HHS Public Access

Author manuscript

Biotechnol Lett. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

Biotechnol Lett. 2015 November ; 37(11): 2147–2150. doi:10.1007/s10529-015-1908-z.

Molecular pharming's foot in the FDA's door: Protalix's trailblazing story

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Abstract

Objectives—This short commentary examines the factors that led to Food and Drug Administration's approval of the first plant-derived biologic.

Results—In 2012, the first plant-derived protein pharmaceutical (biologic) was approved for commercial use in humans. The product, a recombinant form of human β-glucocerebrosidase marketed as ELELYSO, was developed by Protalix Biotherapeutics (Carmiel, Israel). The foresight to select this particular therapeutic product for development, flawless production pipeline, and serendipity seem to provide the key in explaining how ELELYSO became the first plant-derived biologic to achieve approval by Food and Drug Administration.

Conclusions—While the circumstances that enabled Protalix and its scientists to become the first to arrive at this historic milestone are perhaps unique, it is anticipated that more biologics will follow suit in winning regulatory endorsement.

Keywords

Plant-Derived Biologics; FDA-approval; Gaucher's Disease; Ebola; Molecular Pharming

The field of "molecular pharming" – the exploration of plants as production platforms for protein pharmaceuticals - has grown and developed since its early inception a couple of decades ago from just a few laboratories that published their first reports on plant-expression of human proteins and vaccine antigens (Hiatt et al. 1989; Mason et al. 1992; Sijmons et al. 1990), to hundreds of laboratories engaged in the research around the world (for comprehensive recent reviews of the field see Ma et al. 2013; Rybicki 2014; Stoger et al. 2014).

In 2012, molecular pharming reached a major landmark with the US Food and Drug Administration (FDA) approval for marketing of taliglucerase alfa (ELELYSO), a recombinant form of human β-glucocerebrosidase (GCD) used in enzyme-replacement therapy of Gaucher disease (GD). This first plant-derived protein pharmaceutical (biologic) to be approved for commercial use in humans was developed by Protalix Biotherapeutics (Carmiel, Israel). The company scored several important additional "firsts" with ELELYSO

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along the way to the momentous FDA approval, including being the first plant-derived biologic to reach a phase III clinical trial, the first to successfully complete it and the first to be the focus of a multi-million commercialization deal with a large pharmaceutical company (Pfizer). Clearly, Protalix never operated in a vacuum and without competition[1]; the company's deliberate, steady and ultimately successful march to the historic milestone remains, so far, unique and worthwhile.

Charles Arntzen, one of the pioneers of plant-derived biologics research, indicated the key factor in Protalix's success, when he was quoted in a *Nature Biotechnology* interview saying that the company put its "focus on the enzyme they are producing and the product niche it will fit into" rather than on its production platform (Ratner 2010). It is emblematic that when Protalix was first mentioned in that journal's annual analysis of the biotech industry it was simply recorded as a producer of a biologic with no mention of anything extraordinary about its production platform: "FDA approached Shire and Protalix Biotherapeutics […] to consider […] using their biologic enzyme replacement candidates" (Aggarwal 2009). The unique nature of their production host is only briefly commented upon in a later analysis after ELELYSO was approved (Aggarwal 2012). Protalix smartly realized that the very aspects that make academicians excited about the technology - the novelty, the potential, the unknown – are exactly the same things that makes regulators squeamish. Moreover, Protalix chose to concentrate its efforts on a less radical plant-platform – plant cells grown in a reactor and emphasized the similarities with well-established fermentation-based technologies. Specifically, Protalix stressed the issue of containment avoiding at once both usual public controversies related to "GMOs" (genetically modified organisms") as well as complying with the entrenched good manufacturing practices set forth by the FDA (Einat Almon, Lecture at "PBVA 2007 - Plant Expression Systems for Recombinant Pharmacologics" meeting, 18–20 June 2007, University of Verona, Italy, and Ratner 2010).

In addition to de-emphasizing the production platform, Protalix's "focus on the product" means that the company concentrated on a limited number of products, and was committed to taking them all the way from preclinical studies, through three phases of clinical trials, to regulator approval and marketing. How fast a biologic goes through this pipeline depends primarily on the biology of the disease and its societal impact and the biology and chemistry of the protein. GD, a recessive hereditary lysosomal storage disorder, is considered an "orphan disease", manifested in only 1:60,000 live births world-wide, but much more prevalent among certain populations (Cox 2010). Defects in both alleles of the single gene encoding the GCD enzyme account for virtually all cases of GD. Moreover, intravenous application of the GCD provides relief of most symptoms in most GD patients. Being the only available treatment to this debilitating and ultimately fatal disease, enzyme-replacement therapy with recombinant Chinese hamster ovary (CHO) cell-derived GCD (marketed as Cerezyme) became the standard of care, despite the extremely high costs of the drug (Cox 2010). Lowering production costs through use of plant cell-cultures as alternative to the CHO cell production system provided important incentive to develop ELELYSO. But a

[1]A plant-made poultry vaccine against Newcastle disease virus (created by Dow Agroscience), was approved for use by the US Department of Agriculture in 2006. The vaccine was never commercially produced or used (Vermij 2006).

particular feature of expression in plants allowed major, perhaps decisive, savings in downstream formulation of the biologic.

To exert its therapeutic potential, GCD has to be taken up by affected macrophages, a process mediated by interactions between terminal mannose residues on the enzyme's glycans and the mannose receptors on the target cells' surface. However, GCD secreted by mammalian cells normally does not have exposed mannose residues and extensive and expensive post-production processes are required as part of its formulation. In contrast, the plant-cell expressed and vacuole-targeted ELELYSO naturally carries high-mannose glycans (Tekoah et al. 2013). Fortuitously, plant glycosylation, typically counted as a disadvantage of the technology, results in a "bio-better" product, which can be offered at a 25% lower price per dose without affecting profit margins (Aggarwal 2012), providing a clear advantage over competing mammalian cell-derived products.

By 2011, Protalix, relying on the medical community's 15 years of prior experience with a comparable, albeit expensive biologic, was able to present an attractive alternative - a plant-produced biosimilar. In close-spaced succession of publications, Protalix demonstrated efficient plant-cell production (Shaaltiel et al. 2007), biochemical and biophysical equivalency between the biologic and its biosimilar (Brumshtein et al. 2007; Shaaltiel et al. 2007), established its safety in preclinical and phase I clinical trials (Aviezer et al. 2009), and determined its efficacy through double-blind phase III clinical trial (Zimran et al. 2011)

Although FDA approval seems likely to have been eventually granted to plant-derived GCD based on these data alone, a serendipitous turn of events probably allowed it to happen faster. In late 2009, the manufacturer of Cerezyme, Genzyme (Boston, MA), reported viral contamination of its production facility. This triggered a warning letter by the FDA, and significant world-wide shortages in supply of the enzyme (Aggarwal 2009). To meet the demand, FDA called upon Protalix to increase production and to provide patients with the drug under an expanded access program (Aggarwal 2012). The ability of Protalix to step up to the plate and the positive responses from patients undoubtedly greased the regulatory cogs leading to the subsequent approval of the drug. Moreover, these events provided Protalix with an easy access to the market previously dominated by Genzyme with groundbreaking deals with the pharmaceutical giant Pfizer and the Brazilian government (Ratner 2010; Reisch 2013). To quote Pasteur, "la chance ne sourit qu'aux esprits prepares" ("Chance favors the prepared mind").

Considering the regulatory flexibility exercised by the FDA (and by similar agencies outside of the US) in regard to marketing approvals where orphan drugs are concerned (Bashaw et al. 2011), approval of plant-derived GCD seems a low-hanging fruit. In fact, a similar breakthrough came very recently with the emergency approval of a plant-derived monoclonal antibody cocktail to save the lives of two gravely ill physicians who contracted Ebola virus while treating patients in Africa (Bishop 2015; McCarthy 2014; Qiu et al. 2014; Rybicki 2014). While remaining anecdotal at this point (Rubin and Baden 2014), it is likely that the very near future will see expansion of this experimental treatment for this extremely lethal viral infection. In contrast, a much higher regulatory bar (e.g. larger scale phase III clinical trials) is set for therapeutics intended for chronic use by a larger cadre of patients

and even a higher bar is set when a prophylactic drug (e.g. a candidate vaccine) is considered as compared to treatment of sick patients. Nonetheless, it took the foresight and ingenuity of the scientists and businessmen of Protalix to tempt a reluctant industry and cautious regulators to have a first bite into this fruit of knowledge and life. With approval of the first plant-derived biologic we hope that a major hurdle was cleared and the fruits of plant biotechnology will no longer be forbidden.

Acknowledgments

TSM's research was funded in part by Work was supported in part by the National Institute for Drug Abuse Program Grant P1 DA031340 awarded to the Mayo Clinic and subcontracted to ASU and in part by the National Institute for General Medical Sciences U54 GM094599.

Glossary Box

Glucocerebrosides	are a group of membrane sphingolipids in which the glucose constitutes the polar head group linked through a glycosidic bond to the hydrophobic ceramide tail. Sphingolipids together with the more abundant phospholipids and cholesterol, are the major constituents of mammalian biological membranes (Ferraz et al. 2014; Platt 2014)
Human β-glucocerebrosidase (GCD)	is a lysosomal enzyme hydrolyzes the β-glycosidic linkage between the glucose and the ceramide. Mutations that make the enzyme inactive result in abnormal accumulation of glucocerebrosides in various tissues leading to Gaucher's Disease
Taliglucerase alfa (ELELYSO)	a recombinant form of GCD produced in carrot cell cultures. "ELELYSO" is the pharmaceutical brand name under which it is sold
Gaucher's Disease	is a rare genetic disorder, which is nonetheless the most common type of lysosomal storage disease. The disease is manifested in individuals that carry two mutant alleles of the gene encoding GCD and is more common in certain ethnic groups (e.g. the incidence among Ashkenazi Jews is estimated to be 1:800 compared to 1:100,000 among the general population, Ferraz et al. 2014; Platt 2014)
Rare (orphan) disease	refers to "diseases and disorders […] which affect small patient populations, typically populations smaller than 200,000 individuals in the United States" (*Rare Diseases Act of 2002*, Public Law 107–280). By today's demographics the term corresponds to prevalence of less than 0.07% (or less than 1 person in 1,500 people)
Biologics	are medical intervention products composed of sugars, proteins, or nucleic acids (often in complex combinations) that are enriched and purified from a variety of natural and

biotechnological sources. Biologics include vaccines, blood and blood components, gene therapy agent recombinant therapeutic proteins, living cells and tissues (http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048341.htm)

Biosimilars are defined in the *Patient Protection and Affordable Care Act* (Public Law 111–148) under the part of the law known as the *Biologics Price Competition and Innovation Act* (Sections 7001–7003 of the above). These sections create a simplified licensure pathway for biological products that are demonstrated to be "biosimilar" to or "interchangeable" with an FDA-licensed biological product. For all practical purposes it is a "generic version" of a "brand-name" biologic

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