Dear Healthcare Professional,

Thank you for your unsolicited request for information. Accompanying this letter is the following information you requested on Purified Cortrophin® Gel. If we can be of any further assistance, please contact our Medical Information department at (844) CORT-GEL (844-267-8435) between the hours of 9:00 AM to 7:00 PM ET (6:00 AM to 4:00 PM PT), Monday through Friday or via email at cortrophinmedinfo@anipharmaceuticals.com.

Purified Cortrophin Gel is indicated in the following disorders:

1. Rheumatic disorders:
As adjunctive therapy for short-term administration (to tide the patient over an acute episode or exacerbation) in:
- Psoriatic arthritis.
- Rheumatoid arthritis, including juvenile rheumatoid arthritis (selected cases may require low-dose maintenance therapy).
- Ankylosing spondylitis.
- Acute gouty arthritis.

2. Collagen diseases:
During an exacerbation or as maintenance therapy in selected cases of:
- Systemic lupus erythematosus.
- Systemic dermatomyositis (polymyositis).

3. Dermatologic diseases:
Severe erythema multiforme (Stevens-Johnson syndrome).
Severe psoriasis.

4. Allergic states:
Atopic dermatitis
Serum sickness.

5. Ophthalmic diseases:
Severe acute and chronic allergic and inflammatory processes involving the eye and its adnexa such as:
- Allergic conjunctivitis.
- Keratitis.
- Iritis and iridocyclitis.
- Diffuse posterior uveitis and choroiditis.
- Optic neuritis.
- Chorioretinitis.
- Anterior segment inflammation.

6. Respiratory diseases:
Symptomatic sarcoidosis.

7. Edematous states:
To induce a diuresis or a remission of proteinuria in the nephrotic syndrome without uremia of the idiopathic type or that due to lupus erythematosus.

8. Nervous system:
Acute exacerbations of multiple sclerosis.

Purified Cortrophin Gel is contraindicated for intravenous administration.

Purified Cortrophin Gel is contraindicated in patients with scleroderma, osteoporosis, systemic fungal infections, ocular herpes simplex, recent surgery, history of or the presence of a peptic ulcer, congestive heart failure, hypertension, or sensitivity to proteins derived from porcine sources.

Purified Cortrophin Gel is contraindicated in patients with primary adrenocortical insufficiency or adrenocortical hyperfunction.

Please see the enclosed Purified Cortrophin Gel Prescribing Information (PI) for detailed information including Warnings and Precautions and Adverse Reactions as well as the appropriate use of Purified Cortrophin Gel.



Thank you for your inquiry.

Sincerely,

Steve Wu, PharmD
ANI Pharmaceuticals Medical Information

# Evaluation of the Potential Immunogenicity of Purified Cortrophin® Gel (Repository Corticotropin Injection USP) 80 U/mL

## Introduction

- This document summarizes the assessment of the potential immunogenicity of Purified Cortrophin Gel
- The active agent in Cortrophin Gel is porcine-derived adrenocorticotropic hormone (ACTH peptide, amino acids 1-39).[1] Cortrophin Gel is of the same class as other, FDA-approved natural-product[2] corticotropins and is chemically similar to endogenous, human ACTH.[4]

**Note that this document is for information purposes only. This document may contain information not included in the prescribing information for Cortrophin Gel. The usage of corticotropin being discussed may not have been approved by the FDA as safe and effective. ANI Pharmaceuticals does not recommend the use of its products in any manner inconsistent with the FDA-approved labeling.**

**To report an Adverse Event for any ANI Pharmaceuticals product, please call 1-800-308-6755 or contact the FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.**

**Email: drugsafety@anipharmaceuticals.com.**

## Background[1]

Purified Cortrophin Gel (Repository Corticotropin Injection USP) is approved by the FDA for the treatment of the following disorders:

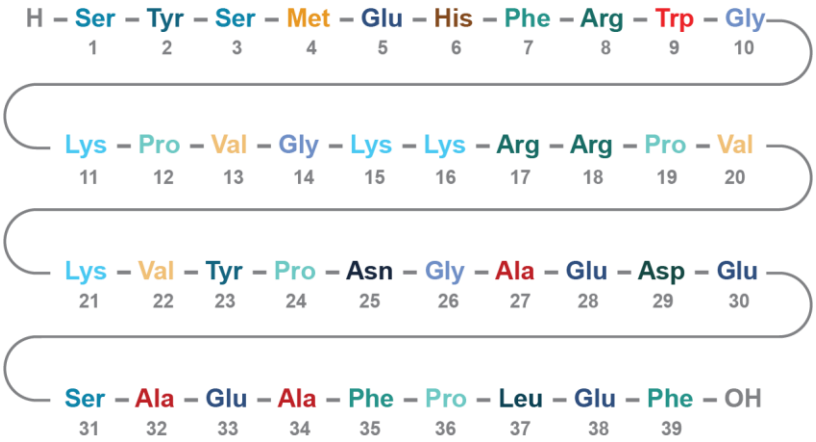
- Rheumatic disorders as adjunctive therapy for short-term administration (to tide the patient over an acute episode or exacerbation in psoriatic arthritis, rheumatoid arthritis, including juvenile rheumatoid arthritis (selected cases may require low-dose maintenance therapy), ankylosing spondylitis, and acute gouty arthritis
- Collagen diseases during an exacerbation or as maintenance therapy in selected cases of systemic lupus erythematosus and systemic dermatomyositis (polymyositis)
- Dermatologic diseases, including severe erythema multiforme (Stevens-Johnson syndrome) and severe psoriasis
- Allergic states, such as atopic dermatitis and serum sickness
- Ophthalmic diseases, including severe acute and chronic allergic and inflammatory processes involving the eye and its adnexa, such as allergic conjunctivitis, keratitis, iritis and iridocyclitis, diffuse posterior uveitis and choroiditis, optic neuritis, chorioretinitis, and anterior segment inflammation
- Respiratory diseases, including symptomatic sarcoidosis
- Edematous states, to induce a diuresis or a remission of proteinuria in the nephrotic syndrome without uremia of the idiopathic type or that due to lupus erythematosus
- Nervous system, such as acute exacerbations of multiple sclerosis

## Composition of Cortrophin Gel

Cortrophin Gel is a porcine-derived purified corticotropin (ACTH) in a sterile solution of gelatin. It is made up of a complex mixture of ACTH, ACTH-related peptides, and other porcine pituitary-derived peptides.[1]

The drug product is a sterile preparation containing 80 USP units per mL and it contains 0.5% phenol (as preservative), 15.0% gelatin (for prolonged activity), water for injection, and the pH is adjusted with hydrochloric acid and sodium hydroxide.[1]

Cortrophin Gel contains the porcine-derived ACTH (1-39) with the following amino acid sequence[1]:




Cortrophin Gel is the anterior pituitary hormone which stimulates the functioning adrenal cortex to produce and secrete adrenocortical hormones.[1]

The potential immunogenicity of Purified Cortrophin® Gel has been assessed based upon an evaluation of product, mechanism, patient factors, and published literature. Additionally, *in silico* predictive analysis of potential T-cell epitopes, T-cell proliferation assay, and *in vitro* assessment for innate immunity were conducted. These assessments were conducted in a manner consistent with the FDA *Guidance's for Industry – Immunogenicity Assessment for Therapeutic Protein Products (2014), Guidance for Industry – Assay Immunogenicity Testing of Therapeutic Protein Products – Developing and validating assays for anti-drug antibody detection (2019),* EMA *Guideline on Immunogenicity Assessment of Therapeutic Proteins (2017)*.

## Study Design

**Immunogenicity Risk Assessment (IRA)**

ANI has taken a wholistic approach to assessing the potential of PCG to illicit an immune response in patients. The evaluation of potential risk for immunogenicity considered both types of impurities, product and process related, to adequately inform the risk assessment and enable determination of the overall immunogenicity risk. The types and the levels of impurities, the methods used for evaluation of impurities, and justification for why such impurity types/levels do not impact the safety, the effectiveness, and the immunogenicity were considered whilst developing the IRA.

The risk assessment served to address patient or product specific factors that can affect immunogenicity, particularly with the multiple indications as proposed in the package insert.

This risk assessment took into consideration the following:

1. Product-specific Factors

    a. Product origin

    b. Primary molecular structure

    c. Impurities

d. Impurities with adjunct ACTH activity

e. Manufacturing

f. Formulation

g. Container closure/product custody

2. Mechanism of Action

3. Patient Factors: Patient factors that were considered included an assessment of dose/duration of treatment/treatment regimen, concomitant medications, endogenous protein homology to test article, and immune responses for the proposed clinical indications.

In addition to these critical items, the IRA was informed by modern tools at the cutting edge of peptide therapeutics including *in silico* predictive analysis of potential T-cell epitopes, T-cell proliferation assay, and *in vitro* assessment for innate immunity. Further discussion of this methodology follows.

**Performance of *in silico* Predictive Analysis**

ANI has identified the peptide components in the drug substance of Purified Cortrophin® Gel that are greater than 0.10% by area on Reversed-phase high-performance liquid chromatography (RP-HPLC). All the peptide sequences were submitted to EpiVax Inc. for in-*silico* assessment of putative T-cell epitopes using EpiVax's proprietary algorithm. Potential immunogenic risk is assigned to each sequence based on total epitope content, non-human epitope content, and abundance.

**T Cell Proliferation Assay**

Peptides that show potential immunogenicity risk from *in silico* predictive analysis were further tested for CD4+ "helper" T cell proliferation using ProImmune Ltd.'s ProMap® Immunogenicity System T cell assay. A panel of peripheral blood mononuclear cell (PBMC) samples from 40 healthy human donors were selected from the ProImmune cell bank. The panel was selected so that HLA class II alleles known to be highly expressed in the global population were well represented.

**Testing for the Activation of Innate Immune Response**

The potential of impurities in Purified Cortrophin® Gel to activate innate immune response was compared to the WHO ACTH international reference standard, as suggested by the FDA. Together with partner HD Biosciences, ANI developed a robust cell-based method that was modified from the pilot work published by FDA.[5] Five cell lines with appropriate positive control ligands that cover TLR1-9 and NOD2 receptors were established for the testing.

## Results

### Performance of *in silico* Predictive Analysis

All but three components were below EpiVax's standard threshold and therefore have low immunogenicity risk. The three peptides above the threshold were determined to have moderate to significant immunogenicity risk. Despite the extremely low levels of these impurities in the drug product, ANI moved forward with assessing the potential for an immune response through cell based testing using a T Cell Proliferation Assay.

### T Cell Proliferation Assay

The three peptides that showed potential immunogenicity by in silico predictive analysis did not elicit a significant stimulation of CD4+ T cells when evaluated by T cell proliferation assay.

### Testing for the Activation of Innate Immune Response

Neither the drug substance of Purified Cortrophin® Gel nor the WHO ACTH international reference standard elicited innate immune response at the concentrations tested (up to 0.8 U/mL). 0.8 U/mL was chosen as the highest test concentration because of material availability of the WHO ACTH standard and cellular toxicity at higher concentrations.

## Conclusions

Purified Cortrophin® Gel is immunogenic. Limited available data suggest that a patient may develop antibodies to Purified Cortrophin® Gel after chronic administration and loss of endogenous ACTH and Purified Cortrophin® Gel activity. Prolonged administration of Purified Cortrophin® Gel may increase the risk of hypersensitivity reactions. Sensitivity to porcine protein should be considered before starting therapy and during the course of treatment should symptoms arise.

## References


1. Purified Cortrophin® Gel. Package insert. ANI Pharmaceuticals, Inc. Published online 2021.

2. Acthar® Gel. Package insert. Mallinckrodt ARD LLC. Published online February 2021. Accessed September 16, 2021. https://acthar.com/Static/pdf/Acthar-PI.pdf

3. Synacthen® Depot Ampoules 1 mg/ml - Summary of Product Characteristics (SmPC) - (emc). Published October 4, 2021. Accessed October 4, 2021. https://www.medicines.org.uk/emc/product/10823/smpc/

4. Upton GV, Hollingsworth DR, Lande S, Lerner AB, Amatruda TT. Comparison of purified human and porcine ACTH in man. *J Clin Endocrinol Metab*. 1970;30(2):190-195. doi:10.1210/jcem-30-2-190

5. Detection of innate immune response modulating impurities in therapeutic proteins. Haile LA, Puig M, Kelley-Baker L, Verthelyi D. Plos One. 2015. Apr 22; 10(4): e0125078.