Allogeneic Platelet and Plasma-Derived Therapeutic C-1101 to Treat Chronic Lumbosacral Radiculopathy

Lynn Pezzanite[1], Teresa Wright[2], Steven Dow[1], Lyndah Chow[1], Peter Linde[1], Jacob Singer[1], Jennifer Newman[2], Vishwesh Patil[2], Jennifer Schmitke[2]
[1]Colorado State University, Fort Collins, CO, [2]Consano Bio, Burlington, MA
Corresponding author: lynn.pezzanite@colostate.edu
Disclosures: Teresa Wright (Consano Bio), Lynn Pezzanite (Consano Bio, EQCell, Vitalfoams), Steven Dow (Consano Bio, EQCell, Laporte, Vitalfoams), Jennifer Newman (Consano Bio), Vishwesh Patil (Consano Bio), Jennifer Schmitke (Consano Bio).

INTRODUCTION: Intervertebral disc disease, a common cause of low back pain, can result in chronic lumbosacral radiculopathy (CLR) when lumbar nerve roots are compressed or irritated, resulting in numbness or radiating pain. Multiple studies have evaluated the potential therapeutic effect of platelet rich plasma (PRP) and platelet derivatives to manage neuropathic pain. C-1101, an allogeneic multi-protein therapeutic comprised of proteins derived from human plasma and platelets, has been developed for epidural injection to improve treatment of chronic painful lumbosacral radiculopathy. C-1101 includes potentially therapeutic anti-inflammatory and reparative components such as human serum albumin, immunoglobulins, growth factors (*e.g.,* platelet derived growth factor isoform AB (PDGF-AB)), and cytokines (*e.g.,* TGF-β). The aims were to assess **1)** immunomodulatory effect on human macrophage cytokine secretion, **2)** impact on cytokine secretion and differential gene expression of three relevant spine tissue derived cell lines (nucleus pulposus, annulus fibrosis, dorsal root ganglia) using a relevant large animal (ovine) model, and **3)** potential efficacy of systemic administration of C-1101 in a rodent model of peripheral neuropathy. The hypothesis was that C-1101 would have a multi-modal mechanism of action involving anti-inflammatory actions and growth factor activity, contributing to a reparative cascade relevant to treatment of lumbosacral radiculopathy.

METHODS: In **aim 1**, human THP-1 monocytic cells were pre-incubated with media, vehicle, or C-1101 (10 or 20 mg/mL), then stimulated with lipopolysaccharide (LPS, 500 ng/mL) and cytokine secretion was assessed at 4 and 24 hours. In **aim 2**, ovine lumbar spines (n=6) were acquired from adult female Rambouillet x Columbia sheep euthanized for other reasons (CSU IACUC #5264). Female sheep were exclusively used as males (rams) present challenges in animal husbandry requiring individual housing and posing risk to personnel; female sheep have become a well-established and widely accepted animal model for musculoskeletal research, particularly in preclinical orthopedic device and orthobiologic development due to similarities to humans in complex traits such as osteogenesis, tendon healing, and disease progression. Intervertebral discs and dorsal root ganglia (DRG) were harvested immediately postmortem. Lumbar intervertebral discs were dissected and digested to isolate annulus fibrosus (AF) and nucleus pulposus (NP) cells. Whole dorsal root ganglia (DRG) were dissociated to form a single cell suspension. Cells were plated in tissue culture (100,000 cells/well, 24 well plates) and subjected to 4 treatment groups for 24 hours (non-stimulated, IL-1β+TNF-α stimulated, IL-1β+TNF-α stimulated + C-1101, IL-1β+TNF-α stimulated + vehicle control). Cells were washed, cultured an additional 24 hours in regular growth media, and conditioned media collected and examined for cytokine secretion (n=14) by ovine multiplex immunoassay. Treated cells were sequenced via mRNA sequencing on an Illumina based platform (Novogene, Inc). In **aim 3**, chemotherapy (paclitaxel)-induced peripheral neuropathy (PIPN) was induced in male C57Bl6/J mice (sham group 10 mice/group, paclitaxel + vehicle or C-1101 15 mice/group) to assess effect of systemic C-1101 administration on allodynia and systemic cytokine levels (Charles River Discovery Services, Finland with IACUC approval #C2570123). Male mice were evaluated initially to reduce overall numbers of animals while achieving sufficient sample size to evaluate behavioral endpoints. Paclitaxel or vehicle (12.5% Kolliphor EL, 12.5% ethanol, 75% saline) was administered intraperitoneally (15 mg/kg/day, days 1-5). C-1101 was administered intravenously (IV 305 or 580 mg/kg/day or vehicle, days 6,7). Acetone cooling test (ACT) was performed at baseline, and daily (day 5-13) to assess allodynia. Cytokine analysis was performed on murine plasma for IL-1β, IL-6, IL-10, TNF-α, IFN-γ (MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel, Custom Premix MCYTOMAG-70K). Statistical analyses were performed via one-way ANOVA in GraphPad Prism Software v9.1.1 (significance $p<0.05$).

RESULTS: In **aim 1**, C-1101 pre-treatment suppressed pro-inflammatory cytokine secretion (IL-6, IL-8, IL-1β, TNF-α) from human monocytes following LPS stimulation (**Figure 1,** IL-1β data shown). In **aim 2**, cytokine analyses revealed C-1101 *in vitro* co-culture enhanced secretion of multiple pro-inflammatory cytokines related to tissue remodeling from AF (IFN-**γ,** IL-1β, TNF-α, IL-1α, IL-4, IL-6, IL-8, IL-10, MIP1α, MIP1β, VEGFα, IL-17A) and NP (IFN-**γ,** IL-1β, TNF-α, IL-1α, IL-8, IL-10, MIP1α, MIP1β) cells compared to untreated controls; no differences were elicited from DRG. Pathways analyses of all three cell types indicated C-1101 treatment downregulated inflammatory pathways such as interferon gamma, complement, extracellular matrix, lymphoid cell interaction and NK cell toxicity compared to vehicle control. Differential gene analyses of DRG indicated upregulation of translation and ribosome activity, which coupled with metabolic reprogramming and cell cycle pathways, suggests neuronal growth and increased turnover (**Figure 2**). In **aim 3**, Paclitaxel induced allodynia evidenced by ACT score compared to vehicle control. Both low (305 mg/kg) and high (580 mg/kg) dose C-1101 reduced paclitaxel-induced allodynia vs. vehicle. Cytokine analyses revealed plasma IL-1β was transiently elevated in C-1101 treatment group on day 8.

DISCUSSION: C-1101 elicited an anti-inflammatory response from human macrophages following LPS treatment and a mixed pro- and anti-inflammatory response from AF and NP cells and to a lesser extent from DRG when administered in an already inflamed environment *in vitro*, with acute upregulation of pro-inflammatory cytokine release, but downregulation of key inflammatory (namely interferons), extracellular matrix and cell signaling gene pathways. In an induced murine model of peripheral neuropathy, C-1101 reduced neuropathic pain and induced a transient and specific increase in pro-inflammatory IL-1β. Pleiotropic activity observed may reflect timing of administration in disease course and condition of the recipient environment in which it was administered (*i.e.,* prior to induction of or in the face of established inflammation). Evaluation of optimal timing, dose, and route of administration of C-1101 *in vivo* are indicated.

SIGNIFICANCE/CLINICAL RELEVANCE:
These findings indicate C-1101 administration may be relevant to mitigate pain and progression of chronic lumbar radiculopathy associated with degenerative disc disease, warranting further investigation *in vivo.*

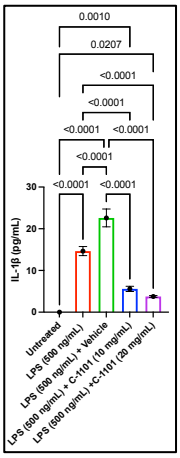

Figure 1 (left) – C-1101 reduced LPS- induced IL-1β release from human THP-1 cells after 24 hours.

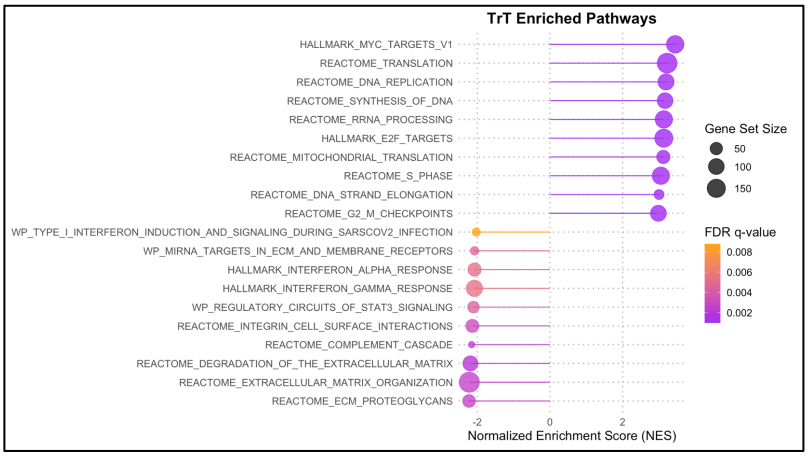

Figure 2 (right) – Pathway analysis of ovine AF cells after C-1101 vs. vehicle indicates IFN suppression.