

P434 Nonclinical Safety Assessment of C-1101, an Allogeneic Platelet- and Plasma-Derived Therapeutic for Epidural Injection

T. Wright¹, J. Newman¹, V. Patil¹, B. Bennett¹, K. Fu¹, N. Aggarwal², G. Jones², W. Grier¹, J. Schmitke¹

¹Consano Bio, Inc. Burlington, MA, ²BioAgilytix Labs, Morrisville, NC

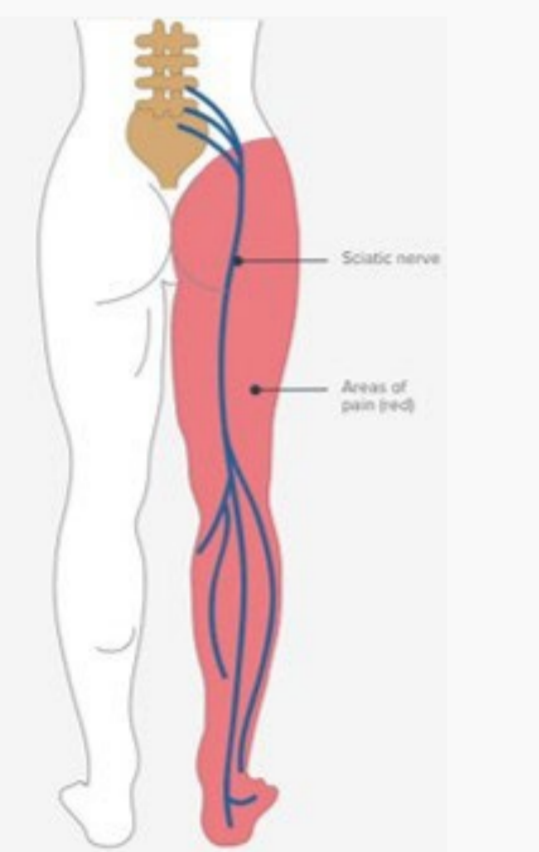
ABSTRACT

C-1101, an allogeneic multi-protein therapeutic comprised of proteins derived from pooled human plasma and platelets, is being developed as an epidural injection for the treatment of chronic painful lumbosacral radiculopathy (LSR). The major components of C-1101 include human serum albumin (HSA) and immunoglobulins as well as cytokines and growth factors that support a mechanism of action involving local reparative actions and inflammation modulation. C-1101 inhibited proinflammatory cytokine release in vitro and reduced neuropathic pain in vivo. Initial in vivo rat studies were conducted to evaluate feasibility of epidural injection, tolerability, and to support selection of the final drug formulation. The toxicology program was designed to evaluate general and local toxicity using a single species, the rat. As C-1101 is an allogeneic human platelet and plasma derived product, the potential for immunogenicity was also evaluated using an in vitro assay in human peripheral blood mononuclear cells. In the pivotal rat 28-day GLP study C-1101 was well tolerated following single or repeat epidural injections with the NOAEL established at the highest dose level evaluated. Pharmacokinetic and toxicokinetic profiles were evaluated in rats using HSA, a major component of C-1101, and demonstrated dose-dependent systemic exposure. Despite the development of anti-HSA antibodies, exposure was not impacted upon repeat dosing in rats. The potential for immunogenicity was determined to be low and similar to intravenous immunoglobulins as evidenced by negligible activation of a CD4+ T cell response. A Phase 1 study has been initiated to evaluate C-1101 after epidural administration to patients with chronic painful LSR.

C-1101 BACKGROUND

C-1101 has been designed to build upon the benefits observed from autologous platelet-based treatments, and to improve upon them by providing a consistent, defined, and controlled product.

- Off-the-shelf therapeutic
 - Consistent biologic product candidate
- Purified protein solution
 - Allogeneic multi-protein therapeutic, derived from platelets in plasma
- Potential disease-modifying Mechanism of Action
 - Demonstrated inflammatory modulation and activation of local cellular repair
- Local delivery to site of injury
 - Transforaminal epidural injection for chronic painful LSR



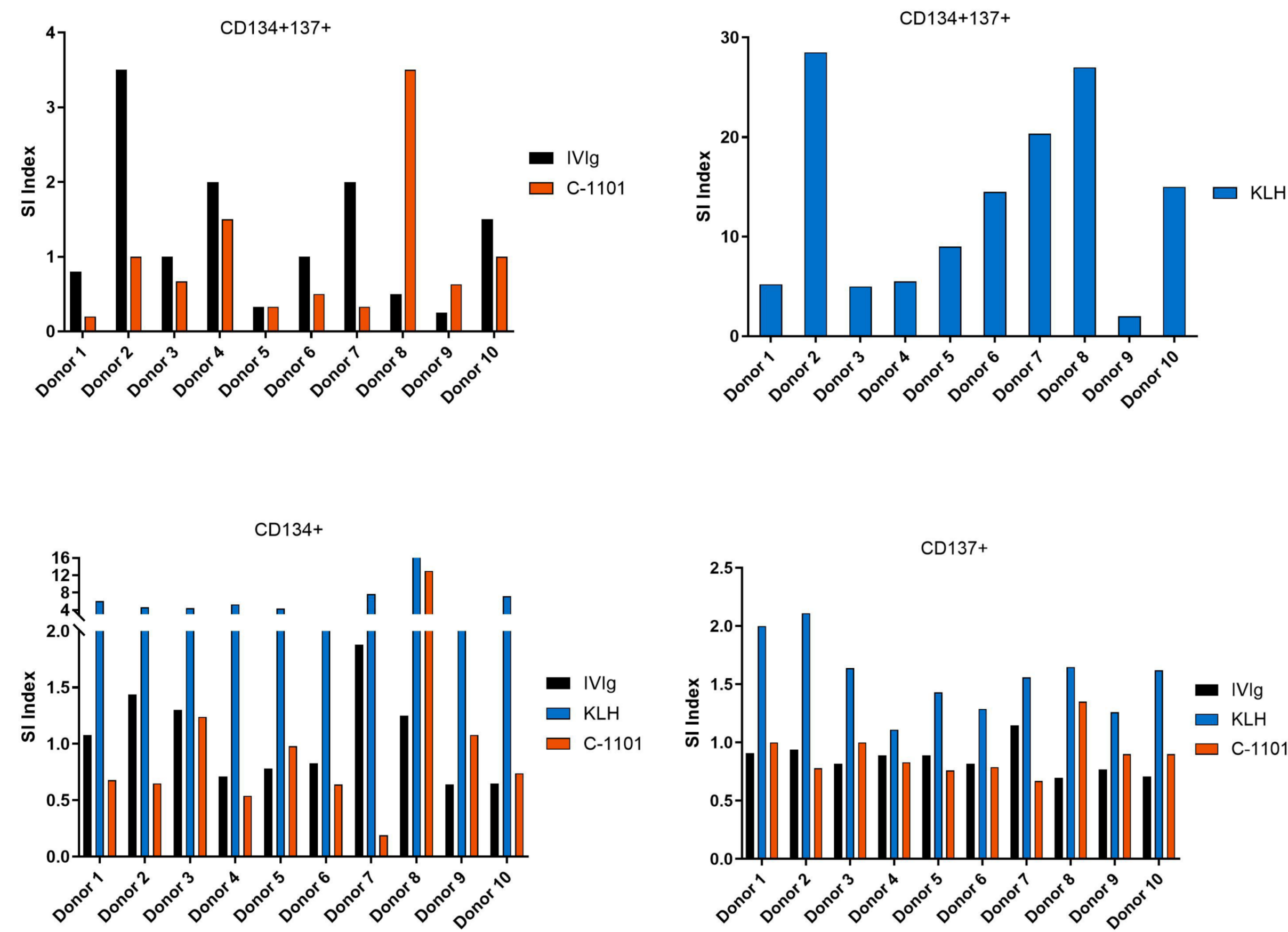
IMMUNOGENICITY RISK

- Immunogenicity was identified as a potential risk from C-1101 administration
- The majority of the proteins in C-1101 are human plasma proteins (albumin and immunoglobulins), growth factors and cytokines which are not expected to elicit an immune response
- Antigens such as rhesus factor D (RhD) proteins from red blood cells (RBC) or human leukocyte antigens (HLA) are identified as potential immunogenic components
- Immune reactions to non-ABO matched platelets are rare as is RhD alloimmunization (Dunbar, 2020)
- T-cell activation is required for development of high specificity anti-drug antibodies and has been used as a screen for potential immunogenicity
- An in vitro T-cell activation study was developed in human PBMC as described in Cohen, 2021

IMMUNOGENICITY ASSAY

- Keyhole limpet hemocyanin (KLH) was used as a positive control, and commercially available intravenous immunoglobulin (IVIg; Gammagard®) was chosen as an allogeneic blood product to compare against C-1101
- The CD4+ T cell response of human PBMC to KLH, C-1101 and IVIg was assessed to determine the potential for immunogenicity
- The stimulation index (SI) was calculated for each compound
- The SI is defined as the ability of the biotherapeutic to increase the fraction of live CD4+ cells that were positive for either CD134+ or CD137+ compared to the fraction of cells positive for these markers in formulation buffer samples

Stimulation Index of CD4 Helper Cells



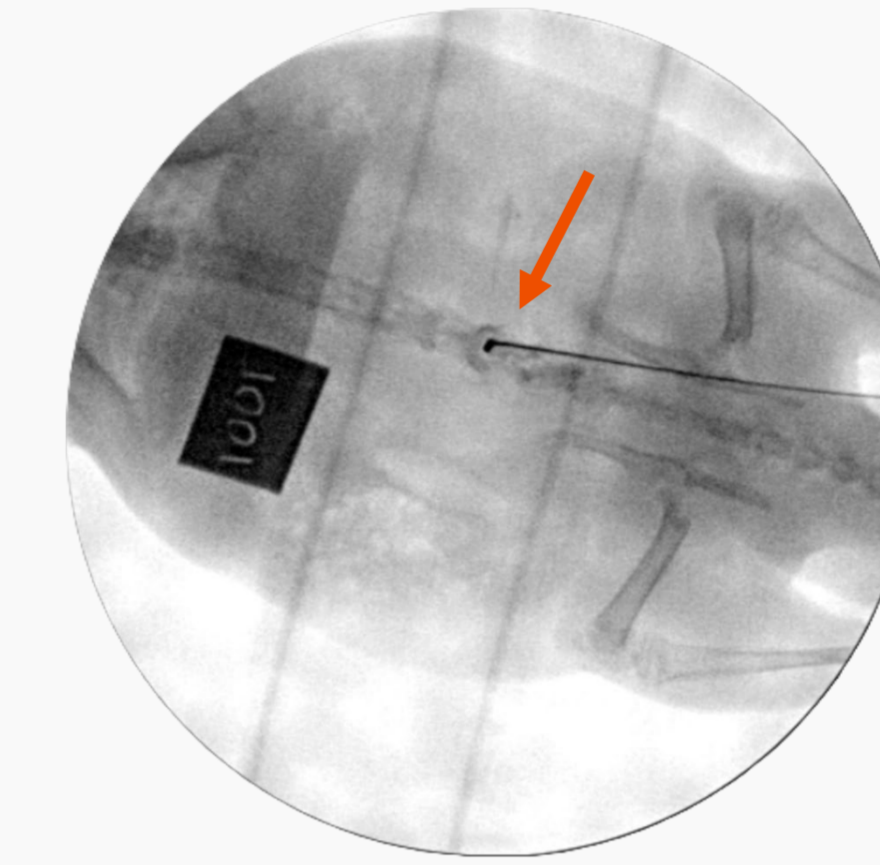
IMMUNOGENICITY RESULTS

- CD4+ activation was comparable for C-1101 and IVIg
- IVIg was chosen as a comparator to C-1101 as it is also a pooled human blood product, with the potential for similar contaminants
- A clinically relevant lot was tested against 10 healthy donor samples (including Rh positive and negative, and blood types O, A, and AB)
- KLH elicited the highest SI score, as expected for a strongly immunogenic protein commonly used as a positive control in immunoassays
- For 9 of 10 donors tested, C-1101 had a similar or lower SI score than IVIg.
- Only Donor 8 had a higher response to C-1101 than IVIg and had an extremely high response to KLH. Donor 2 had a very strong response to KLH and IVIg, and lower response to C-1101
- C-1101 does not strongly activate T-cells and its immunogenicity may be similar to other blood-derived products, such as IVIg

PILOT EPIDURAL RAT STUDIES

- Rodents were expected to be an appropriate model as human plasma and platelet proteins have been shown to be pharmacologically active and repeat dosing well-tolerated (Bucheit, 2023)
- Pilot studies were conducted to select the formulation buffer and assess feasibility of epidural administration and confirm the maximum feasible dose volume of 75 µL
- Rats were dosed epidurally under fluoroscopy as is planned for clinical trials
- Tolerability of repeat administration of C-1101 in rats was assessed by administering repeat subcutaneous injections of C-1101 on Day 1, 15 and 29. No adverse findings were observed (clinical observations, clinical pathology and gross necropsy)
- Human serum albumin (HSA) was selected to assess toxicokinetics and anti-drug antibodies of C-1101. HSA comprises over 50% of C-1101 and is an appropriate marker of distribution and potential immunogenicity. Validated methods were developed for use in the GLP toxicology study

Rats were anesthetized and prepared for epidural administration. Prior to dosing, the needle was primed with vehicle or test article. The needle was inserted into the epidural space and the location of the needle verified using fluoroscopy. Once the needle was appropriately placed, the animals were dosed with C-1101 or vehicle followed by a flush over 30 ± 5 seconds

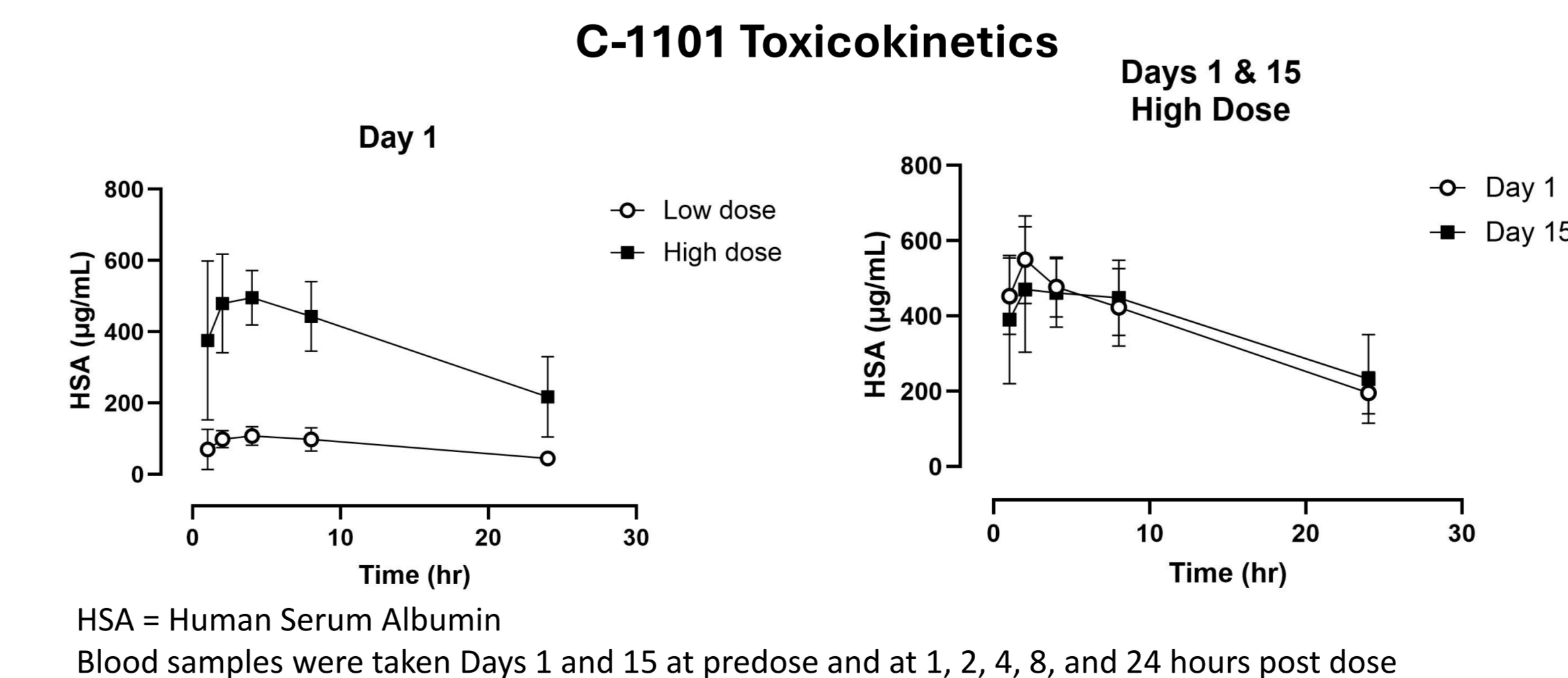


TOXICOLOGY STUDY DESIGN

- Animals received either vehicle or C-1101 epidurally, on Day 1, with some animals receiving a dose of vehicle or high dose C-1101 on Day 15
- Animals were necropsied on Day 15 or 29, 2 weeks after dosing
- Endpoints included: mortality, clinical signs, body weights, body weight gains, ophthalmology examinations, clinical pathology parameters (hematology, coagulation, and clinical chemistry), bioanalytical and toxicokinetic parameters, anti-HSA antibody analysis, organ weights, and macroscopic and microscopic examinations.

Group	Treatment	Dose Level (mg)	Dose Regimen	Animals on Study		Necropsy (Main)	
				Main	TK	Day 16	Day 29
1	Vehicle	0	Day 1 & 15	10M/10F	--	6M/6F	4M/4F
2	C-1101	Low	Day 1	6M/6F	4M/4F	6M/6F	--
3	C-1101	Mid	Day 1	6M/6F	--	6M/6F	--
4	C-1101	High	Day 1	6M/6F	4M/4F	6M/6F	--
5*	C-1101	High	Day 1 & 15	10M/10F	4M/4F	6M/6F	4M/4F

*A total of 10/sex Main Study animals were dosed on Days 1 and 15. A cohort of 6/sex were terminated on Day 16 and a cohort of 4/sex were terminated on Day 29 following a 14-day recovery.



TOXICOLOGY STUDY RESULTS

- The administration of C-1101 at all dose levels was not associated with any mortality, clinical observations, changes in body weight parameters, ophthalmology, macroscopic observations, or organ weight changes in either the Day 16 or Day 29 intervals
- Systemic exposure to HSA following epidural injection of C-1101 was independent of sex. Plasma C_{max} and area under the concentration versus time curve from the start of dose administration to 24 hours postdose (AUC_{0-24hr}) values were similar between males and females
- The majority of rats administered C-1101 developed anti-HSA antibodies which did not affect systemic exposure and were not associated with adverse findings
- On Day 16, C-1101-related findings were inflammatory in nature and were noted in the lumbar DRG and injection site. Minimal to moderate mixed cell inflammation was noted in the lumbar DRG in C-1101 treated animals with associated minimal to mild increased macrophage cellularity on IBA-1
- C-1101-related minimal to mild mixed cell inflammation in the lumbar DRG persisted after a 14-day recovery period in Group 5 males and females, at a lower incidence and severity compared to Day 16, suggesting a partial recovery after a second dose of C-1101
- These findings were all considered non-adverse due to low severity, lack of involvement of DRGs and/or nerves themselves, and lack of associated clinical observations.
- The no-observable-adverse-effect-level (NOAEL) was the highest dose of C-1101 given as a single or repeat epidural dose

CONCLUSIONS

- These minimal to mild inflammation observed histologically were all considered non-adverse due to low severity, lack of involvement of DRGs and/or nerves themselves, and lack of associated clinical observations.
- The no-observable-adverse-effect-level (NOAEL) was the highest dose tested given as a single or repeat epidural dose
- The NOAEL determined in the GLP toxicology provides a >15-fold margin to the planned clinical starting dose on a mg/kg basis
- The overall safety assessment of C-1101 supports administration of C-1101 in the planned clinical trial
- A Phase 1 clinical trial is initiating; CTN number CT-2025-CTN-04714-1

REFERENCES

- Bucheit T, Huh Y, Breglio A, et al. Intrathecal administration of conditioned serum from different species resolves Chemotherapy-Induced neuropathic pain in mice via secretory exosomes. *Brain Behav Immun.* 2023 Jul;111:298-311. doi: 10.1016/j.bbi.2023.04.013. Epub 2023 May 5. PMID: 37150265; PMCID: PMC10363329.
- Cohen S, Myneni S, Batt A, et al. Immunogenicity risk assessment for biotherapeutics through in vitro detection of CD134 and CD137 on T helper cells. *MAbs.* 2021 Jan-Dec;13(1):1898831.
- Dunbar NM. Does ABO and RhD matching matter for platelet transfusion? *Hematology Am Soc Hematol Educ Program.* 2020 Dec 4;2020(1):512-517. doi: 10.1182/hematology.2020000135. PMID: 33275681; PMCID: PMC7727583.

ACKNOWLEDGMENTS

The authors would like to thank Kari Climer, Daniel Miley, and Leslie Kuiper at Charles River Laboratories, Mattawan, MI for their work on the rat toxicology study.