

# The New Albumin-Free Recombinant Factor VIII Concentrates for Treatment of Hemophilia: Do They Represent an Actual Incremental Improvement?

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## Abstract

The goal of eliminating the low levels of infectious disease risk from hemophilia treatment has resulted in the development of multiple generations of recombinant factor VIII (rFVIII) products. The ideal product should be devoid of human and animal proteins, which may transmit infectious agents. These products should also maintain molecular integrity, hemostatic efficacy, similar immunogenicity, and acceptable side effect profiles as compared to plasma-derived factor VIII. Currently available first-, second-, and third-generation rFVIII products include Recombinate; Kogenate FS/Helixate FS and ReFacto; and Advate, respectively. During the evolution of rFVIII products, either full-length or B-domain-deleted factor VIII were transfected into immortalized cell lines. The B-domain-deleted product, ReFacto, has resulted in an additional method to monitor factor VIII levels. The third-generation products offer the theoretical advantage of being produced without human and/or animal proteins. Upon initial introduction into the marketplace, the newer products have a higher cost. However, when analyzing historical trends, the prices of these products are almost equivalent to first-generation products within 3 years of licensure. Thus, the initial cost of the product may be a minimal issue in the medical decision process when selecting rFVIII replacement therapy.

## Introduction

The desire within the hemophilia community to eliminate low levels of residual infectious disease risk, with an ultimate goal of "zero risk" in newly created recombinant factor VIII (rFVIII) products, has driven the "recombinant revolution"<sup>1</sup> in the factor VIII (FVIII) pharmaceutical industry. The stimulus that fuels this high level safety standard emanates from plasma product contamination first seriously noted in the early 1980s with human immunodeficiency virus (HIV) and in later years with other viruses, such as hepatitis C (HCV) and hepatitis B (HBV).<sup>2</sup> The tragedy unleashed by pathogen transmission was fully appreciated by the hemophilia community in the mid-1980s as greater than 75% of patients with hemophilia were HIV positive.<sup>3</sup> Furthermore, by the early 1990s, approximately 74–90% of patients with hemophilia were infected with HCV.<sup>4</sup> Consequently, in order to obtain "zero risk" rFVIII products, these products must be devoid of human and animal proteins that may transmit a known, or unknown, infectious agent while maintaining molecular integrity, hemostatic efficacy, similar immunogenicity, and acceptable side effect profiles as compared to plasma products. This article will address differences between rFVIII concentrates, specifically focusing on safety, clinical effectiveness, cost, and product availability, as these issues influence the choice of selecting FVIII replacement therapy.

## Recombinant FVIII Product Development History

The "recombinant revolution"<sup>1</sup> resulted from the need to manufacture safer FVIII products. Prior to this change and as early as 1840, FVIII produced from blood-derived sources had been the mainstay of FVIII replacement treatment. Lane reported that a whole-blood transfusion had saved the life of a 12-year-old boy with hemophilia A by halting post-surgical bleeding.<sup>6</sup> In the modern era, Dr. Judith Graham Pool (1964) and her colleagues discovered that the precipitate of fresh frozen plasma yielded high concentrations of FVIII.<sup>5</sup> This product is known as cryoprecipitate. For the first time in hemophilia treatment it was possible to achieve FVIII levels approaching 30%, which is the approximate level necessary to obtain normal hemostasis. The cryoprecipitate provided greater than 80 U of FVIII per liter of infused volume. Thus, blood banks nationwide were able to offer effective treatment for hemophilia A. These improvements in the management of hemophilia led to dramatic increases in patient life expectancy.<sup>2,3,6,7</sup>

## Keywords

Hemophilia A, recombinant factor VIII (rFVIII), Advate, Refacto AF, cost-analysis, treatment decisions

Another transition point in hemophilia therapy occurred in the early 1970s when it was discovered that by pooling thousands of plasma donations, plasma concentrates of high-potency dried replacement factors (as high as 100 U/mL) could be produced. This preparative change promoted self-administration and home treatment which revolutionized the treatment of hemophilia and permitted many patients to live a near normal lifestyle. The first antihemophilic factor (AHF) licensed was Hemofil in the late 1960s. However, the result of pooling plasma donations from approximately 25,000 donors to obtain 1 lot of FVIII concentrate escalated the transmission of blood-borne infections, especially viral contaminants such as HBV, HCV, and HIV.<sup>2</sup>

As a result, the first attempt in a long series of innovations to reduce contamination in FVIII concentrates started in the mid 1980s. Heat treatment of plasma concentrates was introduced in 1984, followed by enhanced viral inactivation techniques and expanded donor screening in the late 1980s.<sup>8</sup>

During this period (1984) FVIII was cloned, which proved to be a pivotal scientific achievement that changed the FVIII concentrate industry. This breakthrough, coupled with recombinant DNA technology, provided the fuel to ignite the “recombinant revolution.”<sup>11</sup> By the early 1990s, the first generation of human rFVIII agents were licensed (Recombinate, Kogenate/Helixate). In the new millennium, a second generation of rFVIII products with enhanced purity were developed. In producing Kogenate FS/Helixate FS and ReFacto, all licensed in 2000, human albumin is eliminated from the last stages of formulation. Today, a third generation of rFVIII products exists that are devoid of animal and human protein additions in the cell culture. Advate (2003) is the only currently licensed third-generation FVIII product and is a plasma/albumin-free product.<sup>9,10</sup> Refacto AF is another third-generation rFVIII product, but is currently in the clinical trial phase of development.<sup>11,12</sup> See Table 1 for comparative summary of first- through third-generation products.

## Recombinant FVIII Manufacturing

Recombinant FVIII, first introduced in the early 1990s, significantly reduced the risk for pathogen transmission. Good manufacturing processes are intricate and complex and, thus, must be rigorously monitored to ensure safety. The FVIII gene is one of the largest proteins to be expressed for therapeutic purposes.<sup>13</sup> The gene has been expressed in full length or with the B-domain deleted (ReFacto and Refacto AF). Once the gene is selected for transfection it is spliced into a vector during the first part of recombinant product processing. The vector is then inserted into Chinese hamster ovary (CHO) or baby hamster kidney (BHK) cells.<sup>14-16</sup> An inoculum, meeting strict criteria of viability, sterility, and growth rate, is then fermented in a bioreactor using a batch-refeed process. In culture, the cells then secrete rFVIII into the medium, which is harvested. The rFVIII-rich media undergoes a stepwise purification process (usually unique to the manufacturer), but generally includes multiple chromatography columns. The purification processes are designed to isolate rFVIII and remove or inactivate any remaining contaminants. The bulk processing is designed to ensure consistency in the quantitative and qualitative composition of all preparations. Single-dose vials are then prepared aseptically, and the liquid is lyophilized to produce a powder which can be reconstituted with a diluent prior to administration.<sup>17</sup>

Recombinate, ReFacto products, and Advate are produced in CHO cell lines, whereas Helixate/Kogenate and Helixate FS/Kogenate FS are genetically engineered in a BHK cell line.<sup>9-12</sup> Furthermore, Recombinate and Kogenate are full-length rFVIII agents, whereas ReFacto products are B-domain deleted. Initially, the B-domain was believed to have an insignificant function; however, *in vitro* it has been shown to interact with chaperone proteins that have a processing role with ultracellular structures, although this remains to be determined definitively.<sup>18</sup> This might impact the production of rFVIII, but should not influence its function *in vivo*.

**Table 1.** Commercial Recombinant Factor VIII (rFVIII) Products

rFVIII Agent	Product Generation	Producing Cell Line	FVIII Molecule	FVIII Stabilizer	Virus Removal and Inactivation Methods
Recombinate	First	CHO	Full-length	Human albumin	Immunoaffinity, ion exchange
Kogenate/ Helixate	First	BHK	Full-length	Human albumin	Immunoaffinity, ion exchange, ultrafiltration
ReFacto	Second	CHO	B-domain-deleted	Sucrose	Immunoaffinity, ion exchange, S/D, nanofiltration
Kogenate FS/ Helixate FS	Second	BHK	Full-length	Sucrose	Immunoaffinity, ion exchange, S/D, ultrafiltration
Advate	Third	CHO	Full-length	Trehalose	Immunoaffinity, ion exchange, S/D and plasma/albumin-free culture medium

CHO=chinese hamster ovary; BHK=baby hamster kidney; S/D=solvent/detergent.

### Attenuation of Infectious Risk

The product stabilizer is the major distinction between first- and second-generation products. The first-generation products, Recombinate and Kogenate/Helixate, are stabilized with human albumin. However, sucrose is used rather than albumin in the final formulation of Kogenate FS/Helixate FS and ReFacto. This modification of the manufacturing process significantly reduces the risk for viral contamination. However, human plasma protein solution (Helixate FS/Kogenate FS) and human serum containing albumin (ReFacto) are still used in the cell culture process but are removed prior to the final formulation.

Viral removal and inactivation methods involve immunoaffinity and ion exchange chromatography for all of the rFVIII concentrates. In addition, the production of second-generation products uses a solvent/detergent (S/D) and either ultra- or nanomolar filtration to reduce transmission of enveloped and non-enveloped viruses.<sup>1,19</sup> The filtration steps are the most effective way to eliminate the small, non-enveloped viruses such as hepatitis A and parvovirus B19.<sup>1</sup> Specifically, Kogenate/Helixate use ultrafiltration; Kogenate FS uses S/D and ultrafiltration; and ReFacto uses S/D and nanomolar filtration.

These purification technologies have essentially eliminated the contamination of plasma-derived concentrates and rFVIII products with lipid-enveloped viruses such as HIV, HBV, and HCV.<sup>20</sup> By utilizing these processes and improving the screening of donors, the total number of hemophilia A-associated deaths decreased 40% between 1995 and 1998, and the number of HIV-related deaths in the hemophilia population decreased 78% between 1995 and 1998.<sup>21</sup> Moreover, between 1987 and 1990 only 37 cases of HIV seroconversion were identified in hemophilia patients. More importantly, none of these patients had been treated with currently available plasma-derived or recombinant factor products.<sup>22</sup> Since 1990, there has not been a case of HIV seroconversion in this patient population due to these newer products.

The third-generation products offer the advantage of being manufactured with a medium that is free of both human albumin and plasma. Therefore, these products do not have contact with human proteins during the manufacturing process. However, Advate utilizes mouse monoclonal antibodies in the immunoaffinity chromatography stage.<sup>9</sup> In comparison, ReFacto AF is purified using a novel synthetic affinity ligand and, thus, has both an animal and a human protein-free manufacturing process.<sup>11</sup>

Newer products have attempted to eliminate human albumin and plasma as well as animal proteins from the manufacturing process due to the theoretical risk that these may transmit a potentially harmful pathogen; however, over the past 50 years, human albumin has been used as a volume expander. There has yet to be a proven case of pathogen transmission associated with albumin administration. This may be due to the production process of ethanol separation and pasteurization, to limited long-term observations of exposed

patients, or to a lack of surveillance resulting in underreporting. Despite these advances, apprehension still exists as prions, such as variant Creutzfeldt-Jakob disease, emerge as a threat to hemophilia patients and other patients using blood and blood derivatives. Immunoaffinity and other chromatography techniques for preventing transmission of prions, are not well defined and utilizing pasteurization and S/D are probably ineffective at preventing the transmission of these types of pathogens.<sup>19,23</sup> In addition, other pathogens, such as parvovirus B19, have been found to be resistant to many purification measures and, thus, there may exist an unidentified, more virulent, small, non-lipid-enveloped virus that could be transmitted even by highly purified concentrates.

### First- and Second-Generation rFVIII Products

Over the past decade, prospective, multi-center trials on previously untreated patients (PUPs) with first- and second-generation rFVIII products have been conducted. Much information exists with regard to safety, efficacy, and inhibitor development. Recombinate, Kogenate, ReFacto, and Kogenate FS have been proven to be effective and exhibit a very low side effect profile.<sup>24</sup> All 4 products' mean plasma recovery in percent IU/kg, mean half-life, and mean dose IU/kg are comparable.<sup>25-28</sup>

Hemostatic efficacy among the various rFVIII concentrates was good to excellent in a previously treated patient (PTP) population. A good-to-excellent response was achieved in 91% of patients treated with Recombinate. Resolution with 1 infusion was reported for 82%, 71%, and 80% of bleeding episodes treated with Kogenate, ReFacto, and Kogenate FS, respectively.<sup>25-28</sup> Hemostatic efficacy was also demonstrated in a PUP population. Almost 90% of bleeding episodes resolved with 1 or 2 infusions in those treated with Recombinate, Kogenate, or Kogenate FS. A slightly smaller percentage of bleeding episodes resolved with 1 or 2 infusions in PUPs treated with ReFacto.<sup>25,26,30</sup>

Although the rFVIII concentrates are all effective, their use has not ameliorated the development of inhibitors that is associated with all FVIII products in a subset of hemophilia A patients.

In the multinational PUP trials with Kogenate, Recombinate, and ReFacto, inhibitors developed in 30%, 31%, and 33%, respectively, of severely affected PUPs. Inhibitors were transient in half of patients who developed inhibitors. However, the Kogenate FS trial had insufficient data to assess the incidence of inhibitors.<sup>24</sup>

### Third-Generation rFVIII Concentrates

Currently, Advate is the only licensed third-generation rFVIII product. At the 45th annual meeting of the American Society of Hematology (ASH), the safety, immunogenicity, and efficacy data were presented from a PTP population in a global clinical program made up of 1 completed and 3 ongoing studies. The median age of recipients was 18 years ranging from 1–65 years with 10% under 6 years, 42% between 6 and 18 years, and 48% greater than 18 years. Severely FVIII-deficient patients with levels less than 1% represented approximately 93% of individuals enrolled. In the completed PTP study,

108 patients received Advate for a median of 117 exposure days. In comparing Advate and Recombinate, pharmacokinetic parameters were similar, with mean half-lives of 12.0 and 11.2 hours, and adjusted recoveries of 2.4 and 2.6 IU/dL per IU/kg, respectively. In addition the products were bioequivalent. Advate was also shown to be as effective. In the 510 bleeding episodes, 86% were rated as having an excellent or good response and 93% were managed with 1 or 2 infusions. In an ongoing study, pharmacokinetic parameters for Advate in 14 pediatric PTPs were equivalent with those noted previously. During surgical procedures Advate was effective. Factor was administered as a bolus in 27 individuals and as a continuous infusion in 17 individuals who underwent 44 surgical procedures. Hemostatic efficacy intraoperatively and postoperatively was rated as excellent/good in 98% and 100% of surgical procedures, respectively. The safety profile in all studies have revealed no deaths or serious adverse events. However, the subjects with greater than 74 exposure days had a risk of developing an inhibitor to FVIII between 0.02% and 5.4%. Thus, Advate is bioequivalent to other rFVIII concentrates, does not carry an increased risk of inhibitor development, and is safe and efficacious for FVIII replacement therapy in many clinical settings for patients with hemophilia A.<sup>9,10</sup>

ReFacto AF is the other third-generation rFVIII product and is currently undergoing clinical trials. At the 45th annual ASH meeting, safety, immunogenicity, and efficacy data of ReFacto AF in an open-label study were presented. ReFacto AF was given for treatment and for prevention of bleeding in PTP population with severe FVIII deficiency, defined as FVIII level less than or equal to 2%. Patients with over 50 exposure days received prophylaxis at least twice a week. Twenty patients completed the study and 2 discontinued. The median dose for prophylaxis was 34 IU/kg with a range from 15.8 to 77.7. Eight-six percent of patients were dosed at least 3 times per week. Bleeding episodes had a median annualized rate of 6.6 bleeds/patient/year. Ninety-seven percent of the investigators assessed the prophylaxis efficacy as "very useful" and "useful." Seventy-nine percent of the bleeding episodes (spontaneous and traumatic) resolved with 1 or 2 treatments. Only 1 patient developed a new FVIII inhibitor after 35 exposure days, with a maximum Bethesda inhibitor assay result of 4–9 BU. However, this patient was found to have low-titer inhibitor history prior to study entrance, which was an exclusion criteria in the study protocol. As ReFacto AF is produced in hamster cell line, no hamster or synthetic affinity ligand antibodies were detected in any patients. This interim data on 22 patients reveals a low incidence of breakthrough bleeds as compared to other rFVIII products. The efficacy of ReFacto AF appears to remain consistent over time, and is efficacious for joint and soft tissue/muscle type bleeding injuries. However, as only a limited number of patients have been monitored, the study is ongoing.<sup>11</sup> In addition, data has shown ReFacto and ReFacto AF have comparable pharmacokinetics with regard to bioequivalence and recovery levels.<sup>12</sup>

## FVIII Activity Measurements

There are 3 types of FVIII assays available to measure FVIII activity. Clinicians treating hemophilia A patients must be aware of the assay(s) that are available to them at their clinical laboratory when making rFVIII concentrate therapeutic choices. The 1-stage assay measures the shortening of the clotting time of hemophilia A plasma when a test reagent is added to a system containing activated partial thromboplastin. It is the most widely accepted assay in clinical laboratories for measuring FVIII potency in therapeutic FVIII products when determining recovery of activity in patients and dosing of product.<sup>31</sup> Another method for measuring FVIII activity is the 2-stage clotting assay, which was developed in the United Kingdom but is seldom used today.<sup>32</sup> The third is a chromogenic assay which consist of 2 stages, and is thought to be a descendant of the 2-stage FVIII clotting assay.<sup>31</sup> The first stage of this assay involves mixing together bovine factor IXa, factor X, and thrombin in the presence of phospholipids and then adding the test substance. Activated FVIII then becomes a part of the intrinsic factor Xase complex, causing the activation of factor X. The second stage consists of adding a chromogenic substrate specific for factor Xa yielding an absorbance that can be measured. The chromogenic substrate assay was used as the standard measurement during clinical trials when ReFacto was developed. The majority of laboratories in the United States use the 1-stage assay. All products can be measured with the 1-stage clotting assay except when ReFacto is tested in this system, the expected measured potency of recombinant factor VIII is 20–50% lower. Currently, ReFacto therapy can be accurately monitored by a 1-stage clotting assay as long as a ReFacto FVIII standard is utilized. This has allowed laboratories in the United States to monitor patients on ReFacto who do not routinely use a chromogenic assay.

The ReFacto laboratory standard used in a chromogenic substrate assay elicits much interlaboratory variation around the world. A multicenter collaborative study performed in Europe by the Danish Medicines Agency found that laboratories using a longer factor Xa generation time resulted in higher ReFacto potencies when assessed against a non-product-specific standard.<sup>33</sup> Using the potency derived from this study, Wyeth (the manufacturer of ReFacto), recalibrated the potency of the in-house standard which resulted in an approximate 20% increase in the quantity of ReFacto protein added to each vial. This was done to bring the Wyeth standard and independent clinical laboratories' chromogenic substrate assay values into closer alignment and to modulate interlaboratory variation. This new standard has been approved by the FDA and was introduced to laboratories around the world in the summer of 2003.

Laboratories were notified by Wyeth that artificially high results may occur if the ReFacto Laboratory Standard (prior to reformulation) is used to monitor FVIII activity levels in patients receiving ReFacto calibrated with the new standard. With the newly recalibrated ReFacto product, the results of the 1-stage clotting assay should be closer to the chromogenic assay.

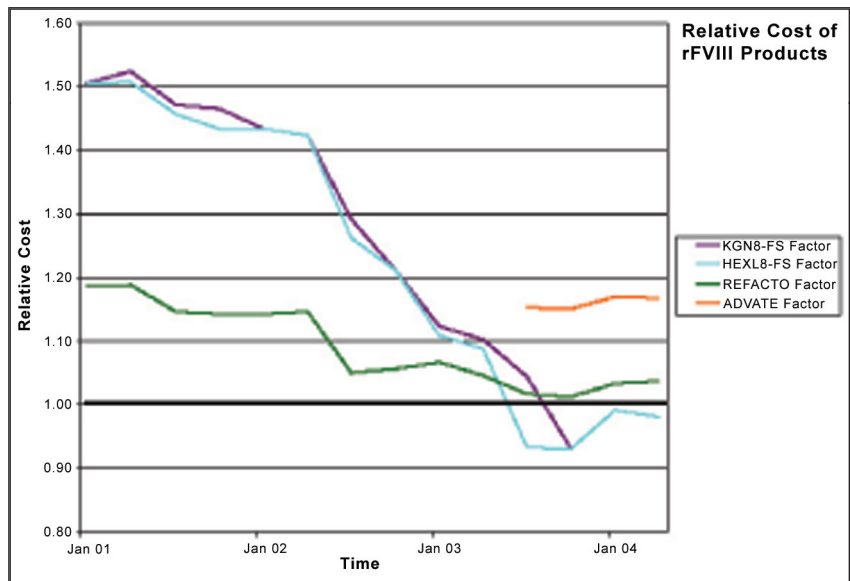
Even though there is 20% more ReFacto protein in each international unit due to the recalibration, the price has remained the same.<sup>34</sup>

### Recombinant FVIII Cost and Availability

Shortages of rFVIII placed an incredible strain on patients and physicians treating hemophilia A. Over a 2-year period between 2000 and 2002, the worldwide hemophilia community was confronted with a shortage of products.<sup>35,36</sup>

This shortage of rFVIII appears to be resolved. However, the possibility of a fluctuating supply of rFVIII in the future should be considered. End user utilization impacts the suppliers' decisions about which products to continue to manufacture and affects the wholesale product price. This is evident for the price of first-generation products (Recombinate at average wholesale price [AWP] of \$1.33/unit), as well as second-generation products (Kogenate FS at AWP of \$1.41/unit, Helixate FS at AWP of \$1.38/unit, and ReFacto at AWP of \$1.36/unit), and the third-generation product (Advate at AWP of \$1.66/unit).<sup>37</sup>

The first-generation pricing is negligibly different (3–8 cents) from second-generation concentrates depending on which product is being directly compared. The second-generation products have only been available over the past 2–3 years, whereas the first-generation products have been around since the early 1990s. Many patients and their families, as well as those who treat hemophilia A, are concerned with the rising costs of healthcare and the restrictions on reimbursements enforced by insurance companies. When a new rFVIII product with similar clinical efficacy and side effect profile, and a theoretical increase in safety margin is introduced into the market, physicians and insurance companies must decide if the increased cost is balanced by a significant improvement in care or reduction in risk. However, it is interesting to note that many places in the United States that are affiliated with hemophilia centers are eligible for public health service (PHS) pricing which is lower than the AWP. Over the last 3–4 years, rFVIII prices have decreased as new rFVIII products have been introduced into the marketplace. Figure 1 shows the relative costs of Kogenate FS, Helixate FS, ReFacto, and Advate in comparison to Recombinate over time. If the relative cost of Recombinate at any given time is 1.0, then during a 3-year period, the relative costs of Kogenate FS and Helixate FS have decreased from 1.5 times that of Recombinate (50% more expensive) to near the cost of Recombinate (equivalent cost). In addition, the cost of ReFacto has similarly decreased from 1.2 times (20% higher than) the cost of Recombinate to nearly the same cost. Advate has only



**Figure 1.** The relative cost of Recombinate is 1.0. This figure shows over time the relative cost, based on Public Health Service Pricing, of Kogenate FS (KGN8-FS), Helixate FS (HELXL8-FS), ReFacto, and Advate compared to Recombinate.

been available for the last 7 months and is currently 1.15 times the cost of Recombinate.<sup>38</sup> If the historical trend of decreasing relative cost illustrated by the second-generation products can be applied to Advate, then the relative price for Advate will decrease to approximately the current cost of Recombinate over a 2- to 3-year period. However, this may occur with a shorter time span depending upon the potential release date of ReFacto AF, another third-generation concentrate with similar advantages.

### Conclusions

The safest possible rFVIII concentrates with the least probable chance of transmitting infectious pathogens continue to be made available for clinical use. Third-generation rFVIII product development has answered many of the concerns of the hemophilia community and of the recommendations of the Medical and Scientific Advisory Council of the National Hemophilia Foundation that manufacturers of the recombinant products should attempt to avoid using human and animal proteins in manufacturing their products in order to reduce the risk of pathogen transmission.<sup>39</sup> However, the current process will never create a “zero risk” because the product is produced in a biologic system with live cell cultures and animal protein and can thus be contaminated with potentially infectious and unidentified pathogens. Additionally, human error may introduce infectious agents into the system. Nevertheless, the safety of FVIII replacement has come a long way over the last 20 years. All newer products should continue to have comparable pharmacokinetics, efficacy, and immunogenicity, but should carry a theoretically improved safety profile in regards to pathogen transmission.

When integrating cost into the decision, the amount of factor required per patient should be considered with these newer

products during their initial introduction into the marketplace. One strategy could be that newly diagnosed children, with low body weight and who are naive to product, may be started on the newest concentrates since the initial overall cost/dose will be substantially less as compared to larger children, adolescent, and adult patients. Furthermore, if the historical trends hold true, then 2–3 years after its release and potentially sooner, Advate and newer products will meet the cost of other products, removing cost from the equation of medical decisions.

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