INTRODUCTION

This is a Botanical Review Team (BRT) Leader’s secondary review of NDA 202292, crofelemer 125 mg tablet for the treatment of HIV-related diarrhea. This application is the second NDA submitted for a botanical drug product¹ under the CDER’s Guidance for Industry: Botanical Drug Products (published in 2004, referred to as “Botanical Guidance” or “Guidance” in this memo)².

The BRT pharmacognosist, Dr. Jin-Hui Dou, has completed a primary review of this application, which was submitted to the NDA file in DARRTS on August 7, 2012. Compared with most botanical preparations in the INDs we have received, crofelemer is a relatively simple botanical derived from a single part of a single plant (latex of Croton lechleri, Euphorbiaceae), containing a class of chemical entities as the major active ingredients (proanthocyanidin - oligomers of catechins). From the BRT’s perspective, there is no issue that should preclude the approval of this NDA. Additional post-approval measures to further strengthen the quality control and to ensure therapeutic consistency of the marketing batches have been recommended in the primary botanical review.

As the new drug divisions in OND are getting familiar with the Botanical Guidance and feel more comfortable handling initial IND submissions of botanical products in the past few years³, late phase development of botanical new drugs remains a rarity and review experience with botanical NDA is limited. As many of the NDA issues unique to botanical products have just emerged with these marketing applications (b) (4) our approaches to these specific regulatory concerns are still

¹ Besides a few OTC monographs that contain botanical ingredient(s), one NDA has been submitted and approved for a new botanical prescription drug in 2006 (NDA-20902, Veregen cream for topical treatment of genital warts).
² For the Botanical Guidance, see www.fda.gov/cder/guidance/4592fnl.htm.
³ with an accumulated total of over botanical INDs in OND divisions as of June 30, 2012.
evolving\textsuperscript{4}. Since we are now setting precedents with the first few botanical NDAs that may affect future applications, it is important to delineate our reasoning for resolving these issues and to document how we arrived at the position for the current application.

**BOTANICAL GUIDANCE & RELATED POLICY ISSUES**

As the second botanical NDA, it helps to summarize again the Botanical Guidance of June 2004 and related policies, as we did in the review of the first botanical NDA. First of all, the Guidance provides that botanical drug products may remain as complex mixtures; both purification and identification of the active ingredients in botanicals are optional and not required. The quality consistency is therefore a more complicated issue than that of non-botanicals when potential active ingredients are numerous and not yet fully identified. The chemical specifications for botanicals are rarely as precise as that of non-botanical drugs and it is often difficult to determine how many of the chemical entities need to be controlled with appropriate analytical technologies (e.g., HPLC fingerprinting). The Guidance recommends that multiple analytical technologies be used to help address this issue. Each individual method may provide only part of the characteristics, but data from several different techniques, when viewed collectively, will usually give an adequate profile. Even so, the nature of botanical and technical limitation often make further chemical characterization very difficult, if not impossible.

To both industry and FDA chemistry review staff, the degree of chemical characterization required to meet the conventional interpretation of identity test often pose a scientific and regulatory challenge and occasionally the technical limit became a contentious issue. From BRT’s experience, attempt to require high degree molecular characterization will usually be futile – it would be technical impossible, financially prohibitive or clinically unnecessary. The strict reading of regulations, e.g., definitions of “identity”; “active ingredient”; “purity”, etc., from a pure small molecule drug perspective appears to be the cause of the concern about inadequate chemical characterization. The interpretation of such regulations therefore needs to be extended from a narrow, chemical mindset to accommodate the complex nature of botanical drug substance. For example, the “identity” of botanicals must include, in addition to the standard chemical analyses, the source of raw materials and other non-CMC data – e.g., identification of species, geographic location of harvesting, processing, and bioassay, if available.

It is thus clear that conventional CMC measures may not be adequate to ensure therapeutic consistency and in the current Guidance the control of botanical drug substance and product is extended to “pre-CMC” steps, i.e., to cover raw material and the good agricultural & collection practice (GACP) of growing/harvesting medicinal plants. At the other end, clinically relevant bioassay and clinical experiences with various doses

\textsuperscript{4} Reference ID: 3172876
or batches may provide the additional support for quality/therapeutic consistency (the “post-CMC” approaches). For approval of this NDA, it is imperative to consider all these data (pre-CMC, CMC, and post-CMC) together to determine whether therapeutic consistency of the marketing batches can be ensured.

The Guidance also stipulates that because many botanicals have been used as medicine in alternative medical systems (e.g., traditional Chinese medicine and Indian Ayurvedic medicine) for long time, the prior human experiences may substitute for animal toxicology studies in the preliminary safety evaluation of IND studies. How these human data, mostly not of modern scientific quality, can be useful to support an NDA application was not clearly described in the Guidance. Instead, conventional non-clinical studies were suggested for the later phase development of most botanical drugs. For this NDA, the sponsor has conducted the full battery of non-clinical toxicity studies and did not request exemption from such requirements because of long history of human consumption (see pharmacology review).

For clinical data to support marketing approval, there should be no difference between botanical and non-botanical drugs. Aside from the concern of therapeutic consistency for marketing batches (see below), there is no botanical specific issue in the clinical development of this product.

Since clinical effects of medicinal plants are usually known from extensive human therapeutic use, most of the well-known botanical products have attracted intensive research interest. Many botanical products are thus not developed completely de novo, with abundant existing knowledge outside of the NDA package. Such information, ranged from chemical characterization to pharmacological studies and clinical experience, may help with new drug development and regulatory review. They should not be dismissed casually. In this NDA, extensive human use of crofelemer to treat diarrhea in central and South America gives us some hint that the therapeutic effect must be fairly consistent with the crude local preparations (see more discussion below). Another example is if publications from experts in the field have reported technical limitation in chemical characterization of crofelemer, imposing regulatory requirements beyond the capability of current technology may not be practical (see “Characterization of Botanical Drug Substance” below).

In the current Guidance, it is noted that since botanicals are all mixtures of different chemical entities, the Agency is revising the policy on combination drug products to facilitate development of botanical new drugs. While many details of the revision remain to be finalized, it is clear that natural mixtures in a single part of a single plants will not be considered as a fixed combination drug product and thus not subject to

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5 These “post-CMC” measures are not covered in the current Botanical Guidance, see also Footnote 4 and Scope of Botanical NDA Review below,
the combination requirement. Combination drug product is thus not an issue for this NDA (latex of a single plant species).

**THE SCOPE OF BOTANICAL NDA REVIEW**

To implement the Botanical Guidance, a new review discipline in the OND was established, and a dedicated review team was assembled to conduct the following botanical review 6:

i) **Biology of the medicinal plants** – identification, potential misuse of related species

ii) **Pharmacology of the medicinal plants** – activity/toxicity in old literature and new testings

iii) **Prior human experiences with the botanical drug substance** – past clinical use and relevance to current setting

iv) **Assurance of therapeutic consistency of marketing batches**

As noted above, the botanical new drugs can rarely have CMC specifications as precise as that of pure chemical drugs. Without the same degree of CMC control as for pure drugs, one critical question for approval of botanical drugs is whether the future marketing batches will have the same therapeutic effects as that observed in clinical trials. This is especially difficult for botanical drugs with unknown number and identities of the active ingredients. We have proposed that one or more of the following be provided to address this unique botanical review issue not yet covered in the current Guidance 7:

a) **Pre-CMC control**
   - Identification of medicinal plant and potential risk of misuse
   - Implementation of GACP.
   - Controls in process for botanical raw material (BRM) will be emphasized
   - cGMP should start at the raw material level

b) **Conventional CMC**
   - Standard CMC characterization of BDS remains the most important method to ensure quality and therapeutic consistency. Analyses using multiple analytical chemical techniques should be conducted as extensively as the technology and practical consideration allow.
   - As for BRM, Controls in processes will be emphasized

c) **Post-CMC Approaches**
   - *Clinically relevant bioassay*

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6 See also the CDER Manual of Policy and Procedure (MaPP) 6007.1.

7 These approaches were presented for discussion at the regulatory briefing of last botanical NDA on September 15, 2006; no objection was raised by the CDER/OND leadership. See also publication by the BRT in *Nature Biotechnology*, October 2008, 26:1077
For botanicals with numerous possibly active ingredients, a clinically relevant bioassay will facilitate the new drug development and ensure quality control in post-approval manufacturing.

- **Sensitivity of Clinical response to dose**
  If dose-response studies showed that clinical effects are not sensitive to dose (flat dose-response), then the batch variations in CMC specifications (but still within the acceptable range) and other uncontrollable uncertainties may be more tolerable.

- **Clinical experience with multiple-batch production**
  Difference in clinical effects of various batches (representative of those within the acceptable ranges of specifications) can be tested in phase 3 studies, similar in concept to that of “multiple-center” trial. A negative “treatment-by-batch” interaction will provide some assurance that therapeutic effects will not be affected by batch-to-batch variations.

Anecdotal claim of benefits in a large population for a long period of time may not be considered as a primary evidence of efficacy. But for symptomatic relief that can be self-evaluated and well-documented, such experience suggests that therapeutic effects are fairly consistent and may not need stringent quality control as are required for non-botanical pharmaceuticals. This could provide some secondary support for therapeutic consistency.

- **Post-marketing confirmation trial**
  Sometime after approval, efficacy trials can be repeated to re-confirm the efficacy of future marketing batches. However, it may not be ethical or feasible to repeat the efficacy studies for certain indications.

These are suggested options and we don’t believe it is necessary for a product to meet every one of these requirements. Available evidence from each of the above panel should be considered together with other information in the overall context. For example, in cases with strong assurance from non-CMC data, the chemical control requirements may be adjusted accordingly. Again, the ultimate objective is consistency in clinical response, chemical quality control is one of the important means, but it is not the end by itself.

**BOTANICAL ISSUES IN THIS NDA**

**Plant Biology of Croton lechleri, Euphorbiaceae**

The biology of *C. lechleri* as a medicinal plant has been described in detail by the BRT’s Dr Dou in his primary review. Since the plant can be easily identified by trained workers because of its distinctive morphology, there is little risk of confusion with similar but incorrect species in the natural habitat. The relatively short list of synonyms (see Section 3.1 of Dr. Dou’s review) also suggests that the taxonomy of this species is relatively straightforward and currently without much controversy or confusion with regard to other similar Croton species. In addition, it is well-known that latex is usually
less variable than other parts of the plant such as the leaf. We recommend that harvesting from the wild growth be restricted to the Eco-Geographic Regions (EGRs), and addition of other EGRs in the future should be evaluated first if the new BRM and BDS meet the specification and bioassay, if available. The applicant has implemented GACP compliance and will develop measures to ensure the forest renewability. These considerations indicate that the variability at the plant and raw material level will be acceptable.

**Characterization of the Botanical Drug Substance (BDS)**

As described by Dr. Dou in his review, the BDS of crofelemer is a partially purified crude plant latex (CPL) of *C. techleri*, containing mainly a mixture of procyanidin oligomers with average molecular weights of 1700-2500 Daltons and average length of 7 monomer units. The oligomers consisted of 3 to 14 (up to 30-40) linearly linked monomers of 4 different catechin like compounds. The following analytical technologies have been applied to characterize the BDS:

- Gel Permeation Chromatography (GPC)
- HPLC with UV/Mass spectroscopy/fluorescent detection
- Acid hydrolysis of BDS for monomer composition
- Mass spectroscopy of BDS
- Proton and $^{13}$C NMR of BDS

The individual techniques and information analyzed are discussed in details in both the CMC and BRT primary reviews (see Section 4.4 of the latter for summary).

The most remarkable feature of the chromatographic data is analytical methods are rooted in the complex nature of the BDS which contains numerous isomers/analogs. Accurate quantification of such a complex botanical mixture is not practical, since it will require each molecule or each group of molecules to be isolated, purified with sufficient amount of pure material as reference standards.

As noted in Dr. Dou’s review, the applicant has submitted data using other techniques to provide details on the identities to compensate and complement the limited resolution on HPLC. The BRT consider these methods collectively have adequately characterized the BDS and the specifications thus established will be sufficient to ensure the correct identity of the active ingredient for crofelemer. Many experts (USDA & industry) have also commented on the complexity of the chromatographic data of

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8 The BRT did a more extensive review on BDS than usual because the CMC reviewers have reached a different conclusion on the adequacy of BDS characterization.
9 The CMC reviewers have required further separation of oligomers peaks of different length on HPLC, and more detail information on the percentages of oligomers of each length.
crofelemer like proanthocyanidin, which is due to interference between large number of oligomers (see References 22 & 25 of Dr. Dou’s review). This most likely represents the current state of the art of analytical technologies, and the limit of the capability to delineate the detail compositions of proanthocyanidin oligomers.

As discussed above, conventional CMC data are not expected to fully characterize the BDS at the level of each individual molecule in the complex mixture. For botanical drugs, we must reply on additional evidence outside of the conventional CMC measures for assurance of therapeutic consistency. The control of active ingredients in the BDS should take into consideration the control at raw material level and the GACP implementation (the “pre-CMC” measures). Analytical chemistry data should be viewed in a broader context for ensuring therapeutic consistency and the standard CMC profile should not be considered in isolation as an approvability issue (see below on Therapeutic Consistency).

It should also be pointed out that without any data to correlate clinical response with minor variations in chemical composition, the degree of characterization to be required for the BDS remains a conjecture. It raises the regulatory questions of whether additional data (e.g., further separation of HPLC peaks) is required for “identity” of the BDS and if the more detailed definition of “identity” is necessary for ensuring therapeutic consistency. Asking for more detail in analytical chemistry data without due regards of other non-CMC support for therapeutic consistency can be construed as an arbitrary regulatory burden. Such requirement for more details should be well-justified as an approvability issue for an effective treatment to manage a serious unmet medical need.

“Identity” of crofelemer as a drug

As noted above on Page 2, the degree of chemical characterization required depends on how the definition of “identity” is interpreted for the botanical active ingredient. Such definitions must include source of botanical raw materials and other non-CMC data to accommodate the nature of complex mixture. For this NDA, identification of species, geographic location of harvesting, processing, and the mechanistic study provide the non-CMC part of the identity test. Together with the chemical characterization the sponsor has performed on BDS, “identity” of crofelemer can reasonably be assured that the botanical drug will generate the intended clinical effects.

Prior Human Experiences with Croton lechleri

Existing knowledge about the pharmacology of C. lechleri and prior experience of human consumption have been described in the primary BRT review. There is substantial research on the CPL of this plant, on the basic pharmacology, potential clinical effects and possible mechanism (see Dr. Dou’s Review). As for most botanical new drugs, the existing knowledge is relevant to this application.

No signal of serious toxicity has been detected in the extensive human exposure to crofelemer in the South America. The sponsor has conducted a full battery of non-clinical toxicity studies and no safety problems were observed.
Wide spread use of formulations derived from *C. lechleri*, known as “dragon’s blood”, for treating diarrhea in the local populations of central and south America has been well-documented (see Sections 3.2 and 6.1 of Dr. Dou’s review). For symptomatic relief of this condition, popularity of the treatment is an indication of its therapeutic consistency. Apparently it does not require tight modern quality control beyond that of crude local preparations for clinical efficacy.

**Ensuring Therapeutic Consistency of Marketing Batches**

For this NDA, we depend on the following to address this concern:

1) Pre-CMC support
   - This is a relatively simple botanical (single part of a single plant) with a class of well-studied active compounds (oligomers of catechins);
   - Identification of the plant is straight forward and there is little risk of confusion with other species
   - The collection of CPL from wild-grown trees will be restricted in EGRs with GACP implementation to minimize variation at the plant level
   - In general, latex is less variable than other parts of the plant, such as leaf.

2) Standard CMC measures
   - Multiple analytical chemical techniques to monitor the chemical composition of BDS; the BRT considers these characterizations adequate for a complex natural mixture, for both regulatory purpose and technical feasibility.
   - Control of manufacturing process will be emphasized

3) Post-CMC evidence
   - Multiple batches of crofelemer have been used in phase 3 clinical trials. Although the data is too confounded by the complicated study design for a formal statistical analysis, there is no sign suggesting certain batches were more or less effective than others.
   - Likewise, the clinical responses did not appear to be sensitive to the 3 doses in clinical trials (see Dr. Gao’s medical review). In studies on the mechanism of action, the inhibition of chloride channels is fully saturated at the dose range of 125-500 mg (see Dr. Gao’s review and Section 5.2 of Drs. Dou’s review).
   - The observations on the batch/dose effects suggest that the clinical response rates are most likely not affected by minor variation in the quantitative composition of proanthocyanidin oligomers and make other uncontrollable variations and uncertainties less critical to clinical response.
   - Wide-spread use of crofelemer to treat diarrhea in central and South America suggests that the symptomatic benefit is fairly consistent in a large population for a long period of time. Very detailed control of the botanical drug apparently was not necessary for such consistency in the indigenous use.
Taking all these into consideration, we have adequate assurance of therapeutic consistency for future marketing batches.

CONCLUSIONS & RECOMMENDATIONS

In conclusion, we found this botanical NDA adequately characterized for a complex natural mixture and therapeutic consistency of marketing batches can be reasonably assured. It is approvable from the BRT perspectives.

Recommendations on labeling and post-approval botanical issues as outlined in the primary BRT review (to ensure renewability of the wild growth and to establish criteria for selection of new cultivation sites, as well as to evaluate potential bioassay, see Sections 1.1.2 and 1.1.3 of Dr. Dou’s review) are concurred.

cc:
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/s/

SHAW T CHEN
08/10/2012

Reference ID: 3172876
BOTANICAL NEW DRUG APPLICATION PRIMARY REVIEW

BY

BOTANICAL REVIEW TEAM

Application Type: NDA 505(b)(1)
NDA Number: 202292
Stamp Date: 12-05-2011
Applicant: Salix Pharmaceuticals
DMF #: NA

Drug Name: Crofelemer
Brand Name: TBD
Priority Designation: Priority Review
PDUFA Date: 06-05-2012 (Extended to 09-05-2012)
Dosage Form: 125 mg tablets
Route of Administration: Oral
Botanical Raw Material: Dragon’s Blood, the latex of Croton lechleri Müll.Arg.
Botanical Drug Substance: Oligomeric and polymeric proanthocyanidins of multiple chain lengths with an average molecular weight range of 1700 – 2500 Daltons and identified monomer units of (+)-catechin, (-)-epicatechin, (+)-gallocatechin, and (-)-epigallocatechin.

Indication(s) requested: Indicated for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy.

Botanical Review Team Reviewer: Jinhui Dou, Ph.D.
Review Completion Date: 08-08-2012
Botanical Review Team Leader: Shaw T. Chen, M.D., Ph.D.

New Drug Review Division: Division of Gastroenterology and Inborn Error Products (HFD-180)
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1. EXECUTIVE SUMMARY

1.1 RECOMMENDATIONS

The botanical drug product (BDP) Crofelemer (125 mg tablet) is indicated for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy. The botanical drug substance (BDS) contains primarily proanthocyanidin oligomers extracted from the red latex of *Croton lechleri* Müll.Arg. [Family Euphorbiaceae] and partially purified.

This Botanical Review Team (BRT) review finds that the control of the botanical raw material (BRM), manufacturing process, and identification and characterization of the BDS are adequate, and the therapeutic consistency of marketing batches can be reasonably ensured. We recommend approving this NDA.

1.1.1 Recommendations on Approvability

- The BRT review has identified no safety or quality issues related to BRM that may affect the approvability of this botanical NDA.
  - The BRM, or Crude Plant Latex (CPL), a red viscous latex of *Croton lechleri* Müll.Arg., is commonly known as Dragon’s blood. Dragon’s blood is one of the most common herbal medicines in Peru. It has been used orally for the treatment of diarrhea and other gastrointestinal (GI) diseases since the 1600s without any safety concerns.
  - The applicant’s Good Agricultural and Collection Practice (GACP) procedures are adequate. *C. lechleri* can be correctly identified according to one or more morphological characteristics to prevent misuse and adulteration from other species.
  - CPL quality can be ensured by properly implementing and enforcing the established GACP procedures to minimize variations in CPL and ensure the batch-to-batch consistency of the BDS.
  - The CPL will be collected from Eco-Geographic Regions (EGRs) as post approval BRM sources, since the CPL batches for clinical trials were supplied from those EGRs. The borders of EGRs will be clearly defined, using global positioning system (GPS), if necessary.
  - BRT recommends tightening the contaminant specification for pesticides, aflatoxins, and heavy metals in the BDS and/or CPL batches.
  - BRT recommends tightening the specification for taspine and total phenolics to include both upper and lower limits.

- The BDS, a partially purified botanical extract, contains primarily oligomeric proanthocyanidins of multiple chain lengths with an average molecular weight range of approximately 1700 – 2500 Daltons (Da) and identified monomer units of (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), and (-)-epigallocatechin (EG). This is in agreement with the identity of crofelemer as reported in literature from the 1990s.
  - Per the Guidance for Industry-Botanical Drug Product, new botanical drug NDA approval does not require further purification of the BDS, nor identification of all the active molecules. The oligomeric
proanthocyanidins fraction used for phase 3 multiple batch clinical trials can be considered as the active BDS without further separation.

- The limitations of the analytical methods (e.g., lack of chromatographic separations between the proanthocyanidin oligomers) are rooted in the complex nature of the BDS which contains numerous isomers and analogs. Accurate quantification of such a complex botanical mixture is not practical, since it would require each molecule or each group of molecules to be isolated and purified in quantities large enough to obtain an adequate amount of reference material.

- The applicant adopted a comprehensive approach by employing modern spectroscopic and spectrometric techniques, Ultra Violet (UV), infrared (IR), mass spectroscopy (MS), and nuclear magnetic resonance (NMR), to characterize and identify the proanthocyanidin oligomers, and the related compounds and impurities, such as and taspine. The MS provides molecular weight to confirm the basic structure of the proanthocyanidins, which are composed of C, EC, GC, and EG monomer units and have degree of polymerization (DP) of .

- To analyze the monomer units in proanthocyanidins, the applicant determined the ranges of procyanidin (PC) and prodelphinidin (PD) ratios and relative molecular weights (MW) using a standard acid hydrolysis method established in 1970s. The data from the acid hydrolysis method is supported by the NMR and MS data. The applicant analyzed the clinical batches and incorporated the PC/PD ratio into the BDS specifications.

- For quality control, .

A comprehensive approach utilizing those methods, with different separation and detection mechanisms and representative of modern day technology, is adequate to control the quality and consistency of the BDS.

- From BRT's perspective, the applicant’s approach utilizes the available current science and technology for the identification, characterization, and quality control of crofelemer as a complex proanthocyanidin oligomer mixture.

- Clinical results from multiple-batch and multiple-dose trials (125-500 mg, bid) suggest that the drug effect is not sensitive to the tested doses, in agreement with that the observation that estimated GI concentrations of the drug will hit the plateau region on the dose-response curve. Thus, the existing minor variations of the BDS (as a result of naturally occurring variations of the chemical profiles of the BRM and purification process) is likely not critical.

- In addition, widespread previous human use of the BRM as herbal medicine for the treatment of diarrhea in the general population also suggests that the efficacy of the drug is not variable.

- Mechanistic studies have demonstrated that the proanthocyanidin oligomers (the major components in the BDS) are functioning through a new anti-diarrheal mechanism with clearly demonstrated dose-response
effects in multiple assays. Crofelemer proanthocyanidins do not cause direct pore occlusion suggesting that the sizes and distributions of the proanthocyanidin molecules may not be fundamentally important for the pharmacological and clinical effects. Thus, controlling all the proanthocyanidin oligomers together in the BDS is appropriate.

- From BRT’s perspective, the applicant’s overall quality control approach, including BRM, manufacturing process, and BDS controls, is adequate and appropriate for approval of the botanical NDA.

1.1.2 Recommendations on Post Approval Botanical Issues

- The applicant should make a concerted effort to prevent the over-harvesting of BRM from the wild grown trees in the current EGRs. The applicant should:
  - continue to evaluate the applicability of BRM collected from other EGRs, and further analyze the chemical profiles of BRM across different EGRs;
  - investigate other means of qualifying additional EGRs, such as developing and using a medically relevant bioassay or post-approval bridging clinical studies; and
  - continue efforts to further qualify BRM collected from cultivation sites,

- The applicant should continue to enforce implementation of the established GACP on CPL quality control, storage, and transportation, to prevent contaminations, including contamination from other botanicals.

1.1.3 Recommendations on Labeling

- **Applicant proposed language:**

  - **Recommended language:**

11.0 DESCRIPTION
1.2 SUMMARY OF BOTANICAL ASSESSMENT

The BRM (crude plant latex or CPL from *Croton lechleri*) is one of the most popular herbal medicines in Peru for treating diarrhea, cholera, and other diseases. The extensive human use of the BRM indicates that crofelemer will be safe at equivalent doses (based on the polyphenolic and proanthocyanidin contents) of CPL.

The applicant’s Good Agricultural and Collection Practice (GACP) procedures are considered adequate to ensure quality and consistency of the CPL. Improvements on CPL quality control will be made in the following areas: collection from more mature trees (≥7 years) and diameter at breast height (DBH) of approximately 30 cm in better defined EGRs, tighter specifications for polyphenolics and taspine, and tighter specifications for contaminants in CPL and/or BDS. *C. lechleri* trees can be easily characterized by their leaves and other morphological characteristics to prevent collection of similar latex from other plant species. Overall, BRT review found no major issues related to the CPL collection, storage, transportation, and related procedures.

BRT did not recommend inspection of the CPL collection or EGR sites. This is consistent with the handling of the previously approved botanical NDA (21902), wherein the BRM (i.e., green tea) extraction site in China was not inspected as part of the pre-approval GMP requirement.

Based on the hundreds of BDS and BRM batches that have been used during the drug development process, nearly all of the BDS and BRM batches produced meet the established or current specifications. Thus, the overall BRM and BDS control is generally adequate and the naturally occurring variations of the CPL compositions do not appear to have a significant impact on the quality of the BDS.

The applicant has employed modern spectroscopic and spectrometric technology (e.g., UV, IR, MS, and NMR) to characterize and identify the structures of the proanthocyanidin oligomers, the major active components in crofelemer BDS. Crofelemer was identified to contain primarily prodelphinidin and procyanidin oligomers.
of multiple chain lengths with an average molecular weight range of 1700 – 2500 Da. The 4 catechin monomer units are 2 PC (C and EC) and 2 PD (GC and EG) units. Crofelemer proanthocyanidins were determined to have \( (b)(4) \) The detected oligomer chains of crofelemer range from 3 to 14 units, with an average length of 7-8 units. The applicant has also used a \( (b)(4) \) method to further characterize crofelemer by the average PC/PD ratios, average chain lengths and molecular weight of the proanthocyanidins. The applicant also characterized and properly controlled the related compounds and impurities, such as \( (b)(4) \) and taspine. BRT considers those methods and data adequate for proving the identity of crofelemer to support approval of this NDA.

The applicant’s comprehensive analytical approach includes several of the commonly applied techniques and methods established by industry, academia, and governmental experts (such as those from USDA) for proanthocyanidins. Because of the vast number of proanthocyanidins, available methods can not separate and identify them one by one, or even by small groups, in many of the cases. Also, accurate quantitation of proanthocyanidins at the molecular level is impossible due to the impracticality of developing purified reference standards. The applicant identifies \( (b)(4) \) The applicant has developed \( (b)(4) \) is acceptable. The applicant has developed a \( (b)(4) \) The applicant views further separation can not be achieved and provided a summary of literature data supporting its position. BRT’s independent review and evaluation concludes that the applicant’s quantitative and qualitative analytical methods are adequate and can be accepted to ensure the quality and consistency of the BDS.

The applicant used a validated \( (b)(4) \) method to analyze 101 BDS batches and provided data on proanthocyanidin oligomer molecular ions and average molecular weight. The data are in general agreement with those generated by other methods. From BRT’s point of view, the \( (b)(4) \) method can be used as a qualitative and quantitative method. A table of characteristic molecular ions can be used as part of crofelemer’s identity test, and the \( (b)(4) \) can be used as an alternative quantitative assay to further strengthen the quality control of the BDS.
The applicant tested multiple BDS and BDP batches in the phase 3 clinical trials, demonstrating that typical quality variations in the BDS and BDP due to the BRM and/or the manufacturing process are insignificant and do not affect the product’s therapeutic consistency. This indicated that the therapeutic consistency from future qualified BRM, BDS, and BDP batches can be ensured. This is further supported by the clinical data showing that both low doses (125 mg, bid) and high doses (500 mg, bid) of crofelemer are similarly safe and effective. Also, herbal medicine use of CPL and of crofelemer’s earlier versions for diarrhea and related GI diseases provides additional support that crofelemer’s anti-diarrheal effect is insensitive to minor changes in the compositions.

From BRT’s perspective, the applicant’s overall approach for CPL and BDS identification, characterization, and quality control is adequate and the NDA is recommended for approval.

Post-NDA approval, the applicant is recommended to further qualify and request Agency’s approval of additional CPL collection areas/regions, such as EGRs and the established cultivation sites, in order to have sustained CPL BRM supply for crofelemer. The applicant is recommended to develop a clinically relevant bioassay to enhance quality control, especially for qualifying new BRM collection regions and changing manufacturing process. In addition, the applicant can use the accepted analytical methods to establish fingerprint libraries of CPL and BDS, which can be used for detecting changes in future CPL/BDS batches, especially with regard to degradants, contaminants or adulterants.
2. INTRODUCTION OF BOTANICAL REVIEW
This botanical NDA review includes the following major sections:
- Medicinal plant biology;
- NDA review of botanical raw material, drug substance, and drug product;
- Previous human use, pharmacology, and toxicology;
- Botanical specific clinical issues; and
- Overall conclusions and recommendations.

Medicinal Plant Biology
Numerous scientific studies of *C. lechleri*, its latex (the BRM), and the main proanthocyanidin oligomer components in the BDS have been reported in the literature. Because the biological, chemical, and pharmacological areas of those studies relate in an important way to the NDA, a review of the literature data is included in the following sections:
- Taxonomy *C. lechleri* and related species;¹⁻⁶
- *C. lechleri* latex, Crofelemer, and their human use and pharmacological importance;⁴,⁷⁻¹²
- General phytochemistry of *C. lechleri*;⁴,⁷,⁹,¹³⁻²⁰
- Proanthocyanidin oligomers and polymers—their presence, usefulness, separation, characterization, identification, and related analyses including catechin monomers ratios.²¹⁻⁵⁷

NDA Review of BRM, BDS, and BDP
This section reviews the quality data provided in the NDA, primarily regarding the quality control of BRM, BDS, and key manufacturing process for characterizing and identifying the BDS and ensuring quality and therapeutic consistency of the BDP. Good Agricultural and Collection Practice (GACP) is the starting point of the BRM quality control. The characterization, identification, and related quality control methods of the BDS, especially of the active proanthocyanidin oligomers, are highlighted and compared with available scientific data reviewed in the previous section.

Previous Human Use, Pharmacology, and Toxicology
Previous human use of dragon’s blood (i.e., CPL) as herbal medicine, non-clinical pharmacological studies, and clinical studies reported in the literature are summarized as reference information for the medical team’s clinical review.

Botanical-Specific Clinical Issues
The clinical section will summarize literature-reported clinical trials of the BDS or similar products to determine whether historical data is useful to support the safety and/or efficacy of the drug for the proposed indication.

The scientific and regulatory considerations for approving a botanical drug, which is often a complex mixture, differ from those for purified small-molecular drugs. In addition to standard or conventional CMC mechanisms that can apply to both purified drugs and botanical mixtures, we may and often required to apply pre-CMC controls.
(e.g., BRM control, GACP) and post-CMC controls (e.g., multiple batch clinical trials, bioassay) to ensure the consistency of the botanical product in terms of both quality and therapeutic effects.

**Overall Conclusions and Recommendations**
BRT assesses whether the applicant’s data is adequate for approval of the NDA in this section. Final recommendations and any pre-approval and post-approval issues are summarized here.
3. MEDICINAL PLANT BIOLOGY
The botanical raw material (BRM) is the latex of *Croton lechleri* Müll.Arg. [Fam. Euphorbiaceae], which is also called dragon’s blood (sangre de drago) or tree’s blood (sangre de grado). Dragon’s blood is an herbal medicine commonly used for the treatment of diarrhea and for wound healing in South America. *C. lechleri*, its latex and partially purified products (such as crofelemer) have been subjects of numerous scientific studies. Those studies and the selected publications on the major active components of crofelemer, will be independently reviewed here. Crofelemer specific data in the NDA that is not available in the public domain will be covered in Section 4.

3.1 DESCRIPTION AND CHARACTERIZATION OF *C. LECHLERI*
*Croton lechleri* is a fast growing tree of secondary forests (i.e., forests disturbed by human activities) in Central and South America, including countries like Mexico, Venezuela, Ecuador, Peru, Brazil, Colombia, and Bolivia.\(^1\)\(^-\)\(^5\) This species generally ranges in height from 3–25 meters and occasionally up to 35 meters with a relatively thin trunk that can be 30 cm or more in diameter. Multiple petiolar glands, floral and fruit morphology, and red latex from the bark, as shown in the following photographs are some of the characteristics of *C. lechleri* that differentiate it from other *Croton* and non-*Croton* species.\(^1\)\(^,\)\(^2\)\(^,\)\(^4\)\(^,\)\(^5\)

The red viscous latex from the tree bark of *C. lechleri*, commonly known as dragon’s blood, has been used as an herbal medicine in its native countries, especially Peru, Ecuador, Colombia, and Mexico, as early as the 1600s.\(^4\)\(^,\)\(^7\) Currently, the red latex/resin
from *C. lechleri* is still the most common household remedy in Peru. The latex/resin has been used both internally and topically for the treatment of bleeding, wounds, inflammation, rheumatoid arthritis, and intestinal ailments such as diarrhea and stomach ulcers, as well as other prescribed medicinal conditions.4,7

The published and accepted taxonomic classification of *C. lechleri* is the following:1-3,6

**Division:** Streptophyta  
**Class:** Equisetopsida  
**Subclass:** Magnoliidae  
**Order:** Malpighiales  
**Family:** Euphorbiaceae  
**Genus:** Croton  
**Subgenus** Adenophylli  
**Section:** Cyclostigma  
**Subsection:** Cyclostigma  
**Species:** Croton lechleri Müll.Arg.

The Plant List, a working list of all plant species established by botanists from several internationally well-known botanical gardens (such as Kew of the UK, The New York Botanical Garden, Missouri Botanical Garden, etc.), listed *Croton lechleri* Müll.Arg. as the accepted name with two synonyms: *Croton draco* var. *cordatus* Müll.Arg. and *Oxydectes lechleri* (Müll.Arg.) Kuntze.6 The relatively short list of synonyms suggests that the taxonomy of this species is relatively straightforward and currently without many controversial or confusing issues with other similar *Croton* species.

The *Croton* genus has a total of approximately 750 species around the world, a relatively large genus in Euphorbiaceae.1-3 In addition to *C. lechleri*, there are several other red latex-producing *Croton* species, such as *C. draconoides* Müll.Arg., *C. draco* Schltdl, *C. urucurana* Baill., *C. xalapensis* Kunth, *C. gossypiifolius* Vahl, *C. erythrochilus* Müll.Arg. and *C. palanostigma* Klotzsch, which have also been used as herbal medicines under the name of Sangre de Drago or Sangre de Grado in their native countries.4,6 *C. perspeciosus* Croizat and *C. erythrochilus* are two species closely related to *C. lechleri*. All three are included in the same section, Cyclostigma, and all are present in parts of its geographical range.1,2,4 *C. lechleri* has the following two distinct characteristics to allow for correct identification: multiple unique-shaped glands on the petiole (as shown in the photographs above) and stipules on the young stems, which are shaped like miniature versions of the leaf (approximately 2 mm) and usually folded length-wise in half.1,2,5 More detailed general descriptions of *C. lechleri* can be found from the publications of Riina R. et al. and van Ee et. al., and others.1,2,5

### 3.2 MAJOR GROUPS OF COMPOUNDS IN DRAGON’S BLOOD

The latex of *C. lechleri* (dragon’s blood) contains alkaloids, polyphenolic compounds (e.g., procyanidins, prodelphenidins), plant sterols, terpenoids, among other chemical components.4,12-20 This botanical review focuses on proanthocyanidins and alkaloids (e.g., taspine), since those two groups are the known active components in dragon’s blood or its products, including Virend® and Crofelemer.4,7,10,16,19
3.2.1 Proanthocyanidins as a Class of Phenolics in General

Proanthocyanidins are major plant secondary metabolites, present as the second most abundant class of natural phenolic compounds after lignin.\textsuperscript{21-23} Comprising a large variety of structures, some proanthocyanidins are ubiquitous in plants while others are restricted to particular families, species, or plant parts, organs, or tissues.\textsuperscript{21-48} Proanthocyanidins are mixtures of oligomers and polymers composed of flavan-3-ol units with a degree of polymerization (DP), linked mainly through (e.g., the structure of Virend\textsuperscript{R} and Crofelemer with R-groups as either –H or –OH, and an average molecular weight of approximately 2100 Da)\textsuperscript{9,16}; however, also exist. The common flavan-3-ols in proanthocyanidins are afzelechin (AF), epiafzelechin (EA), (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), and (-)-epigallocatechin (EG), as the structures show below.

![Diagram of flavan-3-ols and proanthocyanidins](image)

Proanthocyanidins containing the single interflavan linkages are B-type, whereas those containing double interflavan linkages are A-type, with an additional ether bond between C2 and O7. The proanthocyanidins that consist exclusively of C/EC are procyanidins, and thus C and/or EC are called procyanidin (PC) monomer units. Proanthocyanidins that contain afzelechin (AF) and/or epiafzelechin (EA) as subunits are called propelargonidins (PP); and proanthocyanidins that contain GC and/or EG subunits are called prodelphinidins (PD). Propelargonidins and prodelphinidins are mostly heterogeneous in their constituent units and co-exist with the procyanidins.\textsuperscript{22,23}

The complexity of prodelphinindin-procyanidin type (e.g., proanthocyanidins having 4 monomer units, C, EC, GC and EG, as does crofelemer) is obvious when compared with the simpler procyanidins which have only two monomer units (C and EC). Even counting only one single interflavan linkages (e.g., the major C4→C8 type) for a given proanthocyanidin oligomer group with DP = n, there are n + 1 possible isomer groups and a total of 4\textsuperscript{n} of proanthocyanidin molecules. For example, for dimers there will be 2+1 (i.e., 3) molecular ions (molecular weight or MW) and 4\textsuperscript{2} (i.e., 16) molecules; for trimers there will be 3 + 1 (i.e., 4) different MW and 4\textsuperscript{3} (i.e., 64) molecules; for tetramers there will be 4 + 1 (i.e., 5) MW and 4\textsuperscript{4} (i.e., 256) molecules, and so on. For procyanidins, each oligomer group with DP = n will have only one MW, and 2\textsuperscript{n} oligomer molecules (i.e., 2\textsuperscript{2} = 4, 3\textsuperscript{2} = 9, and 4\textsuperscript{2} = 16 molecules of dimers, trimers, and tetramers respectively), exponentially less than those of mixed prodelphinidins. The theoretical number of
proanthocyanidin oligomer molecules from the 4 monomer units with DP = 3-14 (and again one interflavan linkage) is calculated at over 300 million. Experts warn that because of the complex and diverse structures of many proanthocyanidin molecules, including oligomers and polymers of various chain lengths and oxidation patterns, it can prove difficult to extract, separate and elute from chromatographic columns, and/or characterize those proanthocyanidins.22,25

As a group of major plant secondary metabolites with a large variety of structures and various DP, proanthocyanidins are found in rather large quantities in plant based foods and beverages, such as green tea, cocoa beans, chocolate, blue berries, grapes (especially the skin and seeds), wine, soy bean coats, and mangosteen, to name only a few.22-37

Proanthocyanidins and other polyphenols play an important role in protecting the plants themselves, such as protection against UV-light exposure, wound healing, and other plant defense mechanisms against microbial infections.25 Their importance in promoting and protecting human health has also been extensively studied and reported, and only a few relevant references are sampled in this review.25,27,38,49-51. A few proanthocyanidin-enriched extracts from various plant species were reported to have protective effects against animal models of gastric and duodenal ulcers, and diarrhea, and some also possess in vitro antiviral (including anti-HIV) and antimicrobial properties.52-57

Total phenolics can be determined by Folin-Ciocalteau (FC) colorimetric method, which is a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. The drawback of this method is that other reductive compounds (e.g., sugars) in the mixture will also be counted as phenols and lead to error in the analytical results.39

The monomer units of proanthocyanidins can be determined by an acid-catalyzed depolymerization experiment in the presence of a nucleophilic agent, such as phenylmethanethiol, phloroglucinol, or other reagents with similar properties.40 The flavan-3-ol arising from terminal units and the derivatives formed from extension units can then be separated and assayed by HPLC. This method provides important information on the nature and proportion of constitutive monomer units and the average DP, which can be calculated by dividing the sum of all units by the sum of terminal units.40

Methods for analyzing proanthocyanidins by liquid chromatography (e.g., Gel Permeation Chromatography and HPLC) have been developed to provide information on the composition of the oligomers grouped by different DP, determine average monomer chain length, and gain insight on the molecular weight distribution.22,24,25,27,30,36,57 Normal phase HPLC methods, often employing a diol-phase column eluted with a gradient of acidified acetonitrile in water, coupled with tandem mass spectrometry, became available in the last decade to detect the molecular weight of oligomers and also gather information
on the sequence of certain relatively small oligomers (with DP < 6) through fragmentation experiments. As the oligomers and polymers become larger, the sensitivity of MS reduces dramatically. Currently, the molecular ions for proanthocyanidins with DP = 14 are the detection limit for polyphenolic compounds in most food products. Published methods successfully separated grape seed extract and cocoa bean extract, which contain procyandin with only C and EC as the two exclusive monomer units. Even for those simpler procyandin oligomers, the HPLC-UV/fluorescent methods may only separate and detect the oligomer groups with DP < 10, and oligomers and polymers with DP = 10 and above will either wash off the column together as a big peak, or as a hump over the baseline.

Despite the reasonable HPLC separations of several botanical extracts containing procyandin oligomers, no such successful cases for prodelphinidin oligomers and polymers with co-existing procyandins were found in literature cited in PubMed by an intensive search by this reviewer. On the contrary, there are many reports showing that HPLC methods provided no separations of prodelphinidin and co-existing procyandin oligomers and polymers. In addition, HPLC methods were also used to analyze the acid hydrolysis products. Intact proanthocyanidins can be analyzed by MS directly without HPLC separation.

MS has become a very important detector for analyzing intact proanthocyanidin oligomers and polymers, with and without column separation. Other methods to analyze the composition of intact proanthocyanidin oligomer mixtures include 13C-NMR, which can determine the ratio based on the differences of carbon signals resulting from the chemical shift caused by the stereochemistry between monomers, such as C, EC, GC, and EG. By integrating the 13C-NMR signals of C-4, which is a –CH– for the extension units and a –CH2– for the terminal units, average DP can be calculated. However, for complex mixtures the signal-noise ratio may not be adequate to accurately integrate those signals.

The quantification of intact proanthocyanidins oligomers and polymers, especially those with 4 or more monomer units (e.g., with both procyandin and prodelphinidin oligomers), is very challenging because of structural diversity of this group of compounds. Their UV/Fluorescent response factors vary significantly by their different monomer units, oxidation patterns, degree of polymerization, and other structural differences. Mass spectrometry is not a good quantitative tool, and the ionization efficiency will decrease significantly as DP increases. In many cases, the oligomers with DP > 14 give low intensity signals, which make routine detection of their molecular ions a challenge, let alone quantitation.

3.2.2 Proanthocyanidin Compounds in C. lechleri Latex and Crofelemer

The composition of the latex/resin of C. lechleri is apparently very complex. The total phenolics, including proanthocyanidins derived from 4 monomer units (i.e., C, EC, GC, EG), are the major constituents of the latex/resin and may account for up to 69-90% of the dry weight in some latex samples collected from Ecuador and Peru. Fresh latex...
reportedly contains 23-26% dry residue and has a density of approximately 1.07 to 1.1 g/ml. The humps above the baseline on the HPLC chromatogram below indicate a large number of co-eluting compounds, the signature of proanthocyanidin oligomers and polymers that can not be efficiently separated by the HPLC methods. The monomers and dimers are efficiently separated by the HPLC methods, and show as sharp peaks. The chromatogram of the latex of *C. lechleri* is similar to that of proanthocyanidins in pine bark extracts. After purification of pine bark extracts, the sharp peaks disappeared to give proanthocyanidins chromatograms as one or a few wave shaped bumps. This is also true for crofelemer which showed one peak or several wave-shaped bumps for the proanthocyanidin oligomers without big sharp peaks on the chromatograms (except minor monomer peaks).

A $^{13}$C-NMR spectrum of dragon’s blood was reported. The authors did not attempt to assign the carbon signals to the respective carbons in the proanthocyanidin molecules. Characteristic signals around 130 ppm indicate the presence of prodelphinidin.

A proanthocyanidin fraction from *C. lechleri*, SP-303 (with a commercial product name Virend®), from CPL was studied as a topical agent for the treatment of genital herpes lesions in patients with HIV/AIDS and the result was reported in 1997. SP-303 was reported as an oligomer with an average molecular weight of 2100 Da. The structure included procyanidin and prodelphinidin oligomers with DP = 6-13. A recent publication reported that crofelemer contains procyanidin and prodelphinidin with DP = 3-30. The authors of the two publications did not discuss determination of the DP.

### 3.2.3 Factors that Impact the Quality of Polyphenolic Compounds

The genetic and environmental factors that determine the proanthocyanidin composition for *C. lechleri* are largely unknown. The environment, season (e.g., rainy or dry season), age of the tree, and other factors can impact the latex yield, water content, and possibly
also the alkaloid content. Dragon's blood of various colors has been reported, but their differences in chemistry and pharmacological effects have not been well studied.4

The biosynthesis pathways of proanthocyanidins are largely unknown.48 A recent study of proanthocyanidin content in *Populus* species and hybrids suggests that genetics (i.e., cross type) is the primary determinant of proanthocyanidin composition, both in chain length and monomer composition in leaf tissue.46 Environmental factors do not play a significant role in determining proanthocyanidin composition. Developmental zone and season are much less important. In contrast, an earlier study cited by the authors suggested that the quantity of proanthocyanidin is influenced by developmental factors as well as genetics and the environment.46

The composition and quality of the latex, such as the BRM from the wounded trunk of *C. lechleri*, is likely less variable than, for example, the components in the leaves, since the leaves have a more clear growing and aging cycle. The literature reported significant variations of the alkaloid contents in the leaves of *C. lechleri*, but taspine, the only alkaloid, is somewhat consistent in the latex.17 Large numbers of leaf and latex samples were analyzed for taspine, however, no test on polyphenolic compounds was conducted.

### 3.2.4 Alkaloids

Nearly all of the plant parts and tissues of *C. lechleri*, except the exudates from the petiolar glands, contain one to several alkaloids.4,17,18,19 Taspine was first isolated from *Leontice eversmanni* Bunge (Berberidaceae) in 1932 and first isolated from *C. lechleri* in 1979.14 It is reported to be one of the active components responsible for the wound healing activity of *C. lechleri* latex.7,13,15

HPLC analyses of 493 latex and 264 leaf samples of *C. lechleri* from 22 sites in northern Peru and Ecuador were reported in an effort to understand the natural variation in alkaloid content for the species. The mean (± SD) total alkaloid content of the leaves was 6.100 ± 2.517 mg/g dry weight, with a range of 1.142–14.617 mg/g dry weight (0.1–1.4%). Based on the variable contents of 6 alkaloids from the leaves, three chemotypes of *C. lechleri* were defined.

Only one alkaloid, taspine, was detected from the latex with much higher concentrations and showed no correlation to the chemotypes defined by the alkaloid contents in the leaves. The mean (± SD) taspine concentration of *C. lechleri* latex across all samples was 90.075 ± 41.902 mg/g dry weight, with a range of 13.858 to 204.942 mg/g dry weight (1.3 to 20.4%). It was reported that when collecting latex samples from trees with high taspine content, this alkaloid could be observed as a flocculent (flocular) white precipitate in the samples. It is commonly known that dry weather, drought, or other stress will cause plants to produce certain compounds at higher than usual levels, especially alkaloids.

The latex from the stem (trunk) of very young trees (i.e., about 1 year old) also contains several other alkaloids that showed some similarity with the leaves.13 A possible explanation is that the trunk bark of a young tree has chlorophylls for carrying out
photosynthesis functions, similar to the leaves and young stems. As a tree grows older, its trunk will be covered by a non-living, protective, cork layer that does not conduct photosynthesis. The color of the latex from the young trees is lighter (i.e., yellowish-orange) than that of the latex from older trees, presumably because the bark of young trees is covered by living cells which contain more water and fewer other components.

3.2.5 Other Groups of Compounds

*C. lechleri* essential oil has been obtained by steam distillation of fresh stem bark from Amazonian Ecuador adult plants (yield: 0.061%), and then chemically characterized by gas chromatography–mass spectrometry. Seventy-four chemicals were detected and identified, with the most abundant groups of compounds being sesquiterpenes (sesquicineole, 17.29%, α-calacorene 11.29%) and monoterpenes.37 One published review also reported additional compounds from *C. lechleri*.4

3.3 SUMMARY

The identification of *C. lechleri* by its appearance is relatively straightforward, thanks to the characteristic petiolar glands and other morphology features of the flowers and fruits. Dragon’s blood, the latex of *C. lechleri*, has been used as an herbal medicine since the 1600s in the Peruvian Amazonian region for the treatment of various ailments, including diarrhea. The historical knowledge of dragon’s blood plus the popular use of this easily available herbal medicine will be helpful to prevent contamination from wrongful collection of other plant species.

Taspine, an alkaloid in the latex, is considered to be one of the active compounds responsible for wound healing, although other groups of compounds (such as the polyphenolic compounds) may also contribute to the wound healing property. There are 6 alkaloids in the leaves of *C. lechleri* with variable concentrations, and this variation was used to characterize three chemotypes of *C. lechleri*. Interestingly, the taspine content in the latex collected from those same *C. lechleri* trees has no association with the chemotypes characterized by the alkaloids from the leaves.13

The compositions of the latex of *C. lechleri* and proanthocyanidins in crofelemer are reported to be quite complex. The structural diversity of proanthocyanidin with co-existing procyanidin oligomers in dragon’s blood and crofelemer makes HPLC separation and quantitation of intact oligomers extremely challenging.
4. REVIEW OF NDA: BOTANICAL RAW MATERIAL, DRUG SUBSTANCE AND PRODUCT

The crofelemer BDS, a partially purified extract of the red latex of *C. lechleri*, contains primarily proanthocyanidin oligomers with an average molecular weight range of approximately 1700 – 2500 Daltons and an average length of 7-8 monomer units. The oligomers consist of 3 to 14 linearly linked monomers; C, EC, GC and EG.

As indicated in the BRT literature review on proanthocyanidin in the previous section, crofelemer proanthocyanidins contain large numbers of closely related molecules, analogs or isomers. Additional molecular details and accurate quantitation, which requires additional separation and purification of the vast number of proanthocyanidins, are currently impractical for a botanical mixture as complex as crofelemer. This BRT review of the NDA focuses on the BRM identification and quality control, and comments on the difficulties of BDS characterization, identification, and quality control, from a botanical rather than a pure chemical perspective. The applicant has employed available spectroscopic and spectrometric techniques to characterize and identify the basic structures of the proanthocyanidins and utilized current chromatography separation methods to qualitatively and quantitatively analyze the proanthocyanidins in BDS as a whole, with overall outcome comparable to what the proanthocyanidin experts can currently do and expect. From BRT’s perspective, the applicant’s approach on BRM and BDS characterization, identification, and quality control is adequate to assure the safety, efficacy, and consistency of the BDP.

The majority of the information related to the BRM is summarized in NDA Module 2.3.S.2.3.1 Crofelemer Crude Plant Latex with a table of specifications. Most of the details related to raw material control are provided under Module 3.2.S.2.3. Control of Materials (including Crofelemer Crude Plant Latex-Botanical Raw Material Information, Botanical Collection Form, Herbarium Voucher Specimen, Sustainable Harvesting Manual, and RMTL Test Procedures). NDA Module 2.3. Quality Overall Summary and Module 3. Quality include all the other BRM, BDS, and BDP related CMC data. The applicant did not submit a separated Drug Master File (DMF). For a complete CMC review related to the BDS and BDP, please see the CMC review by Nina Ni, Ph.D.

4.1 BOTANICAL RAW MATERIAL AND QUALITY CONTROL

The NDA 3.2.S.2.3 Control of Materials, Crofelemer Crude Plant Latex-Botanical Raw Material Information, provides descriptions and characterizations of the botanical source, *C. lechleri* and CPL. The morphological characteristics of *C. lechleri* such as the leaf (with petiolar glands), flower/inflorescence, and fruits, were described previously (with photos adapted from published literature, and also cited in the NDA). See more morphological description and taxonomy related information of *C. lechleri* in the “Medicinal Plant Biology” Section of this review.

4.1.1 Good Agriculture and Collection Practice (GACP) of the BRM

The applicant references the Good Agriculture and Collection Practice (GACP) guidelines by the World Health Organization (WHO). Field manuals on how to identify
C. lechleri, CPL collection procedures, pesticides/defoliant questionnaire, training of the field collectors, CPL storage and transportation, have been provided in reasonable detail.

Description and Characteristics of C. lechleri
The key morphological characteristics of C. lechleri are the leaf (with petiolar glands) and other distinct features in other parts of plant, the flower/inflorescence, and fruits (see the table below). When the tree stem/trunk is cut, the tree produces red and viscous latex with unique organoleptic characteristics, which is the CPL BRM. Based on the work of Riina R. et al. and van Ee et. al., and others, different parts of C. lechleri were described and their features were summarized as in the following table (adapted from the NDA).¹²⁵

General Information and CPL Collection
The applicant has taken a practice of cutting down the mature trees (7 years or older with diameter at breast height or DBH, ~ 30 cm)
The applicant will implement GACP procedures and only collect CPL from trees of ≥ 7 years with DBH approximately 30 cm. Narrative descriptions of CPL and photographs of CPL collection are provided.

Each batch of CPL is

Variations of CPL related to growth conditions (e.g., season, soil/water, how CPL was collected) in terms of total collection volume and chemical profiles were briefly described. The study indicated that CPL volume is related to rainfall and the yield is higher from December to May. There were minor variations of the latex noticed, such as color (red blood, wine-purple, and ochre) and density. The color and density of the latex is likely associated with rainfall and/or the age of the tree, i.e., latex collected from younger trees and/or after a heavy rainfall is likely lighter in color and lower in density. Based on applicant’s experience, CPL batches collected did not have significant variations to impact the quality of the BDS of Crofelem

The applicant provided data on 222 batches of crofelem BDS. Other earlier batches produced by Shaman Pharmaceutical (SP) during 1991-98 were not compared directly with those batches, because of different manufacturing process and different specifications. On the other hand, SP-303, the BDS used in pre-1998 non-clinical and clinical studies as a topical treatment of genital herpes lesions was reported to be similar to the crofelem batches used in clinical studies.

EGRs for the Production of the BRM/CPL
The applicant has established EGRs and proposed to use all EGRs post-NDA approval. Clinical batches BDS/BDP were prepared from CPL batches collected from EGRs. The applicant has not evaluated whether the CPL produced from EGRs will be equivalent to those batches that have been used during the new drug development process, especially phase 3 clinical trials. The CPL BRM control from EGRs is largely adequate with all the subsequently produced BDS batches
meeting the proposed specifications. EGRs can be accepted as the BRM collection regions post-NDA approval.

Since CPL has been collected from *C. lechleri* trees in the wild, BRT has requested the applicant to provide more detailed information to better define each of the EGRs, such as using GPS to define the borders. Further qualification of EGRs should continue post-NDA approval to avoid raw material shortages and undue burden to the environment of EGRs.

**Initial Processing of the CPL**

**CPL Sources and Suppliers**

There has been no pesticide use on the *C. lechleri* tree, thus BRT does not consider the pesticide residue level to be a serious safety concern or an approvability issue. The suppliers have conducted pesticide use surveys and the CPL also tested for 8 pesticides which were found in the EGRs for other crops. The applicant reported that all 8 pesticides used in the EGRs were below the limit of detection. After discussions with ONDQA reviewers and the division, BRT requested the applicant to further control pesticides by testing the BDS with currently accepted compendial methods for the 8 pesticides, as well as providing the testing methods and full testing reports on CPL. A BRT Information Request (IR) to the applicant related to the pesticides in CPL BRM and BDS were sent out through division Project Manager on June 07, 2012.

**Renewability and Reproducibility of *C. lechleri* and CPL**

The applicant committed to cutting down only mature trees at least 7 years old with DBH of ~ 30 cm. *C. lechleri* is a fast growing tree that matures in 4 years, with each tree producing thousands of seeds. Based on the yield of the BDS from CPL (approximately and each tree producing of CPL on average, it is calculated that each mature
tree will produce approximately \( \frac{3}{4} \) of BDS. With the dose of crofelemer at 250 mg/day, each patient will need \( \frac{3}{4} \) mature \( C. \) lechleri trees per year. The average yield of BDS from CPL could be \( \frac{3}{4} \) times higher than the minimum yield, however, and then trees per patient per year may be adequate. The applicant did not provide estimation on how many mature trees are available for CPL collection each year in EGRs \( \frac{0}{0} \). Although it is unlikely that there would be immediate shortage of BRM/CPL, the CPL resource issue in the long run should be further addressed by the applicant post-NDA approval.

The applicant had introduced “Sustainable Harvesting of Sangre de Drago and Sangre de Grado Educational Material” since the late 1990s to educate the collector and the local people on how to conduct re-plantation/re-forestation of \( C. \) lechleri, including the manual propagation of \( C. \) lechleri in green houses \( \frac{0}{0} \). The applicant has provided no data to show whether cultivation conditions will have any impact on the quality of CPL and BDS.

The procedures for re-forestation and cultivation of \( C. \) lechleri have been established, but no thorough evaluations on CPL from those sources have been conducted by the applicant. A preliminary study of one batch of CPL collected from a cultivation site was comparable to one batch from wild collection, and both met the established CPL specifications. Further physicochemical analyses of additional CPL and subsequent BDS batches will be required to qualify new CPL sources (e.g., EGRs and cultivation sites). A clinically relevant bioassay or even bridging clinical trials may be needed to confirm that the BDS batches are consistent in terms of both quality and therapeutic effects. The applicant is requested to seek Agency’s approval before collecting CPL from new sources for marketing production of BDS batches. The applicant currently plans only to use CPL collected from wild trees from EGRs \( \frac{0}{0} \).

**Non-Croton Species Producing Red Latex/Dragon’s Blood**

There is a non-Croton species used in the region (e.g., \( Pterocarpus officinalis \) Jacq., family Leguminosae/ Fabaceae) that also produces red latex. Since \( C. \) lechleri is well known and the field collectors have been trained on the characteristics of \( C. \) lechleri, misuse of this non-Croton species can be prevented based on established GACP.

The name Dragon’s blood has also been used to represent the latex/resin from \( Daemonorops draco \) (Willd.) Blume (family Areaceae/Palmacea), \( Dracaena cambodi ana \) Pierre ex Gagnep. (family Asparagaceae/Dracaenaceae), or closely related species. \(^1,3\) These non-Croton species are less of an issue for CPL quality control because they grow in Asia and are primarily used as herbal medicines in Asian countries.

Overall, the characterization of \( C. \) lechleri illustrated by the applicant and other GACP procedures are adequate for allowing collection of CPL from correctly identified \( C. \) lechleri.
4.1.2 BRM Specifications
Since no pesticides will be used on *C. lechleri*, and the BDS was manufactured through limited testing of pesticides in either CPL or BDS is justified. Besides pesticides and heavy metals, aflatoxins are another group of contaminants that need to be controlled. Although CPL was reported to have antimicrobial activities and microbial contamination has not been reported by the applicant as a problem under the general uncontrolled storage/shipping conditions for over 2 years, BRT requested the applicant to test aflatoxins in BDS and/or CPL. The applicant revised the BDS specifications on heavy metals and aflatoxins to incorporate the Agency request. The applicant will also test pesticides in the BDS and has not made final decision on the need for routine testing of pesticide residues in CPL. The applicant’s initial specifications for CPL were submitted in the NDA as the following table.

<table>
<thead>
<tr>
<th>TEST</th>
<th>ANALYTICAL PROCEDURE</th>
<th>SPECIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Visual</td>
<td>Brown, viscous liquid</td>
</tr>
<tr>
<td>Identification</td>
<td>UV Spectroscopy</td>
<td>(8) (4)</td>
</tr>
<tr>
<td></td>
<td>STP RMTL 002</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>pH Method</td>
<td>(8) (4)</td>
</tr>
<tr>
<td></td>
<td>STP RMTL 002</td>
<td></td>
</tr>
<tr>
<td>Total Phenolics</td>
<td>Folin-Ciocalteau Method</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STP RMTL 002</td>
<td></td>
</tr>
<tr>
<td>Content of Taspine</td>
<td>HPLC (anhydrous basis)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STP RMTL 002</td>
<td></td>
</tr>
<tr>
<td>Content of Croflemer</td>
<td>HPLC (anhydrous basis)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STP RMTL 002</td>
<td></td>
</tr>
<tr>
<td>Chromatographic Purity</td>
<td>HPLC (area normalization method)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STP RMTL 002</td>
<td></td>
</tr>
</tbody>
</table>

The applicant uses the Folin-Ciocalteau (FC) Method to measure the total phenolics in CPL. The reason of setting the specification of total phenols in CPL is not given. A practical range is preferred than an open-ended specification, since the total phenolic compounds should not be too low. Also, taspine is a useful chemical marker of CPL from *C. lechleri*, a practical range may be useful for early detection of possible adulterations.
The applicant accepted BRT recommendation and revised the CPL specifications on total phenolic and taspine.

### 4.1.3 Discussion on BRM Quality Control

Overall, the natural habitat of *C. lechleri* in the Amazonian rainforest region has been less prone to contamination related to human activities (e.g., industry pollutants and use of pesticides). *C. lechleri* is a common and characteristic tree in the region with popular herbal medicine use, and thus trained field collectors could properly identify the tree and collect the CPL. There has been no botanical issues and collection of similar latex from other plant species can be effective prevented by properly trained field collectors. The applicant is able to follow Agency’s recommendations and properly address potential contamination issues, such as pesticides, heavy metals, and aflatoxins for CPL and/or BDS, by GACP and testing procedures.

Considering that the phenolics are the major components of the BDS and taspine is the characteristic alkaloid in CPL, BRT recommended the applicant to establish both low and high limits for taspine and total phenolic (FC method) specifications. The sponsor has accepted the recommendation and updated the BRM specifications.

The applicant used only CPL collected from the wild in the EGRs in the clinical trials, and thus those EGRs are acceptable as the CPL production region post approval. The acceptance of CPL from the EGRs and cultivation farms of *C. lechleri* needs to be further studied post-NDA approval by chemical analysis and/or clinically relevant bioassays. The reasons are that both environmental changes and agricultural conditions, such as concentrated mono-species cultivation on a farm, may significantly alter the biology of *C. lechleri* and the chemical composition of its latex, and should be further investigated before mass production. Re-forestation of young *C. lechleri* trees in the immediate area after cutting down mature trees for CPL production in accepted EGRs, but trees left alone to grow without further maintenance care (e.g., fertilization, irrigation, pruning, etc.), is considered close enough to the natural habitat. The CPL collected from those re-forested *C. lechleri* trees can be considered as the equivalent of wild grown trees for future CPL collection.

### 4.2 BOTANICAL DRUG SUBSTANCE (BDS)

Crofelemor BDS contains primarily proanthocyanidin oligomers with an average molecular weight range of approximately 1700 – 2500 Daltons and an average length of 7 monomer units (Module 3.2.S.1.1). The oligomers consist of 3 to 14 (and expected to be up to 30) linearly linked monomers; C, EC, GC and EG, with GC/EG The determination of the highest polymer although not a critical issue for the BDS quality control, is not clearly stated. It was reported in the literature that the estimated highest DP of proanthocyanidin from plants is approximately This BRT review highlights critical aspects of characterization of the BDS, which is still a complex mixture of many closely related molecules, to assure consistency in terms of both quality and therapeutic effects.
4.2.1 Crofelemer—A Single Molecule or a Complex Mixture?
A related product, SP-303, was reported in the 1990s with the same as crofelemer provided in the regulatory submissions (e.g., IND 51818 and the NDA) and reported in publicly available references. Although the structure is essentially the same, the descriptions of crofelemer active BDS, the DP and average molecular weight of the proanthocyanidins, also have some differences. Crofelemer is often described as a “purified” “isolated” “molecule/compound,” “a proanthocyanidin oligomer,” or “a polymer,” which in many ways suggesting it is a purified small molecule drug. The nature of crofelemer as a complex mixture has not been discussed in detail in the literature. Some statements, such as “crofelemer oligomers consist of 5–11 linearly linked monomers” with an average molecular weight of 2100-2200 Da (with structure showing degree of polymerization) indicate that crofelemer is a complex mixture. Despite the various names used and controversial descriptions as a single molecule, the proposed structure and average molecular weights of 2100-2200 are consistent in all references and regulatory submissions, suggesting that they are all mixtures containing mainly proanthocyanidin oligomers.

The complexity of crofelemer is confirmed by the MS data of the proanthocyanidin oligomers in crofelemer. The theoretical number of all possible oligomer molecules from the 4 monomer units with is calculated at over 350 million. The MS data indicated that there could be realistically hundreds to thousands of proanthocyanidin oligomers in crofelemer, if not more. Cocoa and grape procyanidins are composed of 2 monomer units (C/EC), which will have molecules with DP = n; while the proanthocyanidins in crofelemer will be molecules from the 4 monomer units (i.e., 2 PC and 2 PD units). Picking trimers as an example, there will be (8) procyanidins (as in the case of grape/cocoa), but will be (64) proanthocyanidins in crofelemer. Similarly, the possible number of decamers (216) in procyanidin is the same as the possible number of pentamers (45) in crofelemer.

The complex nature of crofelemer proanthocyanidin oligomers determines that separation of those closely related and large number of oligomers will be extremely challenging, particularly as DP increases. Even for the simpler procyanidin-containing botanical samples, such as cocoa and grape seeds, the best available HPLC methods can only separate oligomers with DP < 10 into several broad peaks (one broad peak or group of peaks per procyanidin oligomer group with the same DP). For the same separation efficiency, the method may be only adequate for separating the proanthocyanidin oligomers of DP < 6 in crofelemer.

For identification and characterization of proanthocyanidins, multiple methods and techniques have been applied, such as acid hydrolysis, HPLC, MS, NMR have been applied, as reviewed in the previous section. The applicant’s process development and efforts on the characterization, identification and quality control for crofelemer BDS is reviewed below.
4.2.2  Process Development and Manufacturing Process of the BDS
Crofelemere is also known as SP-303/NP303, the two earlier versions of BDS derived from CPL, but manufactured at different sites and through somewhat different manufacturing processes. The major components for crofelemere and SP-303/NP303 are all proanthocyanindin oligomers, and those three BDS can be considered as more or less equivalent. The manufacturing process development was outlined in NDA Module 2.3.S.2.6 and further detailed in Module 3.2.S.2.6.2 (below).

### Overview of Crofelemere Drug Substance Manufacturers and Batch Disposition

<table>
<thead>
<tr>
<th>Years</th>
<th>Manufacturer</th>
<th>Use of Drug Substance Batches</th>
</tr>
</thead>
</table>
| 1991 – 1997 | Shaman Pharmaceuticals, Inc. in San Carlos, CA and South San Francisco, CA | • Nonclinical studies (e.g., primary pharmacodynamics, pharmacokinetics, single- and repeat-dose toxicity, genotoxicity)  
  • Used to manufacture drug product for clinical studies:  
    Phase 2:  SP-303-II-05, SP-303-II-08b, and 51818-201  
  • Stability studies (drug substance and drug product) |
| 1997 – 2007 | Shaman Pharmaceuticals, Inc. in South San Francisco, CA (Step 2) | • Nonclinical studies (e.g., pharmacokinetics, repeat-dose toxicity, genotoxicity, reproductive and developmental toxicity)  
  • Used to manufacture drug product for clinical studies:  
    Phase 1:  51818-102, 37554-103  
    Phase 3:  37554-210  
  • Stability studies (drug substance and drug product) |
| 2007 – Present | (0)(4) | • Nonclinical studies (e.g., safety pharmacology, drug interactions)  
  • Used to manufacture drug product for clinical studies:  
    Phase 1:  CFEE1091  
    Phase 3:  NP303-101 (ADVENT)  
  • Registration batch stability studies (drug substance and drug product) |

The current manufacturing process for crofelemere consists of its

Through the current process,
4.2.3 Acid Hydrolysis of the BDS

Acid hydrolysis of the proanthocyanidin oligomers in the clinical lots of crofelemer BDP was conducted following a reference method.²⁷

A representative HPLC chromatogram of the hydrolysis product is provided below (NDA 3.2.S.3.1 Elucidation of Structure and Other Characteristics, Figure 29).

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The identified by HPLC-MS were assigned by their molecular weights and retention times as the following:

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(such as those procyanidins in cocoa and grape seed), the applicant’s method is considered to be equivalent or even better than the methods developed for procyanidins.

From BRT’s perspective, the applicant’s comprehensive approach for BDS characterization, identification, and quality control to assure batch-to-batch consistency represents what the modern day analytical technology can offer for such complex botanical mixture. In conclusion, BRT recommends accepting the applicant’s analytical methods and the established specifications as the foundation for approving this botanical drug.

4.3 BOTANICAL DRUG PRODUCT (BDP)

The botanical drug product (BDP) is a tablet for oral administration which are film-coated and contain 125 mg of crofelemer BDS. Crofelemer (125 mg, tablet), is indicated for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy. For complete review of the crofelemer drug product, please see Dr. Nina Ni’s CMC review. Consistency issues related to the quality and therapeutic effects and associated approvability issues will be discussed in other sections of this review.

4.4 SUMMARY

To minimize the variation of complex BDS that cannot be adequately controlled by standard CMC measures, both the starting botanical raw material and the manufacturing process must be controlled.

The applicant established GACP for BRM (the CPL from *C. lechleri*) collection, storage, and transportation. Based on the historical CPL batch records and corresponding BDS data, the existing BRM control is adequate. As requested by the BRT, the applicant will also make further improvements to the BRM quality control by the following measures: tightening the ranges of size and age of *C. lechleri* to be harvested, defining the EGRs more precisely (and restrict the EGRs to the ones with previous collection of CPL for the manufacturing of clinical BDS batches), tightening control of the potential contaminants in CPL and/or BDS, and also making improvements of certain CPL specifications (total phenolics and taspine). The applicant’s CPL quality control is appropriate and acceptable for approving this NDA.

The overall comprehensive characterization by the acid hydrolysis experiment, NMR, and multiple HPLC-UV/fluorescent/MS methods can provide unambiguous identity based on monomer units and PC/PD ratios, molecular weight and average monomer units of the proanthocyanidin oligomers, and overall chromatographic profiles. From BRT’s perspective, the level of characterization and identification is inline with the best established methodologies and technologies of modern day analytical science, and is also appropriate and acceptable for approving this botanical NDA.

This BRT review made the following observations and conclusions on BDS identification and quality control:

- The applicant conducted acid hydrolysis and proposed acceptance criteria of [value] for the ratio of PC to PD, mean DP,
and mean acid hydrolysis conversion yield, respectively. The conversion yield was recently changed from the previous. Certainly, change from can be accepted automatically. BRT has no objection if the lower end is also accepted, at least temporarily, since the conversion yield is not a parameter for measuring total proanthocyanidins, and there are better methods for that purpose (e.g., the reverse phase HPLC-UV method, FC method). It is important to save enough retain samples of the clinical batches for other important tests and future references.

- The applicant used a validated [0-4] the proanthocyanidin oligomers were determined to have an average molecular weight of 2100 Da. A chart of detected molecular ions is provided by the applicant, and can be used as an identity test for crofelemer. This method can also be used as an assay test of total proanthocyanidin oligomers in crofelemer.

- Proanthocyanidin oligomers with DP = 200, although expected, were not directly detected by MS. Those proanthocyanidins will be included in the “total proanthocyanidin peak” recorded by the HPLC methods and adequately controlled as part of the “total proanthocyanidins”.

- Applicant’s reverse phase HPLC-UV method was used to analyze 222 batches of BDS with acceptable quality consistency with a specification [0-4] w/w) established comparing with a reference BDS batch. The method was also used to control the related components [0-4].

- The applicant newly developed normal phase HPLC-Fluorescent/MS method was able to partially separate oligomer groups with area percentages for each of the proanthocyanidin oligomer group with [0-4]. No literature reported method gave better separations for proanthocyanidins as complex as crofelemer. Because of the large number of molecules in crofelemer and lacking of any purified oligomer standards, accurate quantitation of each oligomer groups (e.g., trimer groups, tetramer groups, etc.) by weight is impossible by available separation and detection techniques.

- By using the three HPLC methods, the proanthocyanidin oligomers, impurities and other related compounds can be adequately controlled. In addition, the overall quality control of the crofelemer also includes CPL quality control (e.g., GACP), manufacturing and process control.

Overall, the applicant’s approach is comprehensive and to the extent that the available analytical technology and methodologies can offer. Based on the existing methods and available data from the applicant, BRT believes that crofelemer can be uniquely identified and adequately controlled by BRM quality, BDS manufacturing process, and BDS identity and quality to assure the consistency of the therapeutic effects of the to be marketed BDP batches.
5. PREVIOUS HUMAN USE, PHARMACOLOGY, AND TOXICOLOGY

5.1 PREVIOUS HUMAN USE AND PHARMACOLOGICAL ACTIVITIES

Dragon’s blood, the red latex from *C. lechleri* (i.e., the BRM/CPL), has been used in Peru and other Central and South American countries for the treatment of gastrointestinal symptoms, like different types of diarrhea, cholera, and gastric ulcers. The oral dose is typically 5-10 drops of CPL three times a day (i.e., approximately 1-2 g/day), diluted in warm water, juice, milk, or alcohol, for up to three weeks.\(^4\)\(^7\) Considering that the CPL contains up to \(b\) the herbal medicine dose covers the crofelemer dose of 250 mg/day. Thus, the crofelemer BDS at the daily dose of 250 mg is supported by the previous human use of CPL.

Crofelemer contains primarily proanthocyanidin oligomers.\(^6\)\(^4\) Different proanthocyanidins in other food or dietary supplement products have been used at similar or higher doses. Although the details of the chemical structures at the molecular level are expected to be different among polyphenolic compounds (including proanthocyanidins) from various botanical sources, those groups of polyphenolics apparently all have antioxidant activities, with plenty of speculative benefits based on its practical use. Similar (but often simpler) oligomeric proanthocyanidins have been regularly consumed orally at up to gram quantities from various plant species, such as grape (skin and seed extract), cocoa beans, green tea, blue berry, and many others used as food, beverages or dietary supplements for their presumed activities for protecting and prompting health.\(^37\)\(^38\)\(^49\)\(^-\)\(^51\)

Dragon’s blood has not been extensively studied clinically, and its efficacy as an anti-diarrheal agent has not been confirmed in well-designed clinical trials.\(^9\)\(^3\) Crofelemer has also been evaluated as a treatment of diarrhea-predominant irritable bowel syndrome patients.\(^58\) SP-303 has also been tested in pharmacology studies in animals (see the nonclinical section of this NDA) and in clinical trials of patients with diarrhea.

5.2 PHARMACOLOGICAL ACTIVITIES AND BIOASSAYS IN THE NDA

As the applicant demonstrated, crofelemer’s antisecretory effect is related to the inhibition of both cyclic adenosine monophosphate (cAMP)-stimulated cystic fibrosis transmembrane conductance regulator (CFTR) chloride ion (Cl\(^-\)) channel and the calcium-activated Cl\(^-\) channels (CaCC) at the luminal membrane of enterocytes (Module 2.7.2.5).\(^4\)\(^8\)\(^-\)\(^10\) The effect of crofelemer on CFTR-mediated Cl\(^-\) secretion was evaluated in multiple *in vitro* studies in human intestinal epithelial cells, Caco-2, and T84 cell monolayers. The effects of crofelemer on CaCC, an intestinal Cl\(^-\) transport channel distinct from CFTR, were also investigated in T84 cell monolayers.\(^9\) In addition, the inhibition of secretory diarrhea by crofelemer was studied in an *in vivo* mouse model, in
which cAMP mediated Cl⁻ secretion resulted in intestinal fluid accumulation and secretory diarrhea (SP303-E-069, SP303-E-070, and SP303-E-074). Primary pharmacodynamic studies of crofelemer are summarized in NDA Modules 2.6.2 and 2.6.3.

The estimated gastrointestinal lumen concentration of crofelemer following oral administration of the 125 mg BID dose was 178 μM (NDA Module 2.7.2.5.1 In Vitro Studies of Crofelemer Mechanism of Action). This concentration was 25-fold and 3.6-fold greater than the IC₅₀ for CFTR-mediated Cl⁻ secretion in T84 cells (IC₅₀ = 7 μM) and Caco-2 cells (IC₅₀ = 50 μM) respectively. This data correlated with the in vitro potency of crofelemer with its clinical efficacy. Dose-dependent effects of crofelemer on Isc of Caco-2 epithelial monolayers upon stimulation of forskolin were observed. The dose-effect curve indicated that the inhibitory effect of crofelemer was sensitive to its concentration below 120 μM, but the effect reached a plateau afterwards (the concentration tested was up 650 μM).

![Graph showing % Inhibition of cAMP-mediated Isc vs SP303 (mM)](image)

The estimated GI concentration of crofelemer (178 μM) after oral dosing (125 mg, bid) falls into the range where crofelemer’s effect is insensitive to dose variations (i.e., on the curve plateau, see above). Thus the therapeutic consistency will not be impacted by the tolerated variations of the BDS/BDP (i.e., still within the specifications). The applicant’s nonclinical studies confirmed that crofelemer has a unique mechanism to cause closure of chloride ion channels. Crofelemer action resisted washout with <50% reversal of CFTR inhibition after 4 hours. This observation suggests that the activity is not caused by direct pore occlusion. Thus, it is likely that the sizes and distributions of the proanthocyanidin molecules may not be fundamentally important for the pharmacological and clinical effects, and that controlling the proanthocyanidin oligomers in the BDS as a whole is appropriate.

The applicant did not propose to use any bioassays as a complementary quality control purpose. However, the knowledge of the mechanism and the existing diarrhea related experimental models suggesting that a clinically relevant bioassay is a possibility. The division medical team discussed the possibility of developing an in vitro pharmacological test as a bioassay for quality control. However, based on the evaluation of the multiple analytical methods that the applicant has developed for this NDA, BRT believes that the quality and consistency can be assured by employing those analytical methods without further support by a bioassay. BRT agrees that a bioassay, can be a valuable tool for supporting post-approval manufacturing changes, such as changes in BRM collection and
cultivation practices and manufacturing process. For example, clinically relevant bioassays can be further developed for qualifying new EGRs and new *C. lechleri* cultivation sites, especially if those sites are outside of the established EGRs.

For further discussion on pharmacology, please refer to the division Pharmacology and Toxicology review by Snithi T. King, Ph.D., Medical review by Wen-Yi Gao, MD, and Clinical Pharmacology review by Kristina Estes, Pharm.D.

5.3 TOXICITIES DATA FROM LITERATURE AND NDA

The previous human use of Dragon’s blood did not suggest that the latex is toxic, given that the BRM is the most popularly used herbal medicine for various symptom relief purposes.

Contradicting data on the mutagenic potential of *C. lechleri* latex have been reported. In *vitro* studies detected that *C. lechleri* latex (collected from Ecuador) had mutagenic activity when investigated on *Salmonella typhimurium* strains TA98 and TA100, either with or without S9 activation. Another Ames test also reported a *C. lechleri* latex sample from Peru had significant mutagenic activity when tested on TA1535 of *S. typhimurium* in the presence of metabolic activation, while a weak mutagenic activity was detected for strain TA98. However, *C. lechleri* latex may actually possess antimutagenic properties when incubated with human cell lines. The latex can reduce the mutagenic activities induced by both indirectly and directly acting mutagens and can also inhibit the proliferation of human leukemic cells. An *in vivo* study detected no carcinogenic activity or promoter of skin tumors in mice treated with *C. lechleri* latex and taspine for 17 months. These positive Ames tests are not significant safety concerns, since the negative result from the *in vivo* mutagenicity study, and certain protective *in vitro* and *in vivo* activities of proanthocyanidins. Please refer to the division pharmacology and toxicology review on whether carcinogenicity studies are necessary.

There is no indication that taspine may cause safety concerns, although alkaloids are typically possess more potent pharmacological/toxicological effects than other botanical ingredients, such as lipid and carbohydrates.

The major and active components in the BDS are the proanthocyanidin oligomers. Proanthocyanidins are a group of compounds that exist abundantly in many plant species, including some commonly consumed food/vegetables (such as tea, grapes, cocoa, among others). The extensive human consumption of proanthocyanidin oligomers from food or dietary supplements has often being reviewed as beneficial without raising serious safety concerns, especially at relatively moderate doses.

For further discussion on toxicology of the NDA, please refer to the division pharmacology and toxicology review.
Overall, BRT has no safety concerns with crofelemer, which contains primarily proanthocyanidin oligomers, a group of compounds widely found in many edible plants. For a complete nonclinical toxicology data and further safety related discussion, please refer to the division pharmacology and toxicology review.

5.4 SUMMARY

CPL, commonly known as Dragon’s blood, has been commonly used orally as an herbal medicine in Peru and other Central and South American countries for the treatment of diarrhea, cholera, and stomach ulcer, and other GI symptoms. The herbal medicine use of CPL does not suggest any individual component is responsible for the anti-diarrheal activities. The effect of CPL as an antidiarrheal agent in herbal medicine use is consistent with the findings the well-controlled clinical trials.

The proanthocyanidin oligomers, the major active components of crofelemer, are similar to the polyphenolics in numerous plant species used as food. Those polyphenolic compounds are considered to be safe with extensive daily dietary exposure or even beneficial due to their potent antioxidant activities.

The previous human use of CPL dose not reveal serious safety concerns. This is in line with the results of the clinical studies, and animal toxicology studies. The effect of crofelemer (and the related product, SP-303) on CFTR-mediated Cl⁻ secretion was evaluated in multiple in vitro studies and animal models suggesting that crofelemer had a new mechanism of action for its antidiarrheal effect. Preliminarily clinical studies of SP-303, including treatment of traveler’s diarrhea, were reported to be effective.

Pharmacology studies in the NDA and data from journal publications indicated that the mechanism of crofelemer’s antisecretory activities probably involve inhibition of both cyclic cAMP)-stimulated CFTR Cl⁻ channel and the CaCC at the luminal membrane of enterocytes, with dose-dependent effects observed in certain in vitro bioassays. Some of the clinical relevant assays may be further developed after approval for quality control purposes, such as to qualifying new CPL sources from new EGRs and cultivated C. lechleri trees.
6. BOTANICAL SPECIFIC CLINICAL ISSUES

6.1 ENSURING THERAPEUTIC CONSISTENCY FOR BOTANICALS

One of most critical and difficult issues for a botanical new drug is how to ensure "therapeutic consistency" in all marketing batches. Physical and chemical consistency of the BDS and BDP falls primarily into the conventional chemistry, manufacturing, and manufacturing control (CMC) area, with a case-by-case consideration of the complexity of the drug substance. The more complex a mixture the BDS is, the closer it will resemble the BRM, and thus more efforts and attention will need to be given to the GACP and related BRM control, which will be discussed below as “Pre-CMC.” The manufacturing process will also need to be tightly controlled. Under this scenario, the BDS and BDP characterization and quality control should be given more room to accommodate the naturally occurring variations, and evaluating a sponsor’s plan to ensure consistency should also take practicality into consideration. In addition, the effects of naturally-occurring BDS variations on clinical response can be assessed through “Post-CMC” measures, which may include one or more of the following: a clinically relevant bioassay, clinical dose-response data, testing multiple batches in phase 3 clinical trials, and past human use for the same condition.

6.1.1 PRE-CMC

The applicant developed GACP procedures for guiding the CPL collection, storage and transportation. The illustrated characteristic features of mature *C. lechleri* trees can be effectively used by trained field collectors to prevent misuse of other *Croton* species or non-*Croton* species that also produce red latex. The applicant collected multiple CPL batches from &b[4] EGRs to manufacture the phase 3 clinical BDS batches. In order to prevent additional variations in the CPL and BDS, only those LB[4] EGRs will be accepted for CPL collection post-approval. Other EGRs and cultivated sources need to be pre-qualified before collection of CPL for manufacturing market batches of BDS. The CPL batches collected from *C. lechleri* will be tested for total phenolic components, crofelemer contents, taspine, among other physical and chemical parameters with specific specifications.

Over 200 batches of BDS have been manufactured from CPL by the applicant. All batches of CPL meeting the established specification were accepted for producing BDS batches, and the subsequently made BDS batches also met the current specifications, indicating that the naturally occurring chemical variations in CPL do not impact the BDS quality in any significant way.

For future re-forestation or cultivation as a mono-species in large concentrated areas, the cultivation sites need to be further evaluated as a post-approval project. From BRT’s perspective, if the GACP is executed properly, i.e., without too much alteration of the natural environment, those cultivations sites inside EGRs LB[4] can be accepted if the physical and chemical profiles of the CPL and subsequently manufactured BDS batches meet their respective specifications. If one can be developed post-approval, a clinically relevant bioassay should be used as an important confirmation factor as well.
6.1.2 CONVENTIONAL CMC

The complex nature of crofelemers proanthocyanidin oligomers along with the huge number of closely related molecules makes characterization of the intact proanthocyanidin oligomers at a molecular level impossible. However, by utilizing a well established acid hydrolysis method and analyzing the depolymerization products by HPLC, the average molecular weight and average monomer units can be obtained as one of the important identity tests for crofelemers BDS. MS and NMR analysis of the BDS also provide valuable identity data of intact proanthocyanidin oligomers.

The applicant developed

The applicant has used the method to test 3 batches of BDS and reported detection of and the MS data with an established MW table can become part of the identity tests for the intact oligomers in crofelemers.

A comprehensive approach utilizing the three HPLC methods described above coupled with can provide the following data for characterization, identification, and quality control of crofelemers:

• The has been accepted by ONDQA team. This method is also used for controlling other related compounds.
From BRT’s perspective, the applicant’s methods reflect the current level of technology and the applicant’s analytical methods use state-of-the-art instrumentation for drugs and foods. Due to the extremely complex nature of crofelemer, the comprehensive approach as described is necessary and adequate for establishing the unique identity of the BDS for quality control.

### 6.1.3 POST-CMC

Multiple BDS and BDP batches were tested in various stages of the clinical development, including a phase 3 clinical trial. The clinical data suggest that oligomer compositions (e.g., DP, size of the oligomers) may not be critical for the oligomers to have the desired pharmacological activities on the Cl⁻ ion channels to be an effective anti-diarrheal agent. The clinical trial suggested that the clinical response is not sensitive to the dose range tested. The multiple-batch trial without apparent differences in clinical effects is supportive that the level of consistency of the BRM and the subsequently manufactured BDS (through the standard manufacturing process) is acceptable.

### 6.2 DISCUSSION AND CONCLUSIONS

From BRT’s perspective, the overall quality control of the raw material, the manufacturing process and the above mentioned conventional CMC quality and identity tests and assays (mainly the three HPLC methods and the acid hydrolysis test) can adequately control the BDS and BDP to ensure the consistency of crofelemer in terms of both quality and therapeutic effects.

The mechanistic study on chloride channel closure also showed that the clinical dose will provide a saturated effect, and is thus not sensitive to minor variations in the BDS.
7. **OVERALL CONCLUSIONS AND RECOMMENDATIONS**

The applicant has developed and implemented GAPC procedures for CPL. No safety concerns have arisen from CPL’s extensive human use, or from clinical studies of crofelemer and similar products derived from the CPL. We recommend further control of the potential contaminants (i.e., pesticides, heavy metals, and aflatoxins) in the BRM and BDS and the applicant is committed to comply with our recommendations. The applicant’s overall CPL BRM quality control is satisfactory. BRT provided other recommendations to the applicant for CPL collection (e.g., clearly defined EGR boarders, particular tree size and age for collection) and quality control (e.g., high and low limits for phenolics and taspine) and the applicant accepted those recommendations.

The applicant adapted the following comprehensive approach by employing modern spectroscopic and spectrometric technology (e.g., UV, IR, MS, and NMR) to characterize and identify the structures of the proanthocyanidin oligomers, and the related compounds and impurities, such as . In addition, the applicant also developed multiple analytical chromatography methods for the quality control of the BDS active components, proanthocyanidin oligomers, and other components, to ensure the quality and consistency of the BDS. BRT review recommends the following:

- Accept the acid hydrolysis method to determine the ranges of PC/PD ratios and relative molecular weight, and establish specifications based on data from clinical batches.
- Accept
- Accept
- Accept

The applicant has established spectroscopic and spectrometric techniques, and the applicant’s analytical chromatography methods represent the current state of scientific methodology available for proanthocyanidin oligomers and polymers. The large number of closely related proanthocyanidin molecules provides great challenges to the scientific community to adequately separate and identify those individually or even grouped according to their DP. However, the applicant has provided enough molecular details to characterize and uniquely identify crofelemer proanthocyanidins. Using a representative BDS batch as a reference standard and multiple HPLC methods with different separation and detection techniques can adequately measure the combined weight and area.
percentages of all proanthocyanidin oligomers together for the quality and consistency of the BDS.

BRT considers that controlling the active proanthocyanidins as whole is appropriate and adequate, since crofelemer’s clinical effect appears to not be sensitive to the dose ranges tested (e.g., 250, 500, 1000 mg/day). The clinically observed anti-diarrheal activity of oligomeric proanthocyanidins in crofelemer appears to be related to its general polyphenolic nature, and does not appear to be dependent on certain individual molecules with particular molecular size(s).

Overall, it is BRT’s evaluation that the applicant’s controls of CPL, BDS, and the manufacturing process are adequate to ensure the safety, efficacy and consistency of the drug product. The applicant’s overall approach is comprehensive and adequate for approval of this botanical NDA.

BRT has the following recommendations for the applicant to conduct additional quality-related studies to improve the quality control of the BRM and BDS post-approval of the NDA:

- Further study the CPL and qualify new EGRs and cultivation sites for sustained harvest of CPL. FDA review and approval will be necessary before incorporating any changes for collection of CPL or using the CPL for commercial production of BDS.
- Evaluate the possibility of establishing a clinically relevant bioassay for qualifying future manufacturing changes and qualifying new CPL EGRs, especially those outside the region of the current proposed EGRs.
- Additional EGRs and cultivated CPL sources may also be qualified by clinical studies of crofelemer for the same or other related GI indications.
- Continue further characterization and analysis of the BDS by existing and/or new HPLC/MS/NMR methods and establish more comprehensive fingerprint libraries.
- Utilize HPLC/MS-MS to establish the sequence of some of the oligomers with relatively low molecular weights (e.g., DP = 3-6) to shed more light on the composition of the crofelemer BDS.
REFERENCES:


41. Zhang LL, Lin YM. HPLC, NMR and MALDI-TOF MS analysis of condensed tannins from Lithocarpus glaber leaves with potent free radical scavenging activity.


This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JINHUI DOU
08/08/2012

SHAW T CHEN
08/08/2012
Application Type: NDA 505(b)(1)
NDA Number: 202-292
Stamp Date: 12-05-2011
Applicant: Salix Pharmaceuticals
DMF #: NA

Drug Name: Crofelemer
Brand Name: TBD
Priority Designation: Priority Review
PDUFA Date: 06-05-2012
Dosage Form: 125 mg tablets
Route of Administration: Oral
Botanical Raw Material: The red, viscous latex of the plant Croton lechleri of the family Euphorbiaceae
Botanical Drug Substance: Oligomeric proanthocyanidins of multiple chain lengths with an average molecular weight range of 1700 – 2500 Daltons and identified monomer units of (+)-catechin, (-)-epicatechin, (+)-gallocatechin, and (-)-epigallocatechin.
Indication(s) requested: Indicated for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy.

Botanical Review Team Reviewer: Jinhui Dou, Ph.D.
Filing Review Completion Date: 02-03-2012
Botanical Review Team Leader: Shaw T. Chen, M.D., Ph.D.

Review Division: Division of Gastroenterology Products (HFD-180)
BRT FILING REVIEW SUMMARY

- BRT has identified no safety and quality related issues that may affect the filing of this botanical NDA, Croflemer (125 mg, tablet). The botanical drug is indicated for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy. Because the unmet medical need and favorable safety and efficacy profile, according to preliminary medical review, the NDA is granted for priority review.

- The previous oral and topical human applications (for the treatment of diarrhea and wound healing, among others) of the botanical raw material (BRM), the red and viscous latex of the plant Croton lechleri Muell. Arg. [Fam. Euphorbiaceae], commonly known as Dragon’s blood, do not raise significant safety concerns.

- The sponsor provided specifications of the botanical raw material (BRM), and outlined good agricultural and collection practice (GACP). Historically, the Dragon’s blood was collected from a standing tree by slashing the bark. The sponsor has taken efforts of re-forestation and cultivation of C. lechleri since the 1990s.

- One review issue is how to determine the acceptability of the proposed geographic Growing Regions (EGRs), of the larger EGRs with more than were used to produce BRM batches for the phase 3 clinical trials. The smaller EGRs were analyzed and accepted, but were not tested to in phase 3 trials.

- The 2nd review issue is whether the BRM quality is consistent among various batches.

- The 3rd review issue is whether the analytical methodology for the drug substance will be accepted by ONDQA chemists. If any of the assays for the major oligomers need to be changed by ONDQA, then new specifications from the newly adopted methods may be necessary.

BRM Quality and GACP Related Filing Checking List
Is there any NDA filing issues identified in this BRT filing review? N
[If Yes, provide detailed descriptions of all filing issues.]

Are the BRM sections in the NDA well organized and eligible? Y
Does the sponsor correctly identify and describe the plant species for BRM? Y
Does the sponsor propose a specification for the BRM? Y
Does the sponsor define and specify the collection/cultivation areas? Y
Does the sponsor address the potential misuse issues of the related species? Y
Dose the sponsor provide training to the field collectors of the BRM? Y
Does the sponsor address environmental and contamination related safe issues? Y

BRT Review Issues to Be Communicated to the Sponsor
1. Provide more detailed and accurate map of the EcoGeographic Growing Regions (EGRs, with GPS locations, if possible).
2. Compare the analytical data of representative batches of Crude Plant Latex (CPL) botanical raw material (BRM) from different EGRs to show levels of naturally occurring variations in their chemical profiles. Particularly, CPL/drug substance/product samples from EGRs should be compared with the samples from EGRs.
3. Provide analytical data of representative batches of CPL from cultivated and wild grown Croton lechleri tree to show levels of naturally occurring variations.
4. Provide a list of historic Crofelemer (or similar products) and drug substance batches manufactured from CPL batches collected from EGRs. If some of the batches from EGRs have been tested in human clinical trials, provide summaries of safety and efficacy data and compare with the clinical data of the phase 3 trials.
5. Provide clarification why one batch (No. RARM061378) of CPL contains Crofelemer content at lower than the specification of NLT [Section 3.2.S.2.3 Control of Materials], “Crofelemer Crude Plant latex - Botanical Raw Material Information”, Table 3 on page 24].

Botanical Team Filing Review Notes:
The sponsor provided botanical raw material (BRM) related documents/information under Section 3.2.S.2.3 Control of Materials, including a 50-page “Crofelemer Crude Plant latex - Botanical Raw Material Information” and a 59-page “Sustainable Harvesting Manual” in both English and Spanish, among others.

The sponsor provided characteristic botanical descriptions, taxonomy and nomenclature of Croton lechleri Muell. Arg. and practical details for distinguishing C. lechleri from related Croton species. One of the characteristic of C. lechleri is that there are multiple petiolar glands which can be easily viewed on the back of the leaves. Photos/copies of authentic specimen and different parts of live trees (including freshly collected CPL) in the field were provided. Physical and chemical properties of CPL were described. Previous human medicinal use (such as wound healing, treatment of diarrhea and other diseases) in South America was described.

Historically, the Dragon’s blood was collected by slashing the bark from a standing tree. Most of the trees were collected from the wild. The sponsor outlined the Good Agricultural and
Collection practice (GACP). The sponsor stated that the plantation should be manually weeded and pruned during the 1st two years after planting.

Ecological habitats, distribution, and density of *C. lechleri* were described. Favorable environmental and climate conditions for *C. lechleri* and CPL yield were outlined. However, the relationships between the growing season/environment/region and the chemical profiles of CPL were not thoroughly studied. The sponsor referenced sampling data to indicate that all CPL from 1991-1997 (from mostly wild collection of trees at various locations throughout the year) were tested and met the pre-defined release criteria. The sponsor stated that all CPL batches used in the production of phase 3 clinical batches of Crofelemer were acceptable and produced releasable batches of Crofelemer drug substance. However, one BRM batch (No. RARM016378) contains only [redacted] of Crofelemer Content (HPLC on anhydrous basis) and was clearly out of specification (NLT [redacted]). If this Crofelemer content has no effect on the drug substance quality, then the BRM specification may be changed. Nevertheless, the sponsor should clarify and determine the appropriate specification for Crofelemer content.

Currently, the sponsor selected [redacted] EcoGeographic Growing Regions (EGRs), namely, [redacted] EGRs were used to produce phase 3 clinical batches of the drug substance. BRM (CPL) from EGRs [redacted] has been analyzed, accepted and used to produce drug substance by the previous sponsor (and may be used in other human trials). The map describing the EGRs are not detailed enough to accurately define the regions. It appears that the EGRs [redacted] EGRs combined.

The sponsor provided BRM Specification (Table 11) in the NDA. The tested items in the specification table include description, identification, [redacted], pH, total phenolics, content of taspine (an alkaloid that the sponsor considers as an impurity for presumed safety concerns), content of Crofelemer, and chromatographic purity. The sponsor discussed the stability of the CPL and stated that no BRM quality issues have been identified from historical production of batches of Crofelemer botanical drug substance (and similar products), suggesting that CPL is stable and reasonably consistent quality wise.

**Discussion and Conclusions:**

The botanical raw material (BRM, code name CPL) of Crofelemer is the red latex of *Croton lechleri*, commonly known as Dragon’s blood. The latex has been commonly used in South America as an herbal medicine. No safety concerns from previous human use have been identified. The sponsor identified one alkaloid (taspine) as unwanted
The doses of Crofelemer (125 mg, 250 mg, 500 mg per day) that have been tested in both patients and healthy volunteers are lower than or comparable to the equivalent doses of Dragon's blood used as herbal medicine.

Quality variations of the BRM (CPL) related to growing conditions (e.g., different EGRs, cultivated vs wild grown, different seasons, etc.) have not been extensively studied. However, the sponsor stated that all historical drug substance (or API) batches (from 1991 to the most recent phase 3 batches) met the pre-specified specifications and released. The preliminary review of available data suggests that the BRM batches have consistent quality and acceptable stability. How to correlate the BRM quality to BDS quality control will be a review issue and pending further review of sponsor provided data. It was noticed that one batch of the BRM does not meet the specification.

The immediate concern regarding BRM quality control is whether all the EGRs can be accepted for producing BRM post-NDA approval. For the 1st approved botanical NDA (21-902), BRT recommend to restrict the raw material production from two local farms. The arguments were that there are very large chemical and genomic heterogeneity among the hundreds of cultivars growing in different climate regions. GACP issues are too important to ignore also because extensive human intervention (e.g., breeding of new cultivars through long history of tea cultivation, use of pesticides and fertilizer, pruning, etc.), which may also impact the chemical profiles of green tea and thus the efficacy/safety of the drug product.

From a pure quality perspective, however, the Croton lechleri species has not been a subject to extensive human intervention, and it is thus expected to be more homogeneous in terms of chemical and genomic profiles among trees grown in various regions (with similar climate conditions, e.g., rainforest). The naturally occurring quality variations among BRM batches and how the BRM quality will impact the BDS/BDP quality and therapeutic consistency should be further evaluated by the sponsor. BRT will provide comments to recommend the sponsor to address those concerns as review issues. From BRT's perspective, the NDA is acceptable for filing.
This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JINHUI DOU
02/03/2012

SHAW T CHEN
02/06/2012
**CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement**

<table>
<thead>
<tr>
<th>NDA/BLA Number:</th>
<th>NDA 202-292/000</th>
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<tbody>
<tr>
<td>Applicant:</td>
<td>Salix Pharmaceuticals, Inc.</td>
</tr>
<tr>
<td>Stamp Date:</td>
<td>December 6, 2011</td>
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<tr>
<td>Drug Name:</td>
<td>Crofelemer</td>
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<tr>
<td>NDA/BLA Type:</td>
<td>505 (b)(1)</td>
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On initial overview of the NDA/BLA application for filing:

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<td><strong>FORMAT/ORGANIZATION/LEGIBILITY</strong></td>
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<tr>
<td>1. Identify the general format that has been used for this application, e.g. electronic CTD.</td>
<td>X</td>
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<td>Electronic CTD</td>
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<tr>
<td>2. On its face, is the clinical section organized in a manner to allow substantive review to begin?</td>
<td>X</td>
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<td>3. Is the clinical section indexed (using a table of contents) and paginated in a manner to allow substantive review to begin?</td>
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<td>4. For an electronic submission, is it possible to navigate the application in order to allow a substantive review to begin (e.g., are the bookmarks adequate)?</td>
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<tr>
<td>5. Are all documents submitted in English or are English translations provided when necessary?</td>
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<td>6. Is the clinical section legible so that substantive review can begin?</td>
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<tr>
<td><strong>LABELING</strong></td>
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<tr>
<td>7. Has the applicant submitted the design of the development package and draft labeling in electronic format consistent with current regulation, divisional, and Center policies?</td>
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<td><strong>SUMMARIES</strong></td>
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<tr>
<td>8. Has the applicant submitted all the required discipline summaries (i.e., Module 2 summaries)?</td>
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<td>9. Has the applicant submitted the integrated summary of safety (ISS)?</td>
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<tr>
<td>10. Has the applicant submitted the integrated summary of efficacy (ISE)?</td>
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<td>11. Has the applicant submitted a benefit-risk analysis for the product?</td>
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<td>12. Indicate if the Application is a 505(b)(1) or a 505(b)(2). If Application is a 505(b)(2) and if appropriate, what is the reference drug?</td>
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<td>The Application is a 505(b)(1).</td>
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<td><strong>DOSE</strong></td>
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<tr>
<td>13. If needed, has the applicant made an appropriate attempt to determine the correct dosage and schedule for this product (i.e., appropriately designed dose-ranging studies)?</td>
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<td>■ Study Number: NP303-101 (Stage 1)</td>
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<tr>
<td>■ Study Title: Randomized, Double-Blind, Parallel-Group, Placebo-Controlled, Two-Stage Study to Assess the Efficacy and Safety of Crofelemer 125 mg, 250 mg, and 500 mg Orally Twice Daily for the Treatment of HIV-Associated Diarrhea (ADVENT Trial)</td>
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<td>■ Sample Size of Stage 1: Total (194 patients), Crofelemer (144 patients), and placebo (50 patients);</td>
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<tr>
<td>■ Arms: Crofelemer 125 mg bid (44 patients); Crofelemer 250 mg bid (54 patients); Crofelemer 500 mg bid (46</td>
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File name: 5_Clinical Filing Checklist for NDA_BLA or Supplement 010908 1
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<tr>
<td>patients); and Placebo (50 patients).</td>
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<tr>
<td>Study Sites: 67 study sites in the United States and 3 sites in Puerto Rico.</td>
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<tr>
<td>Location in submission: The study report for NP301-01 is in Volume 5 of the submission.</td>
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**EFFICACY**

14. Do there appear to be the requisite number of adequate and well-controlled studies in the application?  
   - Pivotal Study #1: NP303-101  
     - Indication: Treatment of HIV-associated diarrhea on anti-retroviral therapy  
   - Pivotal Study #2: N/A  
     - Indication: N/A  
   X This study consisted of two stages with independent patient enrollments. This reviewer believes that the two stages can be considered two adequate and well-controlled studies.

15. Do all pivotal efficacy studies appear to be adequate and well-controlled within current divisional policies (or to the extent agreed to previously with the applicant by the Division) for approvability of this product based on proposed draft labeling?  
   X To the extent agreed to previously with the applicant by DGIEP

16. Do the endpoints in the pivotal studies conform to previous Agency commitments/agreements? Indicate if there were not previous Agency agreements regarding primary/secondary endpoints.  
   X

17. Has the application submitted a rationale for assuming the applicability of foreign data to U.S. population/practice of medicine in the submission?  
   X

**SAFETY**

18. Has the applicant presented the safety data in a manner consistent with Center guidelines and/or in a manner previously requested by the Division?  
   X

19. Has the applicant submitted adequate information to assess the arythmogenic potential of the product (e.g., QT interval studies, if needed)?  
   X

20. Has the applicant presented a safety assessment based on all current worldwide knowledge regarding this product?  
   X

21. For chronically administered drugs, have an adequate number of patients (based on ICH guidelines for exposure1) been exposed at the dose (or dose range) believed to be efficacious?  
   X Lack of 1 year exposure in any patient. Extended study is ongoing; thus, there may be patients with exposure > 1 year at time of safety update submission.

---

1 For chronically administered drugs, the ICH guidelines recommend 1500 patients overall, 300-600 patients for six months, and 100 patients for one year. These exposures MUST occur at the dose or dose range believed to be efficacious.

File name: 5_Clinical Filing Checklist for NDA_BLA or Supplement 010908

Reference ID: 3073878
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<tr>
<td>22. For drugs not chronically administered (intermittent or short course), have the requisite number of patients been exposed as requested by the Division?</td>
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<tr>
<td>23. Has the applicant submitted the coding dictionary(^2) used for mapping investigator verbatim terms to preferred terms?</td>
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<tr>
<td>24. Has the applicant adequately evaluated the safety issues that are known to occur with the drugs in the class to which the new drug belongs?</td>
<td>X</td>
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<td>25. Have narrative summaries been submitted for all deaths and adverse dropouts (and serious adverse events if requested by the Division)?</td>
<td></td>
<td>X</td>
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<tr>
<td><strong>OTHER STUDIES</strong></td>
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<tr>
<td>26. Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?</td>
<td></td>
<td>X</td>
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<tr>
<td>27. For Rx-to-OTC switch and direct-to-OTC applications, are the necessary consumer behavioral studies included (e.g., label comprehension, self selection and/or actual use)?</td>
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<td>X</td>
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<td><strong>PEDIATRIC USE</strong></td>
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<tr>
<td>28. Has the applicant submitted the pediatric assessment, or provided documentation for a waiver and/or deferral?</td>
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<tr>
<td><strong>ABUSE LIABILITY</strong></td>
<td></td>
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<tr>
<td>29. If relevant, has the applicant submitted information to assess the abuse liability of the product?</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td><strong>FOREIGN STUDIES</strong></td>
<td></td>
<td></td>
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<tr>
<td>30. Has the applicant submitted a rationale for assuming the applicability of foreign data in the submission to the U.S. population?</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td><strong>DATASETS</strong></td>
<td></td>
<td></td>
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<tr>
<td>31. Has the applicant submitted datasets in a format to allow reasonable review of the patient data?</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>32. Has the applicant submitted datasets in the format agreed to previously by the Division?</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>33. Are all datasets for pivotal efficacy studies available and complete for all indications requested?</td>
<td>X</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\(^2\) The “coding dictionary” consists of a list of all investigator verbatim terms and the preferred terms to which they were mapped. It is most helpful if this comes in as a SAS transport file so that it can be sorted as needed; however, if it is submitted as a PDF document, it should be submitted in both directions (verbatim -> preferred and preferred -> verbatim).

File name: 5_Clinical Filing Checklist for NDA_BLA or Supplement 010908
<table>
<thead>
<tr>
<th>Content Parameter</th>
<th>Yes</th>
<th>No</th>
<th>NA</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>34. Are all datasets to support the critical safety analyses available and complete?</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>35. For the major derived or composite endpoints, are all of the raw data needed to derive these endpoints included?</td>
<td>X</td>
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</tr>
</tbody>
</table>

**CASE REPORT FORMS**

| 36. Has the applicant submitted all required Case Report Forms in a legible format (deaths, serious adverse events, and adverse dropouts)? | X   |    |    |         |
| 37. Has the applicant submitted all additional Case Report Forms (beyond deaths, serious adverse events, and adverse drop-outs) as previously requested by the Division? | X   |    |    |         |

**FINANCIAL DISCLOSURE**

| 38. Has the applicant submitted the required Financial Disclosure information? | X   |    |    |         |

**GOOD CLINICAL PRACTICE**

| 39. Is there a statement of Good Clinical Practice; that all clinical studies were conducted under the supervision of an IRB and with adequate informed consent procedures? | X   |    |    |         |

**IS THE CLINICAL SECTION OF THE APPLICATION FILEABLE?** Yes.

If the Application is not fileable from the clinical perspective, state the reasons and provide comments to be sent to the Applicant. N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Sponsor should be requested to provide the “coding dictionary” consisting of a list of all investigator verbatim terms and the preferred terms to which they were mapped.

{See appended electronic signature page}

Reviewing Medical Officer

{See appended electronic signature page}

Clinical Team Leader
This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WEN-YI GAO
01/19/2012

ANIL K RAJPAL
01/19/2012