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KEYNOTE (GREEN)

Emergent treatments for β-thalassemia and orphan drug legislations

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In many countries, β-thalassemia (β-THAL) is not uncommon; however, it qualifies as a rare disease in the US and in European Union (EU), where thalassemia drugs are eligible for Orphan Drug Designation (ODD). In this paper, we evaluate all 28 ODDs for β-THAL granted since 2001 in the US and the EU: of these, ten have since been discontinued, twelve are pending, and six have become licensed drugs available for clinical use. The prime mover for these advances has been the increasing depth of understanding of the pathophysiology of β-THAL; at the same time, and even though only one-fifth of β-THAL ODDs have

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become licensed drugs, the ODD legislation has clearly contributed substantially to the development of improved treatments for β-THAL.

Keywords: Beta thalassemia; Orphan drugs; Global health

Introduction

β-THAL is a group of serious autosomal recessive inherited diseases caused by mutations that affect the synthesis of the β-globin subunits (or chains) of adult hemoglobin. The deficit in β chains causes red cells to be small (microcytic) and abnormal, with consequent hemolysis; the α/non-α chain unbalance causes precipitation of α-globin–heme complexes, which hinders the maturation of erythroid cells (ineffective erythropoiesis).[1] Hemopoietic expansion is stimulated by the severe anemia that results from the combination of hemolysis and ineffective erythropoiesis, creating a vicious circle. Ineffective erythropoiesis is the main feature of this pathology, which, in turn, leads to anemia and iron overload, increased risk of thrombosis, organ damage, and increased mortality.[2] Since the first molecular cloning and characterization of the human β-globin gene cluster in 1980, more than 350 β-THAL mutations have been identified so far, ranging from silent mutations (silent β), to mutations that cause a quantitative reduction in β-globin chains (β^+), to the most severe mutations, which result in complete absence of β-globin chain synthesis (β^0).[3]

From a clinical point of view, patients are classified as having either transfusion-dependent thalassemia (TDT), when blood transfusions (BTs) are needed regularly from childhood; or non-transfusion-dependent thalassemia (NTDT), when BTs are needed only occasionally or not at all.[4] In TDT, the extra iron supplied by BT would inevitably entail iron overload and, therefore, iron chelation therapy (ICT) is mandatory. It has also become increasingly clear that ICT might be indicated even in NTDT, because ineffective erythropoiesis suppresses hepcidin, with consequently excessive iron absorption.[5]

β-THAL was first described in the medical literature more than 90 years ago by Cooley and Lee.[6] Currently, allogeneic hematopoietic stem cell transplantation (HSCT) is the only established and definitive curative option for β-THAL.[7] Unfortunately, it is limited by the availability of a matched donor and clinical conditions; it also carries with it the risk of acute and chronic graft-versus-host disease (GVHD). Gene therapy (GT) pre-empts GVHD-related risks and is being evaluated as a new option in patients with β-THAL.[8]

Approximately 1.5% of the global population are thalassemia heterozygotes or carriers.[9] The relative resistance against malaria of β-THAL heterozygotes is the evolutionary explanation for their high frequency in the area extending from the Mediterranean basin to the Middle East, the Indian subcontinent and South-East Asia.[10] Recent migrations have also introduced β-THAL in North America and northern Europe, where, based on US and EU legislation, it meets the criteria for being regarded as a rare disease.[11] According to the Orphan Drug Act, passed in 1983 in the US and EU Regulation (EC) No 141/2000, any medicinal product ('drug') aimed to treat a rare disease is potentially eligible for ODD.[12,13] ODD is granted by the US Food and Drug Administration (FDA) and by the European Medicine Agency (EMA) to a new drug, to a previously unlicensed drug, or to a new use of an already licensed drug, when used to treat a rare and severe disease. Although the US and EU laws differ in some details, their objectives are similar: they aim to provide incentives to invest in disease areas that might not be otherwise commercially attractive. Incentives are of two types: on the one hand, there are 'push' incentives, which reduce the cost and uncertainty of R&D (e.g., tax credits, protocol assistance, etc.); on the other hand, so-called 'pull' incentives increase the likelihood of profitability once a product is marketed (e.g., a predefined extra period of market exclusivity).[14]

In this paper, we investigate all the drugs to date that have received ODD from the FDA and/or EMA. Through this set of drugs, we have opened a window that helps to explore the evolution over time of therapeutic approaches for β-THAL, to assess factors that have been attractive for investors and factors that might have been determinants of successful drug development.

The review process

Publicly available data on 'drugs' that received ODD by the 31 December 2020 for the treatment of β-THAL were retrieved from the databases of the FDA and the EU Community,[15–17] using the following keywords: <beta thalassemia>, <β-thalassemia>, <iron overload>, and <Cooley's disease>.

We collected data of clinical trials (CTs) from the EU Clinical Trials Register, Clinicaltrials.gov and PubMed, through the following search strategy[18,19]:

EU Clinical Trials Register: <Disease name > AND < ODD description (or related references: acronyms or active pharmaceutical ingredient) > OR Advanced search: Orphan Designation number;

Clinicaltrials.gov: Advanced Search: Condition or disease < Disease name > AND other terms < ODD description (or related references: acronyms or active pharmaceutical ingredient)>;

PubMed: Advanced search: all fields < Disease name > AND < ODD description (or related references: acronyms or active pharmaceutical ingredient) > OR < clinical trial number (NTC number or EudraCT Number) >. Filter: article type < clinical trial>.

The start dates of the first CT related to each ODD were collected; for CTs quoted in PubMed but not on Clinicaltrials.gov or the EU Clinical Trials Register, a proxy of 12 months earlier than the publication was assumed as the start date.

Concomitant and previous development programs were scrutinized for each drug to categorize the ODD as: 'new active substance', when not previously licensed, either as a single ingredient drug or as part of a combination product[20,21]; 'new indication', when already licensed for other indications; or

'new formulation', when already licensed in different formulations.

The freely accessible database DrugBank (https://go.drugbank.com) was used to classify each drug as an Advanced Therapeutic Medicinal Product (ATMP), biological, or chemical. ODDs and R&D programs in common with sickle cell disease (SCD), the other major hemoglobinopathy, were investigated in ODD databases and sponsors' pipelines. Observations to confirm the development status of each ODD were followed up to 31 December 2021. Accordingly, ODDs were classified as: (i) 'licensed', when licensed by the agency at issue; (ii) 'in development', if either the research and development (R&D) process was active (i.e., CTs were ongoing, planned, or concluded within a 3-year period, or the resulting ODD remained active in the pipeline of the sponsor, as determined from its website; (iii) 'discontinued', when the R&D process had failed, either because licensing was refused by agencies or the ODD had been officially withdrawn by the sponsor, or abandoned. ODDs were considered abandoned if no update had been published on EU Clinical Trials Register and Clinicaltrials.gov, and either in the literature (PubMed) or on the official website of the sponsor over the past 3 years, or if CTs were published, but the development had been declared completed by the sponsor, or the product had been removed from the pipeline for thalassemia/iron overload, or the sponsor was inactive or declared bankrupt.[22]

Data of sponsors' profiles (listed/private/nonprofit) were provided by Evaluate Pharma (https://www.evaluate.com), whereas data on financial statements and the status of the sponsor (active or inactive because acquired or declared bankrupt) were from Orbis – Bureau van Bijk (https://orbis.bvdinfo.com) and Dun & Bradstreet (https://www.dnb.com/). In addition, sponsors were categorized as global or regional major enterprises, specialty, biotech, and other research and nonprofit organizations (e.g., research institutions, foundations, social enterprises, etc.). Nationality of the sponsor reflected the country where the headquarters were located. Sponsors were also sorted into: large enterprises (>250 employees or US$50 M turnover), medium enterprises (50–249 employees or US$10–50 M turnover), and small enterprises (<50 employees or US$10 M turnover).[23,24]

Whereas the overall picture of ODDs granted by the FDA and EMA encompassed the whole period (1983–2020), comparative analyses were carried out focusing on ODDs granted during the

TABLE 1

Characteristics of ODDs for β-thalassemia granted by FDA and EMA from 2001 to 2020.

Characteristic	FDA (*N* = 22)		EMA (*N* = 18)		FDA and EMA (*N* = 12)		Total (*N* = 28)	
	N	%	*N*	%	*N*	%	*N*	%
Total	**22**	**79%**	**18**	**64%**	**12**	**43%**	**28**	**100%**
ODDs in common with SCD	11	39%	7	25%	5	18%	13	46%
ODD status								
Active	9	41%	8	44%	5	42%	12	43%
Discontinued	7	32%	7	39%	4	33%	10	36%
Approved	6	27%	3	17%	3	25%	6	21%
Therapeutic approach/mechanism								
Gene therapy and gene editing	4	18.2%	4	22.2%	3	25.0%	5	17.9%
Increasing HbF	4	18.2%	3	16.7%	2	16.7%	5	17.9%
Improving ineffective erythropoiesis	4	18.2%	2	11.1%	2	16.7%	4	14.3%
Iron homeostasis	10	45.5%	9	50.0%	5	41.7%	14	50.0%
Development stage (ongoing or completed)								
Preclinical	2	9.1%	1	5.6%	0	0.0%	3	10.7%
Phase I	2	9.1%	3	16.7%	2	16.7%	3	10.7%
Phase II	12	54.5%	11	61.1%	7	58.3%	16	57.1%
Phase III	2	9.1%	2	11.1%	2	16.7%	2	7.1%
Phase IV	4	18.2%	1	5.6%	1	8.3%	4	14.3%
Pharmaceutical formulation								
Oral	13	59.1%	8	44.4%	6	50.0%	15	53.6%
Subcutaneous	5	22.7%	5	27.8%	3	25.0%	7	25.0%
Intravenous	4	18.2%	5	27.8%	3	25.0%	6	21.4%
Frequency of administration								
Daily	11	50.0%	8	44.4%	6	50.0%	13	46.4%
Weekly	2	9.1%	4	22.2%	1	8.3%	5	17.9%
Monthly	4	18.2%	2	11.1%	2	16.7%	4	14.3%
One shot	4	18.2%	4	22.2%	3	25.0%	5	17.9%
N/A	1	4.5%	0	0.0%	0	0.0%	1	3.6%
Type of product								
ATMP	4	18.2%	4	22.2%	3	25.0%	5	17.9%
Biological	5	22.7%	6	33.3%	3	25.0%	8	28.6%
Chemical	13	59.1%	8	44.4%	6	50.0%	15	53.6%

BB-305 has been licensed only in the EU with Conditional Marketing Authorization, a procedure implemented by the EMA to grant a positive opinion for medicines based on less comprehensive clinical data than normally required, whereby the benefit of immediate availability of the medicine outweighs the risk inherent in the fact that additional data are still required. In our analysis, we considered this ODD as licensed because it has been positively evaluated by at least one of the two regulatory agencies. Listed: company whose shares are bought and sold on a stock market.

period 2000–2020, when both legislations were active. Data were organized by year of designation and features (Table 1). Each item was double-checked by two authors; discrepancies were overcome through discussion involving a third author. The frequency of items was reported as numbers and percentages. Correlations between variables were assessed with a chi-squared test (significance level: $P < 0.05$) using Stata software (StataCorp 2017, version 15.1).

Trends in, and types of, ODD over time

Therapeutic strategies have evolved over time, with an increasing number of ODDs over the past 5 years (Figs. 1, 2).

Since 2001, with both the US and EU legislations active, there have been 28 ODDs: 22 FDA and 18 EMA, with 12 in common (43%) (Table 1). There are several possible reasons for the discrepancies between the FDA and EMA data. First, eight of the ten ODDs granted only by the FDA were from large companies, six of which were based in the USA; conversely, five of the six ODDs granted only by the EMA were from small or medium companies based in Europe. Second, the FDA and EMA have different criteria for determing an ODD. For instance, two different formulations of the same active pharmaceutical ingredient (API), deferiprone and deferasirox, were granted ODD in the US but not in the EU. Third, premature R&D failure might occur during the time between the designation by the first agency (two ODDs in the US and two in the EU). Finally, three ODDs in the US and two in the EU were granted only recently (in 2020).

At the time of analysis, ten out of 28 ODDs (36%) had been discontinued (two at the preclinical stage and eight in Phase II); twelve (42%) are active (one in the preclinical stage, three in Phase I and eight in Phase II). Six ODDs, relating to four APIs, are now licensed for the treatment of β-THAL (five in the US and three in the EU): of these, four are in Phase IV and two in Phase III. Overall, different therapeutic approaches have been proposed, addressing different targets within the pathophysiology of β-THAL (Fig. 3).

Iron homeostasis

Historically, iron overload has been recognized as a major component of the pathophysiology of β-THAL, particularly in TDT and later in NTDT. For decades, deferoxamine mesylate had been the standard iron-chelating therapy, long before orphan drug legislation was passed. Subsequently deferiprone received ODD in the US, and deferasirox received ODD in both the US and in EU: because of their convenient method of administration, both drugs have largely replaced deferoxamine. Deferasirox film-coated and deferiprone twice-daily formulations were then developed to improve patient compliance. Other oral chelating agents have also been granted ODD; however, all have since been discontinued (deferitrin and deferitazole in Phase II, and (*S*)-4′-(HO)-DADFT-PE at the preclinical stage). Although conventional iron-chelation therapy remains imperative for TDT, novel avenues have been investigated for NTDT in which iron overload has also been documented. Ineffective erythropoiesis is associated with release of erythroferrone, which suppresses hepcidin, thus allowing intestinal ferroportin to increase iron absorption, ultimately causing iron overload, mainly in the liver.[25–28] In keeping with the crucial role of hepcidin, administration of synthetic hepcidins is beneficial in β-THAL animal models.[29–31] However, of several drugs that had earned ODD a hepcidin mimetic was abandoned at the preclinical stage; PTG-300 and LJPC-401 (also tested in hereditary hemochromatosis) were discontinued because they failed in Phase II. Another possible approach is to increase the hepatic synthesis of hepcidin by suppressing expression of the gene *TMPRSS6*, which was achieved successfully in preclinical models.[32–34] Currently, two agents (SLN124 and IONIS TMPRSS6-LRx) are in clinical Phase I (NCT04559971) and Phase II (NCT04059406), respectively.

More recently, a new class of agents was introduced, targeting ferroportin directly. VIT-2763 blocks hepcidin–ferroportin binding, preventing internalization and degradation.[35] After a successful Phase I trial in healthy volunteers, VIT-2763 is being studied in NTDT in a Phase II trial (EudraCT: 2019-002221-29; NCT04364269).[36]

Human apotransferrin, extracted and purified from human plasma through a state-of-the-art method, was granted ODD. A Phase II trial (NCT03993613) is now enrolling patients with NTDT. Modulating the ability of transferrin receptor 2 (Tfr2) to control erythropoiesis is another potential approach. In animal models, the combination of Tmprss6-ASO + Tfr2 single-allele deletion resulted in significantly higher hemoglobin levels and reduced splenomegaly.[37] However, no ODDs have been granted as yet.

Increasing HbF through epigenetic manipulation

To date, the most widely used fetal hemoglobin (HbF) enhancer is hydroxyurea (HU), which has become the standard of care for SCD worldwide and is included in the Essential Medicines List of the WHO (WHO-EML). Although HU is featured in management protocols for both NTDT and TDT. its clinical benefits have been insufficiently investigated in β-THAL.[38] Surprisingly in our view, there has been no ODD for HU for β-THAL.

Sodium 2,2 dimethylbuyrate (HQK-1001), originally investigated at the School of Medicine of Boston University and then developed by a small biotech company, focused specifically on hemoglobin disorders, was able to stimulate HbF expression and red blood cell (RBC) production in Phase II trials in both β-THAL and SCD; however, further development was abandoned by the sponsor.[39–42]

EPI01 comprises a combination of decitabine (5-aza-2′-deoxycytidine), a hypomethylating agent that inhibits the chromatin-modifying enzyme DNA methyltransferase 1 (DNMT1), and tetrahydrouridine (THU), an inhibitor of cytidine deaminase (without it, decitabine would be rapidly degraded). The hypothesis was that EPI01 might control the action of genes, such as *BCL11A,* which silence the β-globin gene in adult erythroid cells. EPI01, initially developed by a small US-based specialty company, completed Phase I for SCD and became part of a deal with an EU-based global pharmaceutical company for its further development.[43,44] However, it is still in the preclinical stage for β-THAL. Decitabine is a chemotherapeutic agent licensed for the treatment of myelodysplastic syndromes and is not free of toxicity.[45]

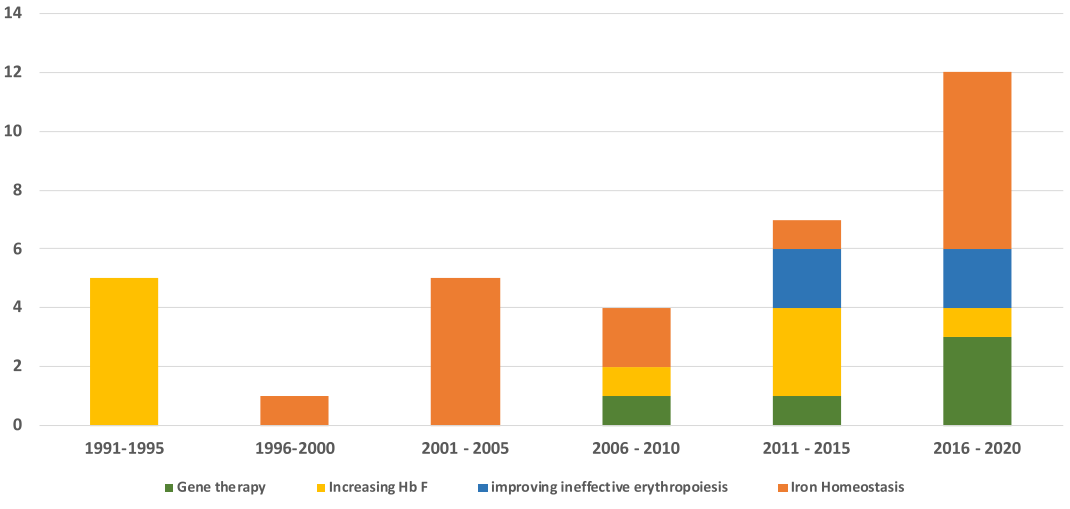

FIGURE 1

Orphan Drug Designations (ODDs) for β-thalassemia (β-THAL) granted by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) over the past four decades (1983–2020). There is a trend toward an increasing number of ODDs over the past 5 years, with changes in the prevalence of the four different therapeutic strategies. Interestingly, the six ODDs granted by the FDA (five butyrate based and one starch conjugate formulation of deferoxamine) between 1983 and 2000 (before EU Regulation No 141/2000), were all discontinued by their sponsors and, therefore, did not impact clinical practice. Abbreviation: HbF, fetal hemoglobin.

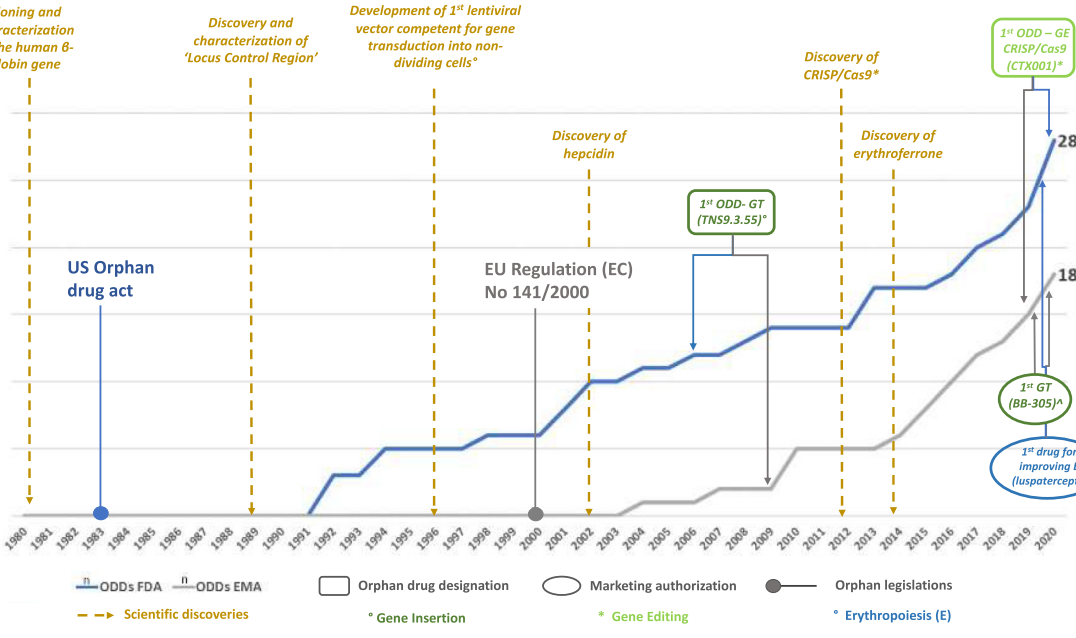

FIGURE 2

Time line of advances in understanding β-thalassemia (β-THAL), gene transfer technologies, and the granting of Orphan Drug Designations (ODDs) and of drug licensing. The notion that β-THAL could be cured through gene therapy arose as soon as the human globin genes were cloned, but a thorough understanding of globin gene regulation took another 20 years. In the meantime, it was found that the reactivation of fetal hemoglobin (HbF) synthesis through partial reversal of the physiological β-γ globin switch, ameliorated the severity of β-hemoglobinopathies by improving the α/non-α chain imbalance. It was also discovered that ineffective erythropoiesis is associated with release of erythroferrone, which suppresses hepcidin, thus allowing intestinal ferroportin to increase iron absorption, ultimately causing iron overload, mainly in the liver. The notion that reducing SMAD2/3 signaling can render erythropoiesis more effective, although the mechanism has not yet been fully elucidated, led to the development of luspatercept.

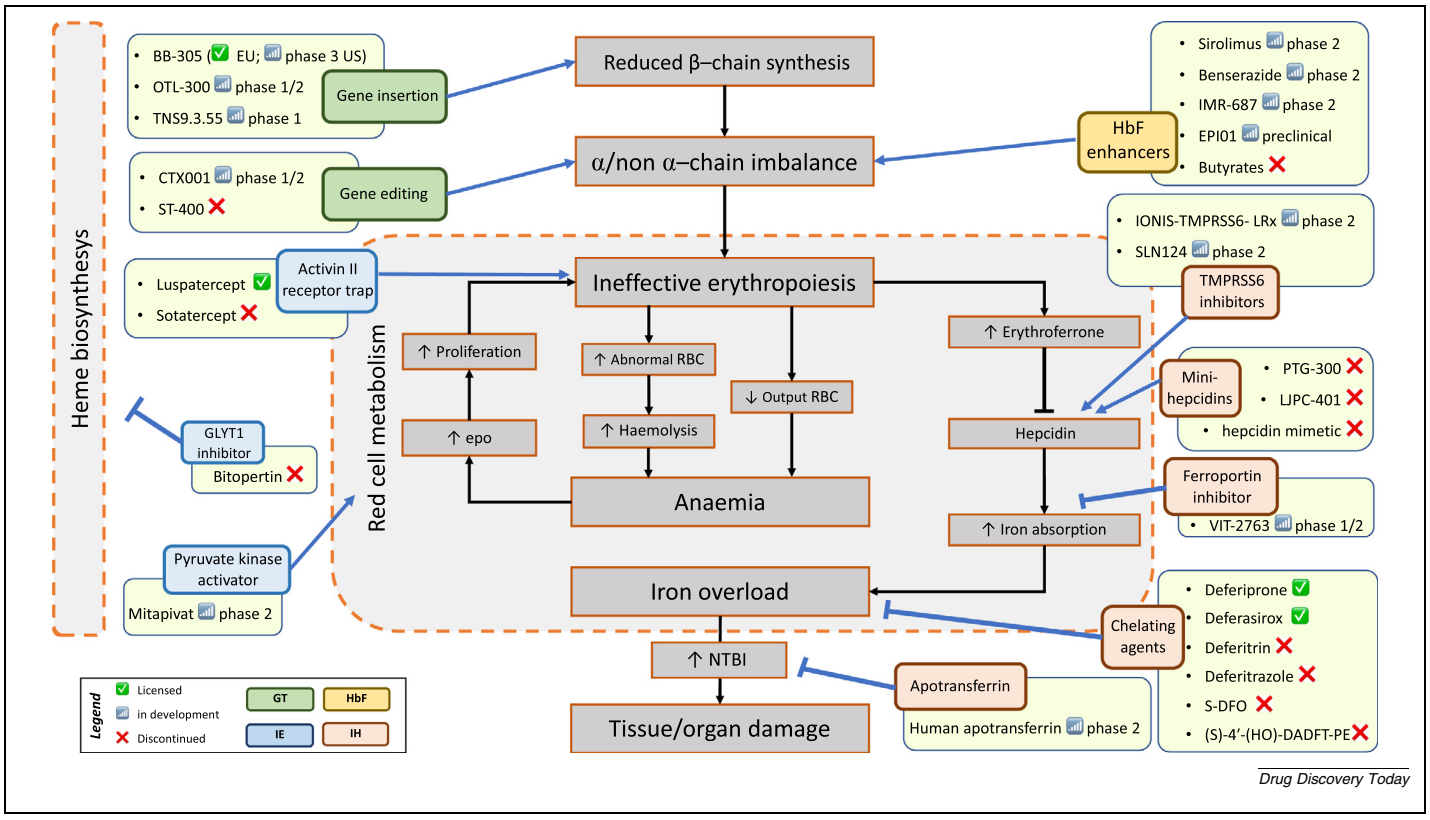

FIGURE 3

Pathophysiology of β-thalassemia (β-THAL) and therapeutic targets of Orphan Drug Designations (ODDs). In graph details all ODDs granted in the US and the European Union (EU) from 1983 to 2020; how the respective drugs work; and the outcome of each ODD. Abbreviations: GT, gene therapy; HbF, fetal hemoglobin; IE, ineffective erythropoiesis; IH, iron homeostasis; NTBI, Non-transferrin-bound iron; RBC, red blood cell.

Sirolimus (also known as rapamycin) is a lipophilic macrolide with immune-suppressive properties, and is already licensed worldwide for the prophylaxis of organ rejection in patients receiving renal transplants.[46] Sirolimus produces a dose-dependent increase in HbF production in erythroid precursor cells from patients with hemoglobinopathies.[47] A small, non-profit social firm dedicated to the development of new therapies for rare diseases is recruiting 26 adults with TDT in a single-arm, open-label, Phase II trial (NCT03877809).[48]

Benserazide hydrochloride is another example of drug repurposing. This DOPA decarboxylase inhibitor currently used for the management of Parkinson's disease in combination with levodopa, was found in preclinical models to increase HbF, possibly by downregulating *BCL11A*, *LSD1*, and *HDAC3*.[49] Sponsored by a small EU-based firm, benserazide received ODD from the EMA in 2014 and patients are being recruited to a Phase Ib escalating-dose regimen study (NCT04432623).

Tovinontrine (IMR-687), a highly specific phosphodiesterase 9 (PDE9) inhibitor, currently also an orphan drug candidate for SCD, is being tested in patients with TDT and NTDT in a Phase II trial (NCT04411082).[50]

Improving ineffective erythropoiesis

Correcting the α/non-α chain imbalance is the logical way to abrogate ineffective erythropoiesis in thalassemia. However, alternatives have been explored.

Activin II receptor traps

The first offshoot of this alternative approach was sotatercept, which was tested in a multicentric Phase II trial (NCT0157163516) in patients with TDT and NTDT. Although there was some improvement in the levels of anemia, the drug was withdrawn by the sponsor.[51] By contrast, luspatercept proceeded to a Phase III trial, showing a reduction in transfusion requirement of at least 33% from baseline in just over one-fifth of patients (compared with 4.5% in the placebo group). Serious adverse events have included thrombosis.[52] Nevertheless, luspatercept was licensed by the FDA and EMA in 2020 for the treatment of adults with TDT and it is now gradually being adopted in clinical practice. Data on long-term efficacy and safety in children are not yet available.

In β-THAL there is an excess of heme which, when not incorporated into hemoglobin, is toxic: therefore, there is a rationale (supported by mouse studies) for inhibiting heme biosynthesis.[53] Bitopertin, an oral reversible, potent, and selective glycine transporter 1 (GlyT1) inhibitor, originally developed for the treatment of schizophrenia was withdrawn by the sponsor at clinical stage.

The maturation of RBCs strictly depends on intracellular cyclic adenosine nucleotides and ATP. Reduced ATP concentrations have been reported in RBCs of individuals with β-THAL. In mouse models of β-THAL, mitapivat, an allosteric activator of the RBC-specific form of pyruvate kinase, increases ATP levels and improves anemia.[54] This small molecule (SM) is being developed by a large US-based biotech company for the treatment of

PK deficiency; in addition, in an open-label Phase II CT in adults with NTDT α- and β-THAL, mitapivat showed improved hemoglobin levels and markers of hemolysis and of ineffective erythropoiesis.[55] Thus, Phase III CTs are in progress for both NTDT and TDT β-THAL (NCT04770753 and NCT04770779, respectively).

Gene therapy

Since orphan legislation came into force, five gene insertion (or addition) and gene-editing approaches have received ODD: four from the FDA and four from the EMA, with three in common. The first was autologous hematopoietic stem cells transduced with lentiviral vector TNS9.3.55**,** encoding the human β-globin gene: this was developed by the Memorial Sloan-Kettering Cancer Center, based on the first successful gene transfer of β-THAL in mice.[56] All subsequent ODDs have been from biotech companies. Betibeglogene autotemcel (LentiGlobin BB305) received 'conditional marketing authorization' from the EMA for patients with TDT, 12 years of age or older, non-β^0/β^0 genotype, and who were eligible for a transplant but did not have a matched sibling donor.

BB305 is similar to TNS.3.55. In two Phase I/II CTs, three out nine patients with a β^0/β^0 genotype, and 12 out of 13 patients whose genotype had at least one β^+ allele, became transfusion independent.[57] Two Phase III CTs involving children and adults with β^0/β^0 or 'non-$\beta^0/\beta^{0\prime}$ TDT are ongoing (NCT02906202 and NCT03207009). Given that insertional oncogenesis had been recognised as a potential risk when BB305 was licensed with the tradename of Zynteglo®, patients who receive this medicinal product are monitored in a registry and must be followed up long-term.[58] Following a case of acute myeloid leukemia in a patient with SCD treated with the same viral vector (*bb1111*), Zynteglo® was suspended by the sponsor in February 2021 and put under safety review by the EMA. In July 2021, the Pharmacovigilance Risk Assessment Committee (PRAC) of the EMA 'found that the viral vector was unlikely to be the cause. In one of the patients, the viral vector was not present in the cancer cells, and in the other patient it was present at a site (*VAMP4*) that does not appear to be involved in cancer development' the suspension of BB305 was therefore lifted'[†].[59]

Although the FDA has similar regulatory tools to expedite the review and licensing of promising drugs, BB305 has not been yet licensed in the USA at the time of the analysis. Thus, the decisions taken by EMA and FDA are not always the same. Given new and cutting-edge advances in drug discovery and development, BB305 is a powerful example of the challenging future of the global dimension of regulatory science.[60,61] Meanwhile, preliminary results from a recent Phase III open-label trial on the non-β^0/β^0 genotype showed a sustained response in term of transfusion independence in 20 of the 22 patients (91%) who could be evaluated, with a median follow-up of 29.5 months (range: 13.0–48.2).[62]

A similar lentiviral vector encoding the human β-globin gene (OTL-300), developed by Orchard Therapeutics in partnership with the San Raffaele-Telethon Institute for Gene Therapy (SR-TIGET) in Milan (Italy), is currently in a Phase I/II CT (NCT03275051).

Lentiviral-mediated gene insertion is a powerful tool, and vectors are being still improved. Recent work in mouse models showed that expression can be optimized by adding important regulatory elements and the ankyrin insulator, so that gene therapy can succeed at a lower copy number of the gene inserted.[63] However, the transduced gene is still inserted essentially at random: although efforts have been made to favor integration into 'safe harbors', the hazard of insertional mutagenesis remains.[64] The gene therapy community has been quick to adopt new technologies that no longer rely on random insertion, but target selectively a gene of interest. By using these technologies, it is easier to inactivate a gene rather than correcting it. Again, a logical way to exploit this approach for the treatment of β-THAL is to reverse the γ–β-globin switch, although by using genetic rather than epigenetic approach, such as by targeted inactivation of *BCL11A*. This gene is a major regulator of the β-globin gene cluster, and its downregulation increases HbF expression. This was the basis for granting ODD to ST-400 [autologous $CD34^+$ hematopoietic stem and progenitor cells transfected with the zinc finger nuclease (ZFN) mRNAs SB-mRENH1 and SB-mRENH2). *In vitro* work showed that this agent provides high-precision, ZFN-mediated editing of the *BCL11A* erythroid-specific enhancer.[65] However, after being tested in a Phase I/II CT (NCT03432364), ST-400 was discontinued.[66]

A similar result can be achieved by using clustered regularly interspaced short palindromic repeats-associated 9 nuclease (CRISPR/Cas9) technology. CTX001 is a cellular product comprising autologous CD341 human hematopoietic stem and progenitor cells modified by CRISPR-Cas9-mediated gene editing: this approach also targets *BCL11A* and is currently in Phase I/II CT (NCT03655678; EU: 2017-003351-38).[67] The use of CTX001 resulted in an early and substantial increase in HbF in 99% of RBCs in two patients (one with TDT and one with SCD), sustained over a 12-month period. CTX001 has been subsequently used in eight more patients (six with TDT and two with SCD), whose early follow-up data are broadly consistent with the findings from the first two patients.[68]

Three of the above gene transfer procedures (BB-305, ST-400, and CTX001) have also been awarded ODD for SCD.

The impact of orphan drug legislations

The primary measure of success of an ODD must whether it becomes a licensed drug available to patients. In this respect, the overall success rate in the case of β-THAL is six out of 28 (21%): BB-305 (gene therapy), luspatercept, and two formulations each of deferiprone and deferasirox. Of course, another measure of success is how widely the new drugs benefit patients, which will have to be assessed through surveys on accessibility and usage at both national and local levels.

[†] On 9 August, 2021, the sponsor of BB-305, announced in a press release that they had decided to shut down its operations in Europe. They explained their decision on the basis that 'they were not achieving appropriate value recognition and market access for BB-305 in Europe'. In addition to the incentives regularly associated with ODD, EMA had gone to great lengths to grant PRIME, accelerated assessment and conditional marketing authorization to BB-305 (which is not yet licensed in the US); for this to happen, European patients and clinical research institutions in Europe had made themselves, their resource and their knowhow available for clinical trials.

Considering ODDs in general, advances in genetics and pathophysiology, incentives from orphan legislations, and accelerated regulatory pathways have stimulated research efforts in the area of rare diseases, increasing the likelihood of successfully bringing new drugs to patients.[69] Over the past few decades, the pharmaceutical industry has increasingly redirected its R&D strategy to include rare diseases[70]: this has happened, at least in part, as a result of the incentives provided by ODD, which can result in increased profits. Indeed, gross margins have been estimated to be 11% higher (85.9% versus 74.8%) in companies specializing in orphan drugs compared with large, medium, or small-sized pharmaceutical and biotechnology companies that do not focus exclusively on them. For each licensed orphan drug, the clinical costs were estimated to be US$166 million, and the capitalized clinical costs US$291 million (total US$457 million); by comparison, these figures for a licensed non-orphan drug were US$291 million and US$412 million, respectively (total US$703 million). However, the situation is more complex than it might appear initially. R&D investments in orphan drug companies as a percentage of sales were, on average, twice those of other companies: 33.9% versus 17.3%, respectively. The operating profitability of orphan drug-specialized companies was half that of other companies (15.6% versus 32.3%). By contrast, with respect to molecular entities only, the capitalized clinical costs per licensed orphan drug were half those of a non-orphan drug.[71,72]

The role of sponsors

Although small- and medium-sized companies would be expected to be most sensitive to incentives, they account for only 25% and 14% of ODDs, respectively, in β-THAL (Table 1). With one exception in gene therapy, the technologically most advanced approaches for β-THAL were developed by large enterprises, as also the case for drugs targeting ineffective erythropoiesis. Among features that we have assessed, the size of the sponsor and the therapeutic approach adopted was the only statistically significant relationship found (Supplementary 1 in the supplemental information online). Although developing drugs for rare diseases is still widely perceived as a no-go area for small companies or not-for-profit organizations, this might not apply to drug repositioning.[73] Indeed, all SM-enhancing HbFs have been developed by small companies (although they had already been tested in other diseases, such as SCD and cancer). Regulatory definition tags a molecular entity as 'new' whenever it has not been previously licensed. With respect to β-THAL, only two drugs fall under this definition: twice-daily deferiprone and film-coated deferasirox. The novelty here is in the formulation only.

Medium companies have been awarded four ODDs: three in iron homeostasis and one in gene therapy. Overall, iron homeostasis has had the highest number of successful ODDs: four out the 14 ODDs (29%) have been licensed. Among the 12 ODDs common to the FDA and EMA, six were granted ODD by the FDA before the EMA; and six by the EMA before the FDA: this does not appear to relate to therapeutic strategy, the size of the enterprise, the type of drug, the year of granting, the country where the headquarter of the sponsor located, or the kind of sponsor (Supplementary 2 in the supplemental information online). In terms of how far therapeutic policies of sponsors have been influenced by investment prospects, we cannot comment on commercial interests and on their changes over time, because our data are from the public domain only: therefore, we do not know why certain ODD applications were discontinued. Fig. 4 shows how the features of sponsors are linked to each other and to different therapeutic approaches.

Types of molecule and pharmaceutical features

Gene therapy is a specific category of pharmaceutical. It comprises a procedure whereby the patient's hematopoietic cells are 'mobilized' into the peripheral blood or harvested from the bone marrow; they are then purified, transduced by a vector, checked for successful vector integration, and finally re-infused into the patient, who has received myelosuppressive treatment, which is required in for the hematopoietic marrow to be repopulated by the transduced stem cells. To designate such a complex procedure as 'a drug' is a misnomer that highlights the deficiency of current regulations: this is particularly because the procedure is highly demanding of the clinical unit hosting the patient, as well as on the company providing the vector and its transduction. The financial implications also need reassessing, given that the high cost of what is in fact an auto-grafting procedure must be added to the price of the 'drug'.

Leaving gene therapy aside, SMs are still more numerous than biologics as β-THAL therapy (Table 1), as also seen in other areas. Iron chelators and HbF enhancers are SMs, as are modulators of biosynthetic or metabolic pathways: exemplified by inhibition of ferroportin or GLYT1, or activation of pyruvate kinase. However, SMs can also target other pathways, such as activin II receptor traps for improving erythropoiesis, or TMPRSS6 inhibition and improvement of hepcidin levels for restoring iron homeostasis (Fig. 5).

Not surprisingly, the experience of a company in terms of developing orphan drugs is an important predictor of their successful development.[74] Two of the six licensed ODDs involved the repurposing of drugs already on the market and comprising the same active pharmaceutical ingredient; at the same time, 13 out 28 (46%) of ODDs for β-THAL have also received ODD for SCD. Although their extension to another disease was clearly suggested by common sense in some cases, algorithms from machine learning might have also helped.

Failed drugs

Of the 28 β-THAL ODDs, ten (36%) have been discontinued (Table 1; Figs. 3, 5): two of which at the preclinical stage and eight following Phase II CTs. The molecule typology is not in itself a predictor of successful drug development, given that a significant number of both SMs (some chelating agents, iron overload modulators, and butyrates), some biologics (hepcidins and analogs), and even a gene therapy (ST-400) have been discontinued or abandoned. ST-400 was abandoned for a lack of efficacy in a landscape in which promising new approaches are showing important results. Similar to other butyrate-derived molecules studied during the 1990s, 2-dimethylbutyrate failed, probably because its dosage is too high in relation to the benefit received.

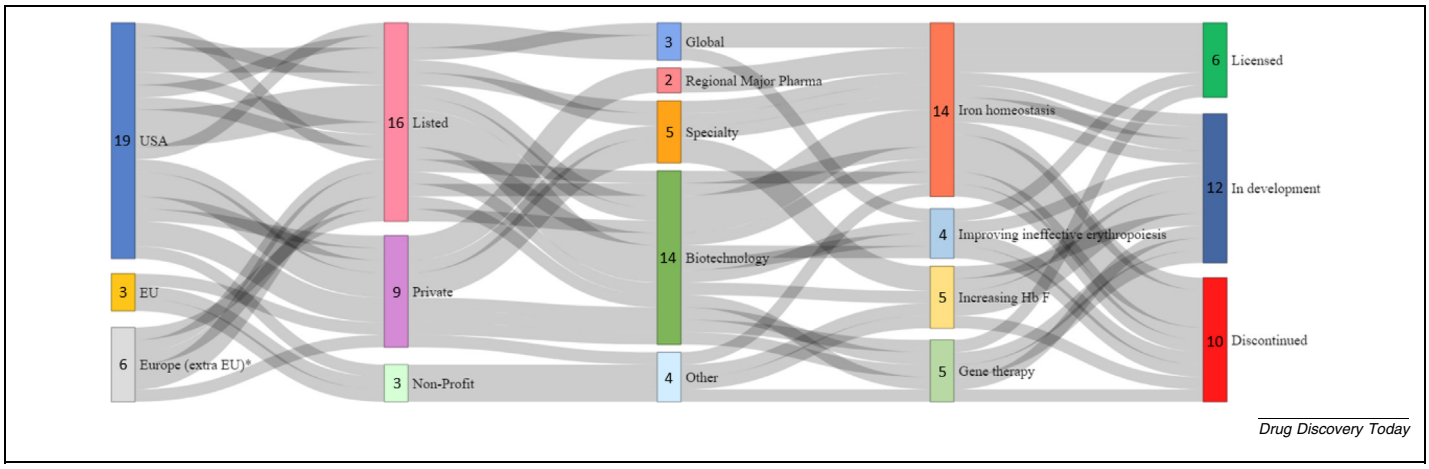

FIGURE 4

Final outcome of Orphan Drug Designations (ODDs) in relation to country of origin, type of sponsor and therapeutic target (2001–2020). Note that 19 (68%) of ODDs were from the USA, three (11%) from the EU, and six (21%) from Switzerland and UK (geographical distribution reflects where the sponsors are based, regardless of where the application was submitted from). No statistically significant associations were found among the variables in this figure. All six ODDs that have obtained licensing were developed by large companies: two global, two regional, and two biotech. However, ODD has also enabled research institutions, foundations, and social (nonprofit) organizations to develop their own programs, including gene therapy. Public–private interactions might also have had a role; however, in this case, as in many others, publicly funded basic and clinical research has been a prerequisite, rather than a partnership, for private enterprise. *Following Brexit, we have to count the UK as non-EU). Abbreviation: HbF, fetal hemoglobin.

The three mini-hepcidins did not effectively reduce iron absorption, whereas the three iron-chelating agents were no better than those already in use. Although bitopertin appeared promising in β-THAL mouse models,[75] it failed to improve erythropoiesis and RBC survival in a Phase II trial enrolling patients with NTDT.[76] Thus, it might be that the hypothesis that inhibition of glycine transport could significantly reduce heme synthesis is misplaced. Sostatercept, which is in use for other disorders, might have been withdrawn by the sponsor for policy reasons because the Phase II CT reported promising results.[51]

Drugs not granted ODD

Seeking ODD status is not mandatory for drug development in the area of rare diseases and, although considered a valuable option, it is the sponsor's decision whether and when to apply for it. Drugs tested for β-THAL that did not result in an application for ODD encompass a range of therapeutic targets and molecules. In some cases, they must have been concerns about the benefit/harm ratio. For instance, thalidomide, a SM in use for the treatment of multiple myeloma, has been tested in patients with TDT and NTDT, also in combination with HU.[77,78] Despite encouraging results in terms of hemoglobin increases and transfusion independence,[79] there have been instances of thrombosis.[80] Another example is ruxolitinib, an oral Jak2 inhibitor already licensed for the treatment of myelofibrosis: it was investigated in a Phase II trial in patients with TDT with spleen enlargement with the primary endpoint being improvement of transfusion regimen (NCT02049450). However, despite an improvement in splenomegaly, no clinically significant reduction in blood transfusion burden was observed, and its development did not continue to Phase III.[81] Polyethylene glycol-conjugated erythropoietin (EPEG), a synthetic and highly stable analog of erythropoietin has been suggested as being helpful in NTDT. However, expansion of extramedullary erythropoiesis, splenomegaly, and possible thrombotic events were reported in a Phase I CT (NCT02950857).

Since the inception of orphan legislations, all drugs licensed for β-THAL in the USA and the EU received orphan designation. In some cases, it appears that sponsors might have rushed to obtain an ODD even for molecules that might not have looked very hopeful, 'just in case' they eventually worked; in other cases, mostly in terms of the repurposing of already-marketed drugs, companies have waited for preliminary clinical data showing a positive risk/benefit profile. In the examples we reported here, the latter approach was chosen mostly by global pharma companies, for which initial incentives put in place by regulators, rather than the market exclusivity provided at the time of approval, might not have been as attractive as it is for small or medium-sized companies or for not-for-profit institutions.

Global health perspective

The management of β-THAL depends on the risk: benefit ratio of new treatments, as well as on their availability and affordability in all countries where most patients live. Given that the prevalence of β-THAL extends beyond the USA and EU, improving the access of patients to new drugs in other countries is the next major challenge for the β-THAL community. The increasing prices of orphan drugs might represent an insurmountable barrier to reaching this objective.[82] The WHO-EML is a register of minimum medicine needs for every healthcare system worldwide, and was introduced based on the principle that essential medicines are those that satisfy the priority healthcare needs of the population[83]: unfortunately, in many countries, the WHO-EML is implemented only in part.[84] For instance, it currently includes deferoxamine and deferasirox, but there are few low-income countries, if any, where these two 'essential medicines' are accessible for all patients. It is no secret that many drugs are not affordable in countries where publicly funded national

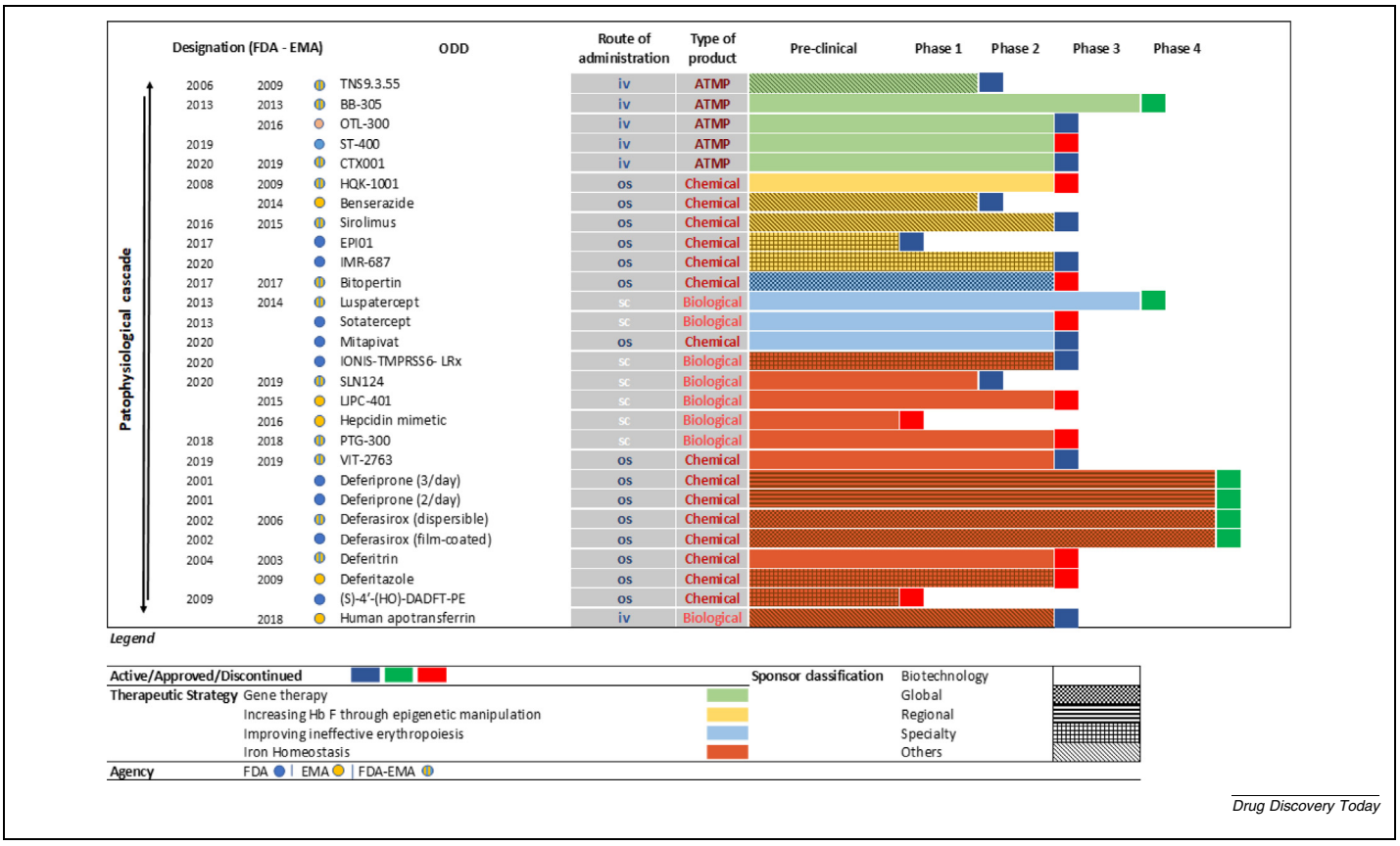

FIGURE 5

Timeline of clinical development and regulatory approval of Orphan Drug Designations (ODDs) for β-thalassemia (β-THAL) (2001–2020). Not surprisingly, the molecule typology as such is not a predictor of successful drug development. A significant number of both small molecules (SMs; some chelating agents, iron overload modulators, and butyrates) and biologics (hepcidins and analogs) have been discontinued or abandoned. By contrast, the route of administration is of significant practical importance: biologicals are parenteral (subcutaneous or intravenous), whereas SMs can be either parenteral or oral, clearly affecting health services and possibly impacting patient compliance. Abbreviations: EMA, European Medicines Agency; FDA, US Food and Drug Administration; HbF, fetal hemoglobin.

health services are not in place and patients have to purchase them themselves.[85] As more orphan drugs are licensed, there is often debate about their inclusion in the EML.[86] On the one hand, it might be appropriate to include them because, to satisfy the health needs of their people, government should know how the therapeutic scenario is evolving. However, as more drugs are added, the gap between 'essential' and real becomes wider. Novel drugs for β-THAL might be a good example of how urgent it is to reconcile inclusion in the WHO-EML with real-life accessibility. This is particularly relevant for gene therapy, which requires skilled personnel, appropriate infrastructure, and public funding.[87] Although we feel strongly that access to this treatment should not be denied *a priori* to anybody, we are aware that the political and financial barriers that need to be overcome are huge. In the meantime, it is our urgent duty to make less complex 'essential medicines' truly available to patients in middle- and low-income countries, where the prevalence of β-THAL is high.

Concluding remarks

β-THAL was identified as a disease entity nearly a century ago and, since then, blood transfusion has been the mainstay of management. Although the importance of iron chelation was already clear, new drugs to confront iron overload and to deliberately address other features of the disease have become available only over the past 30 years, after orphan drug legislations were passed. Our analysis shows that small–medium enterprises and public institutions have not always taken advantage of the incentives provided by orphan drug legislations to the extent that one might have expected. The type of molecule and the pathophysiological target as such have not proven to predict successful drug development. By contrast, the specific expertise of an individual company, whether with respect to a molecule or to a methodology, has had a major role in the successful development of an ODD.

Recently, the prospects for a definitive treatment of β-THAL through allogeneic bone marrow transplantation or gene therapy have taken center stage. These prospects should not detract from the importance to also continue unrelenting efforts to ameliorate treatment protocols for the large proportion of patients who, for a variety of reasons, cannot currently access bone marrow transplantation or gene therapy. There is a vital need to design and conduct CTs of drug combinations to optimize the long-term management of β-THAL. This approach might also help to close the gap between the current prevailing standards of care and the possible future mass roll-out of gene therapy.

Authors' contributions

Enrico Costa: Conceptualization, Data curation, Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Maria Domenica Cappellini:** Data curation, Validation, Writing – review & editing. **Stefano Rivella:** Data curation, Validation, Writing – review & editing. **Adriana Chilin:** Data curation, Validation, Writing – review & editing. **Eva Alessi:** Data curation, Formal analysis, Validation, Writing – review & editing. **Massimo Riccaboni:** Data curation, Validation, Writing – review & editing. **Hubert G.M. Leufkens:** Conceptualization, Writing – review & editing. **Lucio Luzzatto:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.drudis.2022.103342.

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