Immunocom Inc.

Immunotherapeutic Plasmapheresis, "Immunopheresis"

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Abstract

Tumor necrosis factor-alpha (TNF) is a major cytokine involved in inflammation caused by bacterial infections and endotoxin release. TNF is cytotoxic to cancer cells and stimulates anti-cancer immune responses. Natural attenuation of TNF activity is through soluble TNF-receptors, sTNF-Rs, (sTNF-R1 and sTNF-RII) to control deleterious inflammatory responses. However, tumors shed sTNF-Rs as a survival mechanism to downregulate TNF anti-cancer immune responses and cancer patients have elevated levels of these receptors. Immunicom has developed a novel ligand affinity device to capture and remove these immune inhibitors from patient's plasma in conjunction with apheresis to promote TNF activity as a cancer treatment. Treatment of canine cancers using Immunicom's LW-02 device together with the Terumo Optia™ apheresis system has been demonstrated to be safe and effective for cancer treatment.

Introduction

Elevated levels of specific proteins called soluble TNF receptors (sTNF-Rs) have been found in cancer patients due to their overproduction by tumor tissue. Historically, after observations that some cancer patient's tumors regressed after undergoing plasmapheresis treatments, it was found that the anti-cancer effects could be specifically attributed to the removal of these sTNFRs. sTNF-Rs inhibit immune responses by binding and blocking the activity of TNF, a molecule that is well known for its anti-cancer effect.

- ❑ **Tumor necrosis factor alpha (TNF) effects are modulated through its soluble receptor (sTNF-R)**
  - Cancer patients have elevated sTNF-Rs
  - sTNF-Rs suppress TNF activity
- ❑ **Removal of sTNF-R stimulates TNF**
  - TNF activity is augmented
  - Enhanced TNF anti-tumor activity and host immune response via immune-cell regulation (dual path)

Brief History of TNF and Apheresis

- ❑ Coley's Toxins – early 1900's - Infections correlated with tumor regressions (William Coley)
  - Injected patients with mixed bacterial strains
- ❑ Lloyd Old – 1970's - Identified "Tumor Necrosis Factor"
  - TNF produced in response to bacterial infections
- ❑ Seder et al– 1980's - Tumor regression observed with Plasmapheresis
  - Nonspecific removal of inhibitory factors in blood
- ❑ Lentz – 1980's – improved outcomes with "Ultrapheresis"
  - Ultrapheresis – removed <150Kda molecular weight (MW) factors - determined by filtration cutoff
  - Identified as soluble TNFα Receptors (TNF-R)
  - Developed antibody based apheresis for TNF-R and IL-2R
  - Issues with FDA (protocol/ GCP regulatory issues in US)

Currently, a device designed to specifically remove sTNF-Rs is being used to treat a limited number of patients in a small clinic in Europe with beneficial results, but the treatment has not been available elsewhere. Immunicom has developed an improved technology for removal of sTNF-Rs for cancer treatment using a proprietary single chain TNF (scTNF) capture ligand as an alternative to the use of animal derived antibodies currently being used in Europe. Immunicom's device has been developed for use as a secondary device with Terumo's Optia apheresis system through which the patient's blood is circulated (Fig 1). The patient's blood is separated into cellular and plasma fractions by a centrifuge and the plasma is passed through Immunicom's device where the sTNF-R's are captured and removed. The treated plasma is then recombined with the cells and then the reconstituted blood is infused into the patient. A typical procedure takes about 2-3 hours (about the same time as for dialysis) and is performed two to three times per week for up to 12 weeks. Overall the levels of sTNF-Rs are reduced by 80% per treatment which results in activating TNF to enhance anti-tumor responses (Fig 2).

Objective

The objective was to test safety and efficacy of Immunicom's scTNF ligand device for immunopheresis removal of sTNF-Rs from the plasma of dogs with cancer.

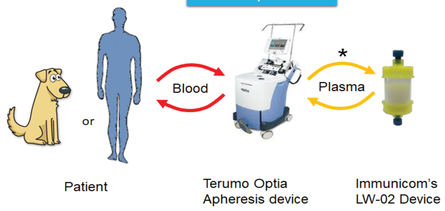
Methods

Recombinant scTNF was expressed in E. coli and purified by column chromatography. The protein (93% pure) was coupled to two different resins of bead bed (Figure 3). The scTNF coupled beads were loaded aseptically into pre-sterilized device housings comprised of a glass barrel with pressure fitted polypropylene caps in which were placed 20-micron polyethylene frits (Biorad) to retain the beads. Dogs were treated to 1.5 times of their plasma volumes. Initially, dogs of various sizes and breeds with different cancers were treated (Part A) and then a second cohort of dogs with melanoma was treated (Part B). Data on this melanoma cohort (Part B) is presented in greater detail.

Pre- and post-apheresis plasma samples were obtained and assayed for sTNF-R and TNF using an MSD (Mesoscale Discovery) multiplex system. Plasma samples were obtained from blood samples and from the device input and exit ports at the start of the apheresis procedure and at 30 minute intervals during the treatment circulation. Additionally, plasma was prepared from blood samples obtained at 30, 60 and 90 min post treatment. sTNF-R captured on the devices was eluted with low pH citrate buffer followed by neutralization with alkaline sodium carbonate and quantitated.

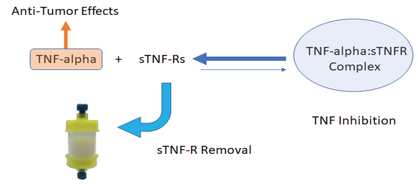
Dogs undergoing treatment were evaluated according to RECIST criteria, with tumor size measurements and CT scans were performed at intervals. Also for each dog a quality of life record was kept including the details.

Fig. 1 **Immunopheresis**



* Plasma treatment is performed to stimulate anti-tumor responses: Blood is circulated through a Terumo Optia™ apheresis system. Plasma is separated from cells by centrifugation and filtered through the LW-02 device, then recombined with the cells and infused into the patient. Specific Immune inhibitors e.g., sTNF-Rs are removed.

Fig. 2 **TNF-alpha Buffer System is a Chemical Equilibrium**



Removal of sTNF-Rs shifts the equilibrium to the left and activates TNF-alpha

Results

Device Development

Fig. 3 **Product optimization to support human studies**

- Optimization TNF Ligand Production
  - Improved Purity
- Proprietary coupling chemistry
  - Leaching reduced below significance
- Improved bead resin bed
  - Efficient Capture of TNF-Rs
- Capacity > 130 micrograms of sTNF-R capture; humans have up to 30 mcg.
- Device meets manufacturing specifications, sterility and endotoxin requirements
- Biocompatibility Testing is Pending

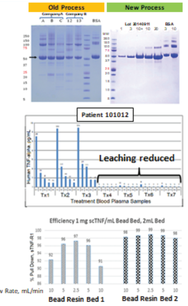
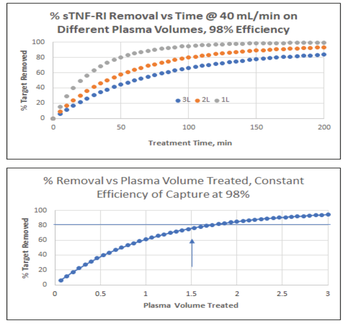


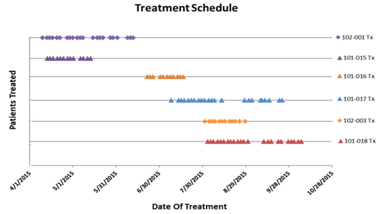
Fig. 4 **Device Performance Characteristics Model**



The amount of removal is dependent on the plasma volume that is treated.

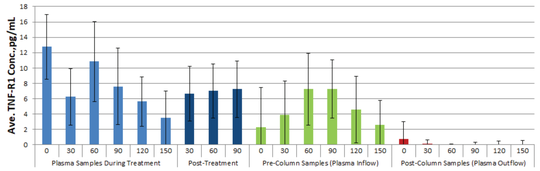
Canine Cancer Study

Fig. 5 **Canine Study on 6 Dogs-Melanomas**



1.5 Volumes of Plasma Processed per Treatment with 18 mL Devices

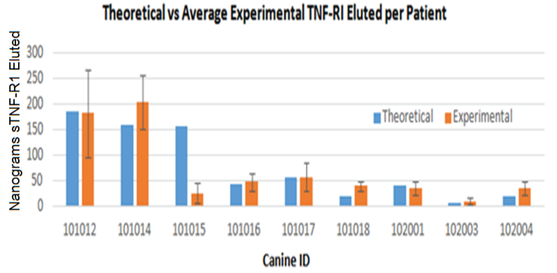
Fig. 6 **Canine sTNF-R1 20 Treatments**



Blood plasma levels show an initial reduction of canine sTNF-R1 followed by an increase at 60 minutes then a linear decrease. Samples taken from the plasma line during treatment prior to device flow through show decreasing levels of canine sTNF-RI.

Note: Canine sTNFR-2 is at or below the level of detection.

Fig. 7 **Device sTNFR Elutions**



Result: Excellent correspondence between theoretical and experimental values.

Fig. 8 **Tumor Growth Results on 6 Patients**

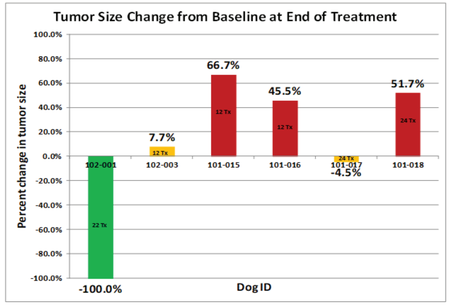


Fig. 9 **Overall Survival**

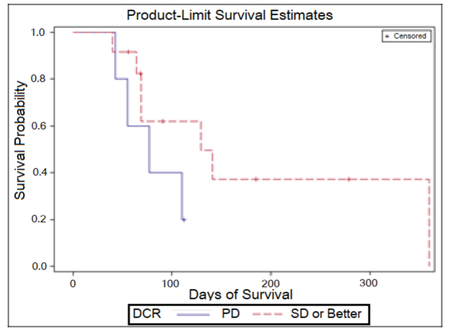
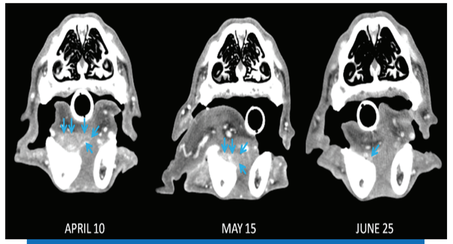


Fig. 10 **Canine Clinical Trial Results**

	Part A (Different Cancers)	Part B (Melanoma)
Status	Completed	Completed
Number of Dogs Treated (evaluable)	12 (10)	8 (6)
Safety, Tolerability, Quality of Life	100%	100%
Best Overall Response (BOR) / Disease Control Rate (DCR)	~ 60% (6/10)	~ 50% (3/6) One Complete Regression
Mean/Median Survival/	~ 81 /~110	~ 132/~164

**Dogs studied were deemed to have aggressive disease that would have likely required euthanasia within 4 to 6 weeks of diagnosis to prevent undue pain and suffering. The Tx prolonged survival with excellent QoL during and immediately post treatment (in contrast to chemotherapy). Part B data was generated with improved column.

Fig. 11 **CT Images of Tumor Regression in Patient 102001 (Sublingual Melanoma)**



A) Baseline mass, 44 x 19.6 x 14mm, with extensive bone damage and break down of the mandibular bone; **B)** tumor mass reduced to 33.3 x 1.2 x 0.5mm; **C)** no tumor is visible, no evidence of progressive bone damage

Conclusions

Fig. 12 **Safety/Efficacy**

With over 300 Treatments in Canines:
No Adverse Events Were Attributable to Immunicom's LW-02 Device

Conclusions:

- ❑ The LW-02 Device is Safely Compatible with the Terumo Optia System
- ❑ LW-02 has demonstrated Safety and Efficacy in Canines
- ❑ Next Step: Human Clinical Safety Studies

Reference

1. Selinsky CL, Howell MD. 2000. Cell Immunol. 200: 81-87.