel- LVV BB305

Based on the data provided, no cases of AML/MDS were reported in clinical trials with betiblogene autotemcel LVV BB305/beti-cel up to the data lock-point (DLP) for this referral.

All of the four reports of MDS and AML, occurred in clinical trial HGB-206, a study evaluating the safety and efficacy of bb1111 in severe SCD. Two reports refer to MDS and two reports to AML, involving

three patients with one of the subject's MDS progressing later on to AML (for formal reasons this was reported as a separate case due to different case numbers). The second MDS case was a female, but MDS was later downgraded to "transfusion dependent anaemia" following further diagnostic tests. Only one of the three patients affected by AML/MDS (including the patient later diagnosed with transfusion dependent anaemia) had integration of the lentiviral vector.

Cytogenetic analysis of post-treatment samples revealed driver mutations (RUNX1/PTPN11) for AML in both patients, the MDS/AML and the AML patient. Pre-treatment samples tested retrospectively for these mutations were negative for both patients

Sickle cell disease population: VAMP4 vector integration site, predisposing risk factors and literature review

One of the main concerns is a potential causal relationship between treatment with bb1111 and the development of haematological malignancies, which is independent of the LVV. Since the LVV used in the bb1111 product is the same as for Zynteglo, any conclusions on the association between treatment with bb1111 and AML development, could have implications for the benefit-risk balance of Zynteglo.

Some of the characteristics of the adverse events of the patients developing AML (time to onset, presence of lentiviral integration in AML blast cells) after exposure to bb1111 raised concerns regarding a possible causal association between the lentiviral vector or other aspects related to the therapy and the event.

The first report of MDS occurred in a male 3 years after treatment with bb1111 and progressed to AML 5 months later. Integrational site analysis in this patient did not detect lentiviral integration into the patient's blast cells.

The second report concerns a case of AML in a female. Following the diagnosis of AML in this patient, investigations for the presence of vector insertions in her blast cells were conducted. The results showed a higher VCN (1.12 c/dg) in peripheral blood CD34+ sample compared to CD34-cells. Further investigations detected the VAMP4 gene as the integration site of the LVV. It should be noted, that VAMP4 has been identified as the most frequent integration site in multiple other subjects in the HGB-206 study. However, this was the only patient where the integration site was present in an expansive clone. It is known that the VAMP4 gene product is involved in Golgi ribbon formation and in the regulation of synaptic vesicle endocytosis but is not associated with cell cycle control, which would have otherwise given rise to concern of potential oncogenesis. Evidence from a literature review on the VAMP4 role also supports its non-oncogenic character.

Further analysis did not show elevated VAMP4 expression in the myeloblast-enriched CD34+PB samples of this patient. Expression patterns were found to be consistent with monosomy 7, which was found by cytogenetic analysis in the patient and which is a characteristic of structural abnormality in AML. A possible enhancer, located in the intron between exon 4 and 5, was 5 kb away from the vector insertion site, which means that the vector insertion did not perturb its sequence. In this context, additional gene expression analysis were performed and data was provided on the transcriptome of the clonally expanded cells, especially for relevant genes near the insertion site (MYOC, EEF1AKNMT/METTL13 and DNM3).

PRAC also assessed if a preference for the integration in VAMP4 in general was seen, and if so, what the possible reasons for this preference could be**.** Fold-changes in gene expression were calculated for genes within a 10-MB flanking region of the VAMP4 locus and for each chromosome. No substantial changes were found in expression in the blast enriched patient CD34+ bone marrow (BM) sample relative to healthy donor (HD CD34+) mobilized peripheral blood cells (mPB). No data comparing the expression of nearby genes in the pre- and post-treatment samples of the patient were presented

(intra-patient fold change). However, in view of sample scarcity, and since the increase in expression levels of these genes in the leukemic cells seems modest and overall expression is still low, the issue was not pursued further.

Investigations into whether any reported oncogenes showed expression changes within 10-MB of the VAMP4 IS, found that no such alterations were reported. When genome-level expression changes were analysed, no substantial perturbations were found to gene expression save for a global shift at chromosome 7 which was consistent with monosomy 7, as detected by SNP microarray. Tracking and tabulation of integration sites was performed on the exploratory research GeneWerk database (DADB, Data Analysis Database). Integration sites in the VAMP4 locus are present in 31/55 (56%) beti-cel treated subjects and 25/35 (71%) bb1111 treated subjects and thus the VAMP4 locus is in 68th percentile among protein-coding genes in HGB-206 that have an IS.

Both cases were analysed in view of pre-disposing risk factors associated with pre-treatment conditions, treatment and family history. Both patients identified with MDS/AML had no family history of cancer and were rather young for haematologic malignancies compared to the general population. Published literature on predisposition of SCD patients for malignancies suggest that the SCD population *per se* is at a particularly increased risk for haematologic malignancies and the SCD population is affected much earlier. The risk for SCD patients is between 4 and 10-fold higher than in the general population.

Recent (unpublished) results reported at the ASGCT conference In May 2021 (lecture by Daniel Bauer, Boston about data by Lettre, Lindsley *et al.*) seem to confirm occurrence of clonal hematopoiesis in the SCD population at a 10-years earlier age than in the general population (age of 29 versus 39 years).

In this context, development of clonal haematopoiesis may play a role, which is one suspected pre-condition for the development of haemato-oncologic malignancies (Gibson et al.2017[8]) and as mentioned above, occurring in SCD patients at an earlier age

Additionally, specific risks apply for the transplant procedure used in both the study drug bb1111 and LentiGlobinBB305-Zynteglo due to mandatory pre-conditioning with the alkylating agent busulfan, which also confers a risk of leukaemia following treatment[9].

<u>MDS case changed into transfusion-dependant anaemia</u>

One additional MDS case was initially reported in the SCD trial HGB-206. However, the diagnosis was subsequently changed to the transfusion-dependant anaemia.

The initial diagnosis of MDS was based on persistent severe anaemia and the isolated finding of trisomy 8. It was subsequently changed into transfusion-dependent anaemia after additional testing on 12 March 2021 At that time, the cytogenetic analysis of bone marrow was negative for Trisomy 8. Cytogenetic analyses have been performed on a number of samples pre-treatment (preconditioning, drug product retains, pre-transduction retains). In all samples analysed to date from pre-treatment, no genetic abnormalities have been detected by the methods indicated. These data suggest that the genetic abnormality was only transiently present in a subpopulation of cells. Based on the presented background information and the data provided, it seems unlikely that that this patient had MDS. However, it is difficult to fully exclude a diagnosis of MDS. Absence of cytogenetic or chromosomal abnormality does not exclude a diagnosis of MDS. To further elucidate this aspect additional

[8] Gibson CJ, Lindsley RC, Tchekmedyian V, Mar BG, Shi J, Jaiswal S, Bosworth A, Francisco L, He J, Bansal A, Morgan EA, Lacasce AS, Freedman AS, Fisher DC, Jacobsen E, Armand P, Alyea EP, Koreth J, Ho V, Soiffer RJ, Antin JH, Ritz J, Nikiforow S, Forman SJ, Michor F, Neuberg D, Bhatia R, Bhatia S, Ebert BL. Clonal Hematopoiesis Associated With Adverse Outcomes After Autologous Stem-Cell Transplantation for Lymphoma. J Clin Oncol. 2017 May 10;35(14):1598-1605. doi: 10.1200/JCO.2016.71.6712. Epub 2017 Jan 9. PMID: 28068180; PMCID: PMC5455707.
[9] Busilvex Summary of Product Characteristics https://www.ema.europa.eu/en/documents/product-information/busilvex-epar-product-information_en.pdf

information on the reasons for downgrading the diagnosis were requested. The MAH referred to the WHO definition of MDS which requires detection of blast cells in bone marrow, which was not the case in the above patient.

In order to obtain further information on this case, the MAH committed to provide follow-up information on test results and clinical course of this patient in future Zynteglo PSURs as new information becomes available or is reported during the relevant PSUR reporting period. This information is expected to include peripheral blood haematology values, testing for trisomy 8, and examination of cytogenetics and morphology of bone marrow cells, when available.

ß-thalassaemia population: predisposing risk factors and literature review

As mentioned above, no case of AML/MDS following Zynteglo treatment have been reported so far.

Clinical data from studies with HPV569 Lentiviral Vector which is the forerunner vector included in the drug product to treat ß-thalassaemia patients in clinical trial LG001 were further analyzed. One subject, treated with HPV569 Lentiviral Vector (Subject LG001-1003) had a highly represented dominant clone, following treatment, present in granulocytes and monocytes but not lymphocytes with the LVV IS within the 3rd intron of the HMGA2 gene. Long-term follow-up of this subject revealed that the clone has remained under homeostatic control, gradually decreasing to ~1% of total nucleated blood cells at ~6 years. Follow-up of this patient for over 14 years did not show any malignancy. Notably, the integration of viral vectors in the HMGA2 gene, a known proto-oncogene, was observed before in other gene therapy studies with no case of leukemia/lymphoma (Negre et al. 2016[10]). The occurrence of clonal expansion(s), as in a case with HPV569 LVV (forerunner drug product of LVVBB305 and bb1111), which integrated at the HMGA2 locus may be considered a typical risk with gene therapies, which substantiates the requirement for close long-term monitoring of the patients.

Concerning background risks of ß-thalassaemia patients in terms of occurrence of haematologic malignancy, published literature is limited and mostly based on retrospective database investigations, only some of which include control groups of the general population. In addition, numerous case series and case reports, also published on this topic are of rather limited relevance for evidence-based evaluation. Although retrospective database investigations have their limitations regarding available level of patient details, at least one from a cohort in Taiwan between 1998 and 2010 (Chung et al 2015[11]) also reflects an increased risk for haematologic malignancies and for cancer in ß-thalassaemia patients (5.32 for haematologic malignancies). To which extent this risk for haematologic malignancy in the ß-thalassaemia population is comparable to the risk in the ß-thalassaemia population treated with Zynteglo is not evaluable due to missing information on severity of the disease in the cohort.

However, thalassaemia patients who are transfusion dependent (TDT population) not only appear to be at increased risk for haematological malignancies when compared to the general population but also when compared to thalassemia patients who are not transfusion dependent (non-TDT population). Even though regular RBC transfusions could result in a reduction of bone marrow stress, frequent transfusions lead to iron overload that is believed to play a role in cancer development. Therefore, the risk for MDS/AML due to the underlying disease can be considered to be present in both SCD and TDT populations, though to a lesser extent in the TDT population. Further to the disease severity, any carcinogenic risk is modified by patient specific co-factors e.g. iron overload, age/gender and potential viral co-infection (due to chronic transfusion regimen) or family history.

[10] Negre O, Eggimann A-V, Beuzard Y, et al (2016) Gene Therapy of the beta-Hemoglobinopathies by Lentiviral Transfer of the beta(A(T87Q))-Globin Gene. Hum Gene Ther 27:148–65

[11] Chung W-S, Lin CL, Lin C-L, et al. J Epidemiol Community Health 2015;69:1066–1070.

It should be noted that all patients currently affected by AML or MDS are SCD patients who had treatment with bb1111 and no case concerned patients treated with Zynteglo/beti-cel.

Comparison of SCD and ß-thalassemia patient population regarding the risk of haematological malignancies

The two patient populations, sickle cell disease and ß-thalassaemia, are considerably different regarding their predisposing risk factors for development of haematopoietic malignancies, concomitant diseases and treatments but share one risk factor applicable to the treatment regimen (myeloablative pre-conditioning treatment).

SCD patients suffer from chronic anaemia, pain and haemolysis due to misshaped (sickled) erythrocytes. RBC proliferation rate is extremely increased due to haemolysis and puts the bone marrow under high oxidative stress. Organs relying on high oxygen consumption suffer under chronic oxygen deprivation due to the sickled erythrocytes having reduced oxygen transport capacity because of malformed haemoglobin. Sickled erythrocytes cause frequent vaso-occlusive crisis resulting in early dysfunction of cardiovascular system, liver and brain damages and an increased haemolysis due to early maturation of the cells. No causal treatment options are available for the underlying disease and treatment mostly includes hydroxyurea and pain management.

ß-thalassaemia patients suffer from marked anaemia and haemolysis, lack of functional haemoglobin as oxygen transporter and the consequences of life-long transfusion dependency associated with iron overload and organ damages caused thereby.

Specific risks affecting the different disease population thus arise from the different concomitant treatment regimen they receive (hydroxyurea/pain management for SCD and iron chelators, chronic adaptive transfusion regime for ß-thalassaemia).

Whereas the role of hydroxyurea for oncogenesis in SCD patients is still under discussion, iron overload is a recognized risk factor for oncogenesis[12]. In addition, toxicities and organ damages arising from chronic treatment with chelators have to be considered for ß-thalassaemia patients. The only available causative treatment for ß-thalassaemia until approval of Zynteglo was allo-HSCT preferably by an HSC from a sibling donor and at rather young age to be ahead of any severe organ damage.

As mentioned above, all AML/MDS events occurred in the SCD population, which has an increased risk for haemato-oncologic malignancy inherent to their underlying disease, also confirmed by investigations in several retrospective database by cohort studies. Though these studies lack some detail on patient level, they do suggest that sickle cell patients generally are at an increased risk to develop haematologic malignancies in their early lifetime compared to the general population in the same age [13]. Literature on ß-thalassaemia reflects that the risk for haematologic malignancy may be increased in the ß-thalassaemia population also[14]. The evidence level of the respective literature, however, might be regarded rather low since the data origins from a few retrospective cohort studies only.

Additionally, the known risk for treatment-related leukemia through the alkylating agent Busulfan for myeloablation may be considered as significantly contributory.

[12] Torti SV, Manz DH, Paul BT, Blanchette-Farra N, Torti FM. Iron and Cancer. Annu Rev Nutr. 2018 Aug 21;38:97-125. doi: 10.1146/annurev-nutr-082117-051732. PMID: 30130469; PMCID: PMC8118195.
[13] Brunson A, Keegan THM, Bang H, et al (2017) Increased risk of leukemia among sickle cell disease patients in California. Blood 130:1597–1599. doi: 10.1182/blood-2017-05-783233
[14] Halawi R, Cappellini MD, Taher A (2017) A higher prevalence of hematologic malignancies in patients with thalassemia: Background and culprits. Am J Hematol.; 92:414–416

Discussion on safety aspects

As post-treatment samples revealed driver mutations (RUNX1/PTPN11) for AML in both patients, the MDS/AML and the AML patient and pre-treatment samples tested retrospectively for these mutations were negative for both patients it can be assumed that the events occurred after the treatment with bb1111. Both mutations are well-known to be associated with treatment related malignancies from treatment with alkylating agents i.e. busulfan in haemato-oncology. Additionally, chromosome aberrations (monosomy 7) were detected, which is also considered to be a myeloablative conditioning related chromosome aberration.

However, some other aspects that could explain the development of the SAEs should also be considered. i.e., oncogenic risk factors associated with the lentiviral vector itself or, the patients pre-treatment.

In view of the integration at the VAMP4 locus in one AML patient, PRAC notes that LVVs integrate preferentially in intronic regions of genes that are being actively transcribed (Demeulemeester et al. 2015[15]) and thus it is considered possible that VAMP4 is a frequent insertion site because this locus is transcribed in hematopoietic stem cells. The information provided about the functions of VAMP4 would however support a non-oncogenic role.

It is known from autologous stem cell transplantation that there is a subsequent period of intense proliferation, as a relatively small proportion of the patient's HSC pool is called upon to reconstitute and maintain haematopoiesis long term. HSC Transplantation causes "proliferative stress" and alters the bone marrow microenvironment, potentially influencing the competitive fitness of HSCs. Clonal hematopoietic expansion has been linked to the development of hematologic malignancies both with and without previous cytotoxic therapy exposure.[16] The level of proliferative stress following the transplantation may be affected also by the dose of HSPCs administered (that is dependent on the percentage of the HSPCs in the graft and the size of the graft). It may therefore be possible that a lower dose of HSPCs is predicted to result in more oligoclonal haematopoiesis and higher proliferative stress on the engrafting cells; also the total cell dose received by the patient (including non-HSPC) must be considered as a potential risk factor that potentially could influence repopulation of the bone marrow niche.

Of note, both subjects who developed AML following treatment with bb1111 received a previous version of the product that had different release specifications. Thus, the bb1111 product used in the earlier cohorts (Group A Study 206), had lower cell dose with lower DP VCN (vector copy number) compared to the currently used bb1111 product. Also, these cell doses are about 50% lower compared to the current doses used in the Zynteglo trials as well as in post-marketing setting. The low total dose as well as the low HSCs cell number in the drug product that was used in the Group A Study 206, in which the two subjects with AML had participated, are likely due to the initially different manufacturing process. This initial manufacturing process used bone marrow harvest as a starting material instead of peripheral mobilized cells. For the production of Zynteglo for TDT, only peripheral mobilized cells are used as a starting material, and higher cell dose and improved transduction efficiencies are applied, resulting in general in higher numbers of transduced long-term engrafting cells in the drug product Zynteglo. Therefore, the risk of proliferative stress possibly caused by the low administered dose for patients treated with Zynteglo may be lower compared to the two SCD patients who developed AML. It

[15] Demeulemeester J, De Rijck J, Gijsbers R, Debyser Z. Retroviral integration: Site matters: Mechanisms and consequences of retroviral integration site selection. Bioessays. 2015 Nov;37(11):1202-14. doi: 10.1002/bies.201500051. Epub 2015 Aug 21. PMID: 26293289; PMCID: PMC5053271.

[16] Wong, T.N., Miller, C.A., Jotte, M.R.M. *et al.* Cellular stressors contribute to the expansion of hematopoietic clones of varying leukemic potential. *Nat Commun* **9,** 455 (2018). https://doi.org/10.1038/s41467-018-02858-0

should be noted that the manufacturing process and drug specifications were improved for bb1111 and the currently applied bb1111 dose is more comparable to the dose used in TDT patients.

The Zynteglo SmPC acknowledges the risk of myeloablative conditioning and does not mandate use of any particular conditioning regimen, even though myeloablative treatment regimen all have a similar risk profile in terms of later potential carcinogenesis. The risk of malignancy related to myeloablative conditioning is currently monitored, in the RMP, with both routine and additional risk minimization measures (RMMs) that direct HCPs (and advise patients) to consider the warnings and precautions of the myeloablative conditioning agent used. The RMMs also carry instructions for long-term monitoring of patients for malignancy, which include routine bloodwork and are the same as those for insertional oncogenesis monitoring. The educational material will further emphasize the information on carcinogenesis through myeloablative pre-conditioning considering that busulfan is a human carcinogen.

Concerning studies with Zynteglo and bb1111 occurrence of cytopenia in the context of malignancy in the 2 populations was analysed. The data reflect that cytopenia is frequent in the target populations and malignancy is limited to the 2 patients currently known. Cytopenia is frequent for two reasons: one inherent to the disease and its conventional treatment and one inherent to the pre-conditioning regimen, which is mandatory for the treatment with bb1111/beti-cel. In this regard, anaemia is not evaluable in terms of any treatment related risk since all patients are more or less anaemic due to their underlying diseases (either SCD or ß-thalassaemia). Neutropenia and thrombocytopenia occur following the conditioning regimen with busulfan and resolve in the majority of patients within the first 365 days. Cytopenia in peripheral blood count is therefore considered a rather weak indicator for malignancy when used as a single parameter only and not suitable as an early warning parameter. It should be noted that lack of clinical benefit applies as a contributing factor in both patients with AML/MDS. Drug products for the 2 subjects who developed AML was manufactured according to the earlier manufacturing process and using previous release specifications. As a result, while in accordance to the previous specifications, both products had lower percentage LVV+ cells than the current improved release specifications for Zynteglo and bb1111. Consequently, these 2 subjects did not achieve an adequate therapeutic response, with low pharmacodynamic parameters in peripheral blood (e.g., low VCN, low HbAT87Q). Both patients remained symptomatic with persistent anaemia and required re-initiation of the regular blood transfusions and additional treatment with HU or HU + darbepoetin. The low cell dose may have resulted in ongoing bone marrow proliferation stress, and therefore an increased risk for development of malignancies. In addition, SCD patients are already exposed to proliferative stress due to the disease, so if there is limited efficacy of the product this is expected to remain.

As a further potential risk factor, PRAC considered the use of hydroxyurea. It is widely used for preventive treatment for sickle cell crisis and has been repeatedly suspected to increase the risk for leukemic malignancy in SCD patients. However, so far studies have not sustained this hypothesis. This potential risk in mentioned in the SmPC for hydroxyurea (Xromi).

In summary, several risk factors have been presented which might have contributed to the development of the two AML cases observed in the bb1111 trials. These confounding risk factors include the underlying disease, the induced proliferative stress associated with myeloablative conditioning and the transplantation procedure, the persistent hematopoietic stress due to the lack of efficacy and concomitant use of hydroxyurea. The risk factors of underlying disease, myeloablative conditioning and proliferative stress associated with the transplantation procedure are considered also to be of relevance for TDT patients treated with Zynteglo. However, similar risks are also applicable for allogenic HSCT.

Based on the current knowledge, a causal relationship of the oncogenic events with integration event at VAMP4 and the direct role of VAMP4 in the development of AML seems unlikely. Of note, the leukaemic blast cells derived from one AML patient did not contain LVV insertions. While in the other AML patient with LVV IS in the VAMP4, analysis of transcriptomic data indicated that this integration event did not impact the functioning of this gene and literature review did not provide evidence that VAMP4 mutations are regarded as being oncogenic.

In conclusion, the occurrence of AML/MDS in the subjects treated with bb1111 in HGB-206 study is likely to be attributed to a combination of various risk factors, such as pre-conditioning regimen, underlying disease, which both play a significant role as well as the low product cell number and poor clinical response that might have led to additional bone marrow stress.

In view of patient follow-up, 1-year-routine haematologic work-up starts currently with year 1 following treatment, but implementation of maintaining for longer a 6-month frequency of ISA for possible clonal predominance is implemented in the follow-up study LTF-303, given the interventional nature of the study, which does not rely on routine clinical practice monitoring. It is therefore further proposed to strengthen the information on haematologic work-up in the SmPC by stating that this should occur at least annually, to allow for more frequent schedules for follow-up when the clinical practice guidelines or patient characteristics (e.g. family background) would recommend it, and not to restrict the HCPs options for follow-up.

2.4. Non-clinical aspects

During the initial MAA for Zynteglo (autologous CD34+ cells transduced with BB305 LVV), the applicant addressed the risk for insertional oncogenesis by means of an *in vitro* immortalization (IVIM) assays, an integration site analysis and an assessment (of the development) of the clonal dominance. These analyses did not give rise to suspect a risk for increased tumorigenic potential. Additional data on ten batches further confirmed that the oncogenic risk can be regarded as very limited. The *in vivo* studies also did not give rise to concerns with regards to oncogenicity. Thus, based on the non-clinical studies, transduction of cells with BB305 is not considered to cause an increased tumorigenic risk.

2.5. Quality aspects

Zynteglo and bb1111 are manufactured according to similar processes, both using LVV BB305 for transduction of the CD34+ cells. The comparison of the manufacturing process and batch analysis data of Zynteglo with information on the bb1111 batches that were administered to the patients that developed AML did not reveal any common quality issues that could increase the risk of leukemic transformation.

All specifications were met with all batches of bb1111 presented. No obvious out-of-trend results were indicated. Information on the dose for all patients (total number and number of transduced cells) has been provided. Obtained values for total cell count, Dose, %LVV+ cells and number of transduced cells are within the range observed with other SCD batches manufactured with the respective process. Regarding the bb111 batches that were administered to patients that developed AML, the only quality aspect that was identified as a potential contributor was their relatively low content of cells expressing markers for long-term engrafting cells. It is also noted that the dose of CD34+ cells/kg received by the two patients that developed AML was approximately 2-fold lower than the recommended minimum dose of Zynteglo. Of note, as discussed above a low number of transplanted cells may possibly increase proliferative stress on the engrafted cells following the transplantation.

The low content of cells expressing markers for long-term engrafting cells was observed in bb1111 batches manufactured from bone marrow according to an earlier manufacturing process, but not in

bb1111 batches manufactured from mobilized peripheral blood according to the current process or in Zynteglo batches, suggesting that it could be specific for the starting material or the earlier version of the manufacturing process.

The properties and specifications of the drug substances used in SCD and TDT were also discussed. Differences in properties of the cell starting material were discussed in view of the different genetic equipment (different mutations) and cellular composition due to different mobilization regimen. Moreover, for the 2 SCD patients developing AML, the cell procurement technique differs between the early SCD process and TDT and later SCD processes (bone marrow aspirate vs. apheresis). The differences also led to differences in the number of cells, which could be obtained for transduction, and in the control strategy for both products with respect to potency testing.

Culture conditions including cytokine stimulation and transduction are considered to not promote a risk of leukaemia / MDS. Cytogenetic analysis failed to detect the presence of AML-related abnormal rearrangements/mutations in pre-transduction and drug product retains as well as in patient samples up to 2 years after transplantation.

Cell recovery after transduction and wash was calculated to 101% in the bb1111 HGB-206 clinical study cohort Group C, which suggest no significant cell expansion. In this calculation however, it seems that cell death due to transduction and cell loss due to wash has not been considered, which nevertheless does not significantly impact the final result.

Overall, based on the data presented no quality deviation of the bb1111 drug product has been found that could have contributed to the occurrence of MDS/AML in the respective patients.

3. Benefit-risk balance

Four events concerning 2 cases of MDS and 2 of AML have been reported in a clinical trial where the drug product bb1111 was administered to patients with sickle cell disease. Out of the 2 MDS cases, one was not confirmed, and one patient progressed to AML later on as such, 3 events in 2 patients were further assessed.

Since bb1111 contains the same lentiviral vector as Zynteglo (betiblogene autotemcel-beticel) any conclusions on the association between treatment with bb1111 and development of AML might have had implications for the B-R balance of Zynteglo. Zynteglo is approved for the treatment of transfusion dependent thalassaemia in patients > 12 years with non-ß0/ß0-genotype and no matched HSC donor available, whereas bb1111 for SCD is currently not authorised in any country.

Sickle cell disease population substantially differs from the ß-thalassaemia population in terms of disease characteristics and\ symptoms, conservative treatment options and long-term complications.

Based on the data and investigations provided through this referral a causal association of the oncogenic event with integration at VAMP4 site of the LVV and the direct role of VAMP4 in the development of AML in one of the SCD cases is considered unlikely. Thorough investigation of possible alternative routes of IS involvement in AML development have been ruled out as far this is possible based on current scientific knowledge and methods.

On the other hand, several risk factors related to the treatment procedure (myeloablative conditioning, HSCT) and drug product (low dose of hematopoietic stem and progenitor cells (HSPCs), relatively low vector copy number (VCN)) potentially translating into lack of clinical effect seen may all have contributed to proliferative stress on HSPCs which may have all contributed to development of AML in two reported cases in SCD patients.

In view of the quality of the bb1111 product received by the AML patients, all release specifications were met.

Data from 63 subjects in 4 clinical development studies for Zynteglo (HGB-204, HGB-205, HGB-207 and 212) were assessed. The data reflect a well-tolerated treatment with mostly non-serious adverse reactions. Fifty SAEs were reported from 29 subjects, of which 13 occurred prior to drug product infusion and were attributed to study procedures, mobilization and apheresis. The remaining 37 SAEs were treatment-emergent and occurred in 22 subjects. There have been no events of splenic rupture in beti-cel-treated subjects (potential risk). One serious adverse event of Grade 3 Thrombocytopenia occurred, 16 events of thrombocytopenia were non-serious and assessed as possibly related or related. Most other events attributed as related or possibly related to drug product were consistent with side effects of the DMSO cryo-preservative used in beti-cel. Delayed platelet engraftment is captured in the safety concerns as an identified risk for Zynteglo and is closely monitored following treatment. Besides one event of epistaxis, no other serious bleeding events occurred so far in context with thrombocytopenia in patients treated with beti-cel.

The only patient treated with Zynteglo in the post-marketing setting took a favourable course with neutrophil engraftment on Day 27. The patient is currently free of transfusion requirements. Blood count revealed Hb at 11.2g/dl and platelets are stable at 29.000 / µl on Day 61 (12-Apr-2021-no platelet engraftment, which is defined as sustained >20.000pts/ µl).

Integration site analyses performed in all ß-thalassaemia subjects continued to be inconspicuous for clonal predominance and no malignancy (leukaemia/MDS/lymphoma or other) occurred within a maximum follow-up time of 71.8 months following treatment (data obtained from 2nd renewal assessment).

Overall, there is no evidence that the vector integration is involved in the development of the two AML events. Other risk factors related to Busulfan use for myeloablative conditioning, underlying disease, as well as poor treatment response might have contributed to the development of AML in two SCD cases. The risk factors that are directly related to the bb1111 drug product (low dose of HSPC, relatively low VCN, lack of clinical effect) are considered low for Zynteglo and unlikely to substantially contribute to an increased risk of AML reported for TDT patients. The risk factors related to the transplantation procedure itself were already considered in the benefit-risk assessment at the time of the initial conditional approval.

Both subjects who developed AML following treatment with bb1111 received drug product made from bone marrow harvest with a low cell dose with compared to the current doses used in the Zynteglo trials as well as in post-marketing setting (product made from peripheral mobilized cells obtained by apheresis). If it can be assumed that the degree of proliferative stress increases with decreasing transplanted cell dose, then due to the higher cell dose and higher percentage of long-term engrafting cells (CD34hi/+) received by patients treated with Zynteglo, a risk of additional proliferative stress on the bone marrow is considered to be lower than for the two SCD patients who developed AML.

Finally, for TDT patients, treatment with Zynteglo offers those patients, who in principal would be eligible for HSCT, but do not have a matched (-related) donor, a causative treatment option with expected life-long effect. Since Zynteglo is based on transduced autologous haematopoietic stem cells no life-long immune suppressive therapy is warranted, which is considered an additional advantage over conventional allo-HSCT treatment, in particular with respect to adolescent patients.

Based on the information provided through this referral it can be concluded that:

- Vector insertion site VAMP4 does not seem to be associated with oncogenicity

- Post-treatment mutations detected in both patients who developed AML are most likely to be related to the myeloablative conditioning and to an underlying risk of haematological malignancy in patients with SCD
- The SCD population has an increased baseline risk for haematologic malignancies
- The SCD population substantially differs from the ß-thalassaemia (TDT) population in terms of characteristics and symptoms of the underlying disease, conservative treatment options and long-term complications
- The TDT population and the SCD population share the risk associated with the myeloablative treatment due to the same pre-conditioning requirements for Zynteglo as for bb1111. This risk was already considered during the CMA assessment of Zynteglo and is covered in the SmPC.
- Both subjects who developed AML following treatment with bb1111 received drug product made from bone marrow harvest with a lower cell dose compared to the current doses used in the Zynteglo trials as well as in post-marketing setting (product made from peripheral mobilized cells obtained by apheresis). Due to the higher cell dose and higher percentage of long-term engrafting cells (CD34hi/+) received by patients treated with Zynteglo, a lower risk of additional proliferative stress on the bone marrow can be assumed compared to the two SCD patients who developed AML.

Taking into consideration all of the data discussed above and that no case of haematological malignancy occurred in the clinical trial TDT-population with beti-cel over a follow-up time of 7 years, PRAC concluded that the benefit-risk-balance for Zynteglo remains positive but recommended amendments to the product information and risk management plan to

- add that patients should also be monitored for myelodysplasia in addition to leukaemia or lymphoma,
- clarify that monitoring of patients should occur at least annually over the period of 15 years
- better inform patients on the risks of the myeloablative conditioning through the educational material
- reflect also that monitoring of patients should occur at least annually also in the registry study REG-501, and extend 6-monthly monitoring in the long-term follow up study LTF-303 up to 5 years, (thereafter monitoring will be carried out on an annual basis).

4. Summary of new activities and measures

4.1. Risk management

The MAH should operate a risk management system described in a Risk Management Plan version 2.0, which has been endorsed as part of the current review procedure.

The revised RMP v2.0 included the following updates related to or derived from the data presented in the referral:

- An update to the post authorisation experience to add information on the single patient treated outside clinical studies;
- An update to section *SVI.2.2 Risks to Patients Related to Administration Procedures* to further clarify the risks of the myeloablative conditioning;

- The addition of myelodysplasia to leukaemia or lymphoma as a potential new-onset life-threatening disease that can be caused by the important potential risk "Insertional oncogenesis";
- Based on the updated protocols, an RMP update on the details of the registry study REG-501 and the long-term follow up study LTF-303 to clarify the frequency of clinical monitoring and follow-up, in view of blood cell count and other analyses (ISA, VCN, globin analysis) as well as study HGB-212 in accordance with the latest version of the protocol;
- An update to the key messages for the educational material in line with changes to Annex IID (see section 5 below);
- consequential changes to the RMP derived from the Product Information update.

4.1.1. Risk minimisation measures

4.1.1.1. Routine risk minimisation measures

Amendments to the product information

The PRAC considered that amendments to section 4.4 of the SmPC were necessary to include the information of this review.

The PRAC considered that routine risk minimisation measures in the form of updates to the product information would be necessary in order to further clarify that monitoring patients for malignancies post treatment with Zynteglo should occur at least annually, especially in the light of the risks associated with the use of myeloablative conditioning agents as part of the therapeutic procedure.

These changes include amendments to section 4.4 of the SmPC.

The Package Leaflet was amended accordingly.

PRAC also recommended to update Annex IID in line with the changes to the RMP to strengthen the key messages for educational material in view of myeloablative conditioning and to be aligned with the changes in section 4.4 of the SmPC on follow-up of patients.

4.1.1.2. Additional risk minimisation measures

The key messages of educational materials for healthcare professionals, patients, and carers have been updated to reflect the data in the referral:

Physician educational material - Guide for healthcare professionals:

- A requirement was added to explicitly mention and explained to the patient the increased risk of a malignancy following myeloablative conditioning
- The recommendation to advise the patients on signs of potential new-onset life-threatening diseases related to insertional oncogenesis was aligned with the respective changes to the PI, together with more frequent options for routine clinical follow-up;

The patient/carer guide:

- The message on recognising the signs of potential new-onset life-threatening diseases related to insertional oncogenesis and seeking urgent medical help if these signs are present was aligned with the PI, together with the more frequent options for routine clinical follow-up.

5. Grounds for Recommendation.

- The PRAC considered the procedure under Article 20 of Regulation (EC) No 726/2004 resulting from pharmacovigilance data for Zynteglo.
- PRAC considered the totality of the data submitted during the referral, regarding the development of acute myeloid leukaemia (AML) in a clinical trial in two sickle cell disease patients treated with the investigational drug product bb1111 transduced with the same lentiviral vector as Zynteglo (betiblogene autotemcel, or beti-cel), including the responses submitted by the marketing authorisation holder in writing. The PRAC also considered the views expressed by experts of the Committee for Advanced Therapies (CAT).
- PRAC noted that based on the extensive review of available information on the integration site found in one of the reported cases of AML the VAMP4 gene is not known to be associated with oncogenicity, therefore a causal association of the oncogenic event with the integration of the lentiviral vector at the VAMP4 site is considered unlikely.
- PRAC also concluded that post-treatment mutations detected in a second AML patient treated with bb1111 in whom the leukaemic cells did not contain the lentiviral vector, are most likely to be related to the myeloablative conditioning. PRAC also considered based on the scientific knowledge about proliferative stress and its impact on patients that increased bone marrow stress due to the low cell number administered and lack of clinical response may have contributed to the development of AML in the reported cases.
- Available non-clinical and quality data also did not point toward an increased tumorigenic risk through transduction of cells with the lentiviral vector used in Zynteglo and bb111.
- PRAC concluded that overall, there is no evidence that the vector integration is involved in the development of the AML events reported with the bb1111, and as such, the risk of AML associated with Zynteglo remains unchanged. As for other gene therapies, insertional oncogenesis remains an important potential risk also for Zynteglo and PRAC recommended that patients should be monitored at least annually also for myelodysplasia in addition to leukaemia or lymphoma (including a complete blood count). Amendments to strengthen the product information in this respect were recommended accordingly.
- PRAC also agreed on revised key messages for the educational materials to strengthen the information on the risks associated with myeloablative conditioning and further emphasize the periodic monitoring of patients for malignancies post treatment with Zynteglo. PRAC also recommended amendments to the risk management plan to reflect these measures and clarify the frequencies for integration site analysis in long-term follow-up studies.

In view of the above, the Committee considers that the benefit-risk balance Zynteglo remains favourable subject to the agreed conditions to the marketing authorisation and agreed amendments to the product information and other risk minimisation measures.

The Committee, as a consequence, recommends the variation to the terms of the marketing authorisation for Zynteglo.