Immunicom Inc.

Immunopheresis™ Technology Overview

Immunicom Inc.

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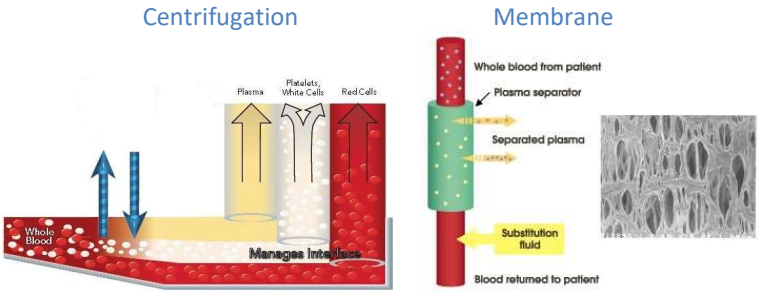
Introduction

Immunicom Inc., an immuno-oncology company located in San Diego, CA that is developing a proprietary subtractive device-based technology platform as a novel approach to treating cancer. Immunicom's immunomodulatory technologies incorporate an extracorporeal, medical device-based "subtractive" approach utilizing its proprietary apheresis column. The lead therapy currently under development focuses on removing sTNF-R immune-suppressive factors to restore TNF-α anti-tumor activity and to reactivate a patient's immune system to potentially safely and effectively reduce tumor burden and otherwise address cancer. This therapy utilizes a proprietary recombinant protein initially developed at University of Stuttgart for which Immunicom has been granted worldwide exclusive rights for extracorporeal use through a license.[1] Additionally, in connection with the development of its sTNF-R ligand Apheresis Immunoadsorption Affinity Column, Immunicom has gained extensive expertise on the design, testing and production of Affinity Columns and has now developed a corporate "platform-based" technology capability that can potentially extend its proprietary "subtractive" apheresis therapeutic approach to other cytokine targets such as sIL-2R and TGF-beta, which are currently under early development by the Company.

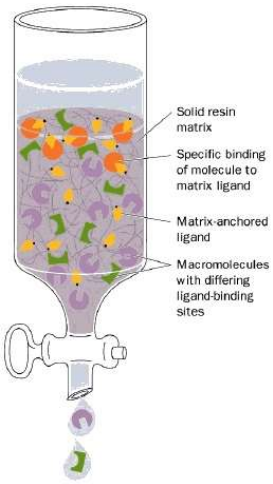
Apheresis Background



Apheresis, originating from the Greek word meaning "to take away" or "to remove" has been used to refer to procedures that involve the removal of whole blood or plasma from patients or donors followed by the removal of specific components prior to returning the remainder to the patient. Separation of blood components is usually accomplished by means of 1) continuous or discontinuous centrifugation, 2) membrane separation, or 3) a combination of the two.

Therapeutic apheresis refers to the removal of components of the blood to treat a disease or condition. The most commonly used therapeutic apheresis procedure is "therapeutic plasma exchange" (TPE), also referred to as plasmapheresis, in which large quantities of plasma are removed and replaced by a replacement solution (plasma or solutes) to reduce the concentrations of circulating, non-cellular components whose removal provides therapeutic benefit. It should be noted that TPE, because it is non-selective, also results in the removal of desirable blood components.

An alternative to TPE is to use selective method employing an affinity chromatography column (device) to perform therapeutic apheresis in a targeted fashion. The affinity chromatography



approach results in a highly selective separation process, which takes advantage of specific binding properties of ligands, receptors or antibodies to tightly bind unique molecular targets in the blood plasma. Using methods analogous to bio-separation processes, peptides, proteins, antibodies or other binding agents are covalently immobilized onto beads in a chromatography device, which removes specifically targeted molecules from the patient's plasma as it is passed through the device, returning all other blood components back to the patient. Since the therapeutic risk of using an affinity chromatography column is expected to be similar to the known and limited risks of commonly used TPE procedures, regulatory approvals to conduct human clinical trials should be facilitated, provided that nothing potentially harmful to the patient is leached from the device during its use, or that removal of a specific molecules have no untoward physiological effects.

Commercially available equipment to perform routine apheresis procedures is supplied by various manufacturers, including Terumo, Baxter/Fenwal, Haemonetics, BBraun, and Fresenius. In the field of therapeutic apheresis, Terumo has captured the majority of market share and has over 5000 Cobe Spectra and newer Spectra Optia machines installed all over the world. Other competing machines include the Baxter/Fenwal CS3000 Plus and Amicus, the Fresenius ASI04, and the BBraun HELP System. Regardless of the system and its manufacturer, all systems can perform targeted apheresis using secondary circuits that can include extracorporeal chromatography devices. Vascular access (typically venous catheterization) and the use of anticoagulation (typically now extracorporeal) are required for the apheresis procedure. Therapeutic apheresis procedures are typically conducted under controlled conditions by skilled personnel, usually in a hospital setting, or in apheresis centers specifically designed for that purpose. Immunicom has partnered with Terumo to further develop the LW-02 device in conjunction with their apheresis units. Terumo has provided Spectra Optia apheresis systems, disposables and accessories to support Immunicom with its first series of preclinical trials targeting solid cancers and melanoma, utilizing the LW-02 affinity chromatography technology for "Immunopheresis™".

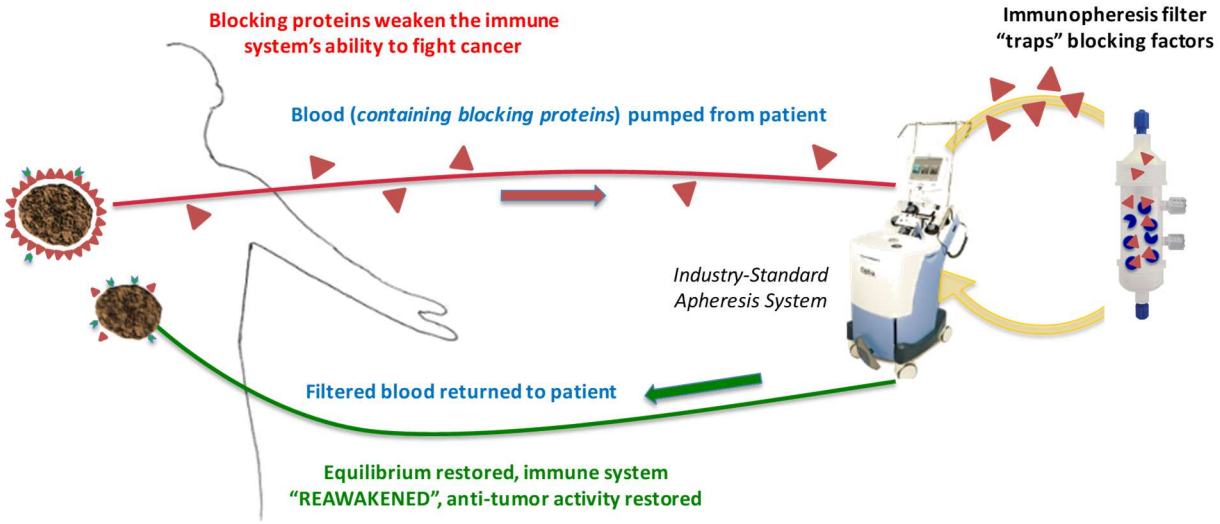
Terumo Optia

Immunicom's Therapeutic Approach

This novel Immunopheresis™ approach marries two core technologies: 1) Plasmapheresis / apheresis (hemodialysis-like technology); and 2) Immunomodulation to attack cancer. These two technologies, each with strong scientific foundations and widespread use, resolve the historical challenges experienced with "subtractive" therapy – an approach that is intended to enhance a patient's natural immune response to destroy cancer while being significantly better tolerated than standard chemotherapy or radiation therapy. Immunicom's "subtractive" immunotherapeutic approach is designed to remove inhibitory factors (soluble tumor necrosis factor receptors; sTNF-R's) from a patient's circulation allowing TNF-α anti-tumor activity. This novel approach also has promise for addressing autoimmune diseases.

Immunicom's Immunopheresis™ approach boosts the natural immune response and pro-apoptotic activity of tumor necrosis factor alpha (TNF-α) against neoplastic cells. This is achieved by

removing soluble TNF receptors (sTNF-Rs) from patients' plasma, thereby potentially disrupting local sTNF-R mediated inhibition of TNF-α activity in tumors and modulating T-cell activity. The system employs Immunicom's proprietary affinity column that employs a proprietary human recombinant protein, single chain (SC) TNF-α ligand, that is covalently linked to a bead resin using proprietary methods, and Terumo BCT's state-of-the-art centrifugal plasma separator, the Spectra Optia®, to reproducibly and predictably remove sTNF-Rs from the systemic circulation.



In comparison to existing therapies, Immunicom's subtractive therapy has the potential for fewer side effects, uses the body's own systems to fight disease (that should be agnostic to a patient's genetic makeup) requiring no personalized tailoring, and could potentially be effective against a broad spectrum of cancers. Importantly, the approach should not produce general systemic toxicity, allowing for faster recovery times and a much-improved quality-of-life. While not necessarily a replacement for standard chemo- or radiation therapies, we believe that Immunopheresis™ can be safe and effective both as a front-line treatment or adjunct therapy.

Immunpheresis™ Background

Immunopheresis™ is a blood fractionation methodology to remove specific blood components to stimulate a natural immune response. Today several forms of apheresis are used clinically to provide therapeutic benefits including the treatment of sickle cell anemia, production of platelets for bleeding disorders and the removal of leukocytes post-transfusion. [2][3] The significant advantage of Immunopheresis™ over all non "pheresis" types of immune therapy is that nothing is put into the patient and the materials removed are only a small portion of the patient's total blood content. This small loss of existing blood components is less stressful than other procedures that are additive or directly damaging to the body. This is particularly true when treating cancer.

Current Status of Immunotherapy

Over the past 5 to 10 years, immunotherapeutic approaches have become a major focus of oncology research given the potential for enhanced efficacy and reduced toxicity compared with standard cytotoxic chemo and radiation therapy [3][5], with some having reached the market as front-line therapeutics. Half of Standard & Poor's top 10 list of promising drugs for 2015 include cancer immunotherapies, indicating significant investment in this therapeutic area for the

foreseeable future. These new therapies include therapeutic vaccines (where the objective is to directly stimulate an immune response), [6] immune checkpoint inhibitors (PD-1, PD-L1 (Keytruda-pembrolizumab, Opdivo-nivolumab), CTLA-4, that target inhibitory molecules of the immune system allowing a more robust immune response), [7] immuno-transplantation (with adoptive T Cell transfer), chimeric antigen receptor (CAR) T-cell therapy, and monoclonal antibodies [8][9]. Experimental T-cell therapies (CAR) developed at the University of Pennsylvania are at the forefront of immunotherapeutic treatments. In a small study of CLL, ~24% of patients showed complete remission, and another ~24% showed improvement with progression. Antibody based approaches, such as monoclonal (Campath, Herceptin, Zevalin, Adcentris), chimeric and even bi-specific antibodies [10], have demonstrated clinical benefit, with several becoming highly successful products; e.g., Erbitux® (cetuximab), Avastin® (bevacizumab) and Yervoy® (Ipilimumab).

While producing very promising results, many of these immunologic approaches suffer from a lack of broader applicability and are generally customized to certain disease subtypes and small subpopulations. Often, the excellent results sometimes seen in initial smaller studies often do not show equal benefit when larger studies are employed. Equally concerning, most of these approaches produce side effects that can be disabling and prevent continued therapy. As an example, IL-2 treatment hasn't been completely positive. The treatment can have debilitating side effects and has killed as many as 4% of recipients. [11][12]

One of the first approved checkpoint inhibitors, ipilimumab (brand name Yervoy), came to market in 2011 and targets a checkpoint protein called CTLA-4. Ipilimumab was also the first drug to extend the overall survival of patients with advanced melanoma. The average survival rate for patients on the drug was about 10 months (four months more than those who got a cancer vaccine) — but some people had responses lasting years and counting. However, the drug causes severe autoimmune side effects in about 15% of patients, and 2% percent (14 people) died as a result of the treatment.

Immunicom believes subtractive immunotherapy can be positioned as a potential first-line therapy and/or adjunct therapy for a wide range of cancers as well as other immunologic diseases

A New England Journal of Medicine study showed that a combination of two immunotherapies — ipilimumab and nivolumab — and nivolumab alone helped melanoma patients live longer than treatment with ipilimumab only. [13], [14] But most patients (95% in the combination group and about 82% in the nivolumab group) experienced intense side effects (colitis, diarrhea, and fatigue). Some were so severe, the patients had to discontinue treatment. Clearly alternate immunotherapeutic approaches are needed that not only have broader applicability but are safe and well tolerated.

Immunopheresis™ Therapeutic Rationale

The idea behind immunotherapy for treating cancer is at least a century old, yet it is only within the past decade that breakthroughs in the field have emerged that now lead oncological research and treatment. These breakthroughs were largely driven by a more complete understanding of the complex nature of the human immune system. Likewise, Immunopheresis™ is also not a novel concept. The hypothesis that removing immune inhibitors from the circulating blood is based on

sound, accepted science, yet no breakthroughs in Immunopheresis™ have occurred despite more than three decades of research. Immunicom believes that the problems encountered by historical studies were largely technical in nature and driven in part by a lack of understanding of the complexity of the immune system. Failure to achieve an FDA approved Immunopheresis™ therapy then was largely due to these technical problems along with poor study design and controls that ultimately left the scientific community to largely abandon the method, until very recently.

To re-establish Immunopheresis™ as a viable cancer therapy, Immunicom has used advanced materials science to resolve all of the historical technical problems that were faced in earlier studies.

Specifically, early therapies often produced inconclusive or tempered results likely due to:

- lack of capture ligand specificity (leading to poor capture efficiency and thus ineffective immune stimulation)

- poor capture technology that led to leaching of materials into the systemic circulation, with risk of hemodynamic instability and/or anaphylaxis

- poorly controlled therapy approaches that either produced limited benefit, or led to uncontrolled tumor lysis (and which led to significant patient morbidity and even death associated with the treatment)

Using our proprietary sc-TNF-α adsorptive substrate, in combination with Terumo's Spectra Optia system, Immunicom has eliminated these historical problems by nearly eliminating column leaching of the capture ligand while substantially improving capture efficiency and concomitantly reducing the risk of tumor lysis syndrome from unpredictable treatment regimens. With its effective, predictable, proprietary Apheresis Immunoadsorption Affinity Column and a state-of-art apheresis system (the Terumo BCT Spectra Optia®), Immunicom believes subtractive immunotherapy can be positioned as a potential first-line therapy and/or adjunct therapy for a wide range of cancers as well as other immunologic diseases.

Immunologic modulation as a means to control cancer

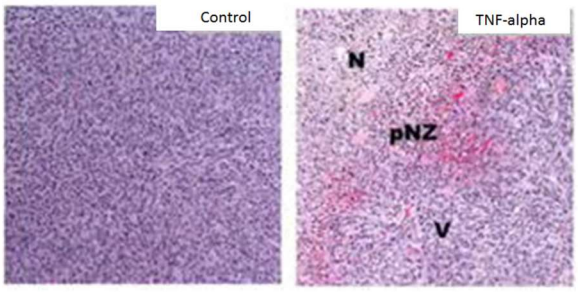
It is well established that the body's immune system is able to "control" cancer. Theories of immunosurveillance and immuno-stimulation, developed over the last half century have helped explain clinical observations of spontaneous remission, even in patients with widespread metastases. [15] Moreover, extensive research has led to the understanding of the role of humoral factors, such as interferons, cytokines and growth factors, as well as intercellular messengers, in immunomodulation of cancer with targeted approaches. For example, significant effort has focused on targets that impact intracellular messaging, such as development of the anti-PD-1/PD-L1 class of agents.

TNF-α as a Potent Natural Mediator of Immune Function

The body's control of inflammation and cellular apoptosis is a very complex and highly regulated process encompassing multiple distinct molecular pathways controlled by a multitude of regulatory proteins. Regardless, there is without scientific doubt, certain key regulators of these processes that includes tumor necrosis factor alpha (TNF-α). TNF-α is a particularly unique and

highly potent signaling protein in that it can drive both local and systemic effects that impact infection control, tissue maintenance and homeostasis. It is this very potency that has limited its therapeutic application as a drug to induce tumor apoptosis and vascular collapse because systemic application creates significant systemic toxicity, severely limiting its clinical use, even when targeted strategies like a TNF-fusion protein or PEGylated TNF, have been used. [16]

But as a naturally secreted cytokine, its immune modulating role is accomplished through three major functional regimes: inflammation and necrosis, cell signaling and intracellular survival, and proliferation and apoptosis signaling. As a systemic pyrogen, TNF-α is involved in local inflammation (heat, swelling, redness, pain and loss of function) through vasodilation, increased capillary permeability and hypotension as well as systemic effects (cachexia, fever, malaise) caused in part by decreased systemic vascular resistance and hypotension and by direct effects on the hypothalamus. It is these functions that also make TNF-α a highly toxic compound when applied systemically or leached into the blood stream in high concentrations. But when used by the body in appropriate levels against cancer, these effects can trigger a cascade of events that ultimately leads to local vascular collapse and tumor necrosis. It is not surprising that this initial and rapid function of TNF-α prepares the surrounding tissues for later invasion by immune cells.



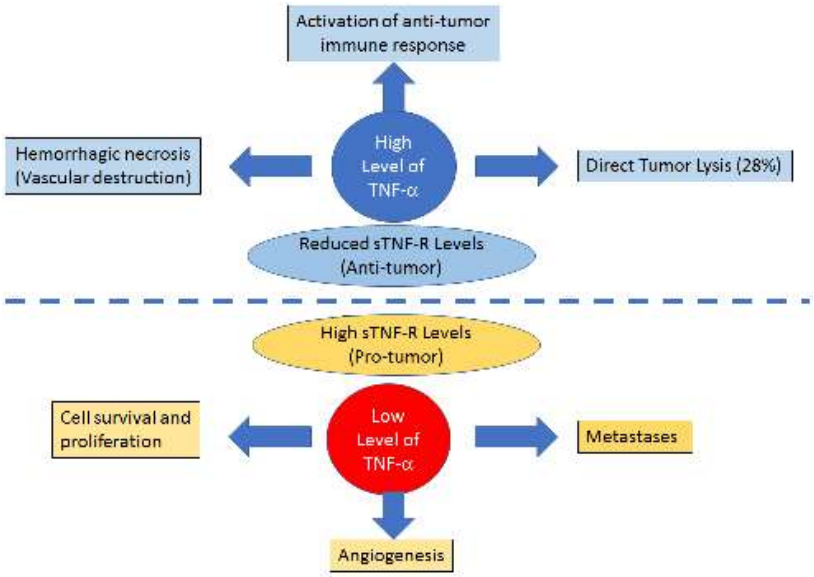
Effect of TNF-alpha on 4T1 Breast Tumor Tissue. Control untreated 4T1 Breast tumor (left panel). TNF treatment shows vascular destruction and extravasation of RBC (right panel). N =Necrotic Region V= Viable Region and pNZ=Perinecrotic Zone. From Reference: Koonce, Nathan A. et al. Combination of Gold Nanoparticle-Conjugated Tumor Necrosis Factor-α and Radiation Therapy Results in a Synergistic Antitumor Response in Murine Carcinoma Models. International Journal of Radiation Oncology • Biology • Physics , Volume 93 , Issue 3 , 588 – 596.

As a strong chemo-attractant for immune cells (particularly neutrophils and macrophages, but also many other immune cells through TNFR-2 binding), the increased capillary permeability likely improves cellular invasion into the region promoting the destruction of infectious agents or, in the case of cancer, enhancing cellular elimination and debri removal (it has also been suggested that this permeability is also likely responsible for the increased uptake and effectiveness of combination therapies using anti-cancer drugs and TNF-α, as is noted in studies using isolated limb perfusion). TNF-α release is further enhanced by IL-1 which is secreted by a number of immune cell types (IL-1 is heavily produced by macrophages, monocytes, fibroblasts, and dendritic cells, but is also expressed by B lymphocytes, NK cells and epithelial cells). TNF-α release as well as IL-1 released by recruited immune cells stimulate adhesion molecules on epithelia cells which also promotes migration of immune cells.

TNF-alpha has anti-cancer activity

Subsequently TNF-α was shown to have both pro-tumor and anti-tumor effects depending on its contextual activity within the tumor microenvironment [42]. Tumor necrosis factor-alpha or TNF-α, as its name implies, is a potent cytokine initially characterized as an anti-tumor agent [41] with **4 defined mechanisms of action: cellular apoptosis, angiogenesis, T-effector cell activation by blocking T-Reg cells, and promoting TAM (Tumor Associated Macrophages) to M1 anti-tumor stage**. In the tumor microenvironment, expression of TNF-α at low levels contributes to angiogenesis, vessel permeability and metastatic potential whereas at high levels and during therapeutic delivery to tumors, TNF-α has shown anti-tumor effects including disruption of vascular integrity through apoptosis, direct tumor killing and induction of anti-tumor immune

responses [42-45]. Support for the beneficial effects of elevated TNF in the clinical setting is well documented. A study of TNF-α expression in 61 NSCLC patients demonstrated expression of TNF-α in 45.9% of cases that directly correlated with a more favorable clinical outcome [46]. Finally, TNF-α can directly affect apoptosis of cells through one of three intracellular pathways. Specifically, TNF-α binding to its receptor can trigger apoptotic pathways through the receptor's death-domain. While its apoptotic activity is the least functional of its molecular roles, The TRADD/FADD activation of the caspase cascade can lead directly to cellular apoptosis. This apoptotic activity is tempered by two other intracellular paths that are also activated by TNF-α binding to its receptor. The TRAF2-JNK pathway is involved in a number of processes related to both cell differentiation, proliferation and is supportive of apoptosis while the TRAF2/IKK/NF-KB pathway mediates transcription of cell survival, proliferation, inflammation and anti-apoptotic processes.



Soluble TNF-α receptors inhibit anti-cancer immune responses

The natural control or attenuation of TNF-α anti-tumor effects is attributed to the presence of inhibitory molecules comprising the soluble shed soluble TNF-α receptors, sTNF-R1 (p55) and sTNF-R2 (p75), that are present in the plasma which bind to and neutralize TNF-α [47-49] [25-26]. In mice, the main TNF-α inhibitor is sTNF-R2 [50]. The cancer promoting activities of these soluble inhibitors was discovered after initial observations of cancer regressions that occurred in patients undergoing plasmapheresis [51,52]. Subsequent studies showed that this observation was attributed to the removal of specific molecular weight plasma components which were identified as soluble sTNF-α receptors (sTNF-Rs) [22,23,39]. The molecular cloning of the cDNA and studies of the recombinant proteins confirmed their anti-TNF-α activity and pro-tumor function [24,26,53]. At low doses of TNF-α, the normal concentrations of these inhibitors can bind and inactivate small amounts of TNF-α. However, increased dosing induces sTNF-R shedding that compensates the ability to quickly reach therapeutic anti-tumor concentrations without toxic effects [48]. For this reason, systemic TNF-α therapy, although effective, has shown problematic toxicities in numerous human clinical trials. Thus, the ability to overcome TNF inhibition to achieve anti-cancer effects requires administration of TNF-α in amounts that are much too close to the maximum tolerated dose (MTD). Due to this adverse risk benefit, systemic therapy using TNF-α has been abandoned. However, isolated limb procedures that block systemic exposure to

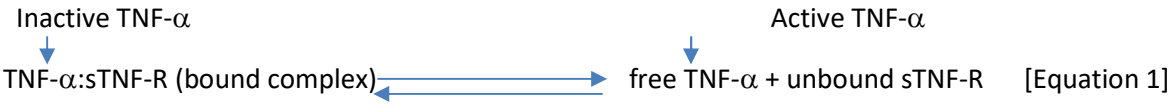
TNF-α are quite effective and are routinely being performed in combination with chemotherapeutic agents [54,55].

New Approaches to TNF-α Therapy

New efforts in TNF-α therapy to surmount sTNF-R inhibition and the untoward effects of induced sTNF-R shedding are in various stages of development. These have involved modification of the TNF-α protein to lessen its toxicity, the development of tumor specific targeting forms of TNF-α and cloaking TNF-α such as binding to PEG or incorporating TNF-α into nanoparticles. These are reviewed in reference [42].

Immunicom's Therapeutic Immunopheresis-- Removal of TNF-α Receptors

Immunicom's focus has been directed toward an alternative approach, the direct removal of the inhibitors sTNF-R1 and sTNF-R2, to promote TNF-α activity by shifting the equilibrium to increase the availability of active forms of TNF-α (Equation 1).



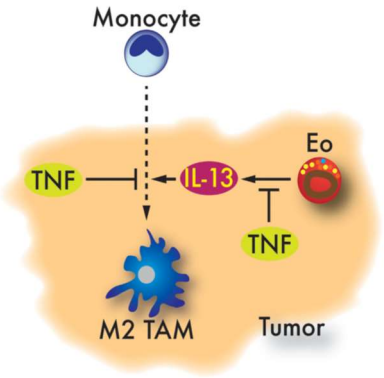
The *in vitro* Keq was found to be 0.59 nM [56] using ELISA assays. This value is less than 0.001, so it is expected this reaction to have mostly complexes at equilibrium, as opposed to having mostly free reactants. The K_{off} values for TNF-RI and TNF-RII at 37°C were 0.021±0.009 and 0.631±0.241 min^{-1} respectively [57]. The dissociation of TNF-α from TNF-R1 was found to be extremely slow.

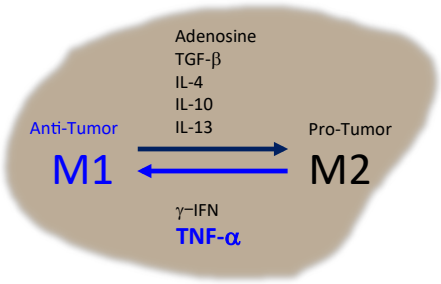
Theoretically, the amount of active TNF-α can be increased by removal of sTNF-R1/R2 (resulting in a shift of the equilibrium of Equation 1 to the right) to promote anti-tumor effects. To do this, we have developed a biocompatible affinity column for the capture and removal of sTNF-R1 and sTNF-R2 from plasma using apheresis technology. The capture ligand Immunicom uses is a patented recombinant single chain TNF-α (sc-TNF-α) which is then chemically linked to a bead substrate support. The sc-TNF-α mimics the structure of the active membrane or soluble TNF-α forms by maintaining a trimerized configuration. In this manner, the sc-TNF-α polypeptide is stabilized, whereas the natural TNF-α monomer ligands must associate into a trimer for binding to the TNF-α receptors and activation. This decrease of sTNF-Rs during treatment is considered to potentiate the activity of tumor resident TNF-α for both transmembrane (tmTNF-α) and free forms (sTNF-α). The former by lowering the amount of sTNF-R that can bind to the tmTNF, the latter by the equilibrium shift toward increased concentrations of sTNF-α.

TNF-α Promoting TAM to M1 Anti-Tumor Stage

Increased sTNF-α blocks the differentiation of monocytes to M2 immunosuppressive cell types and blocks the immunosuppressive pro-tumor activity of IL-13.

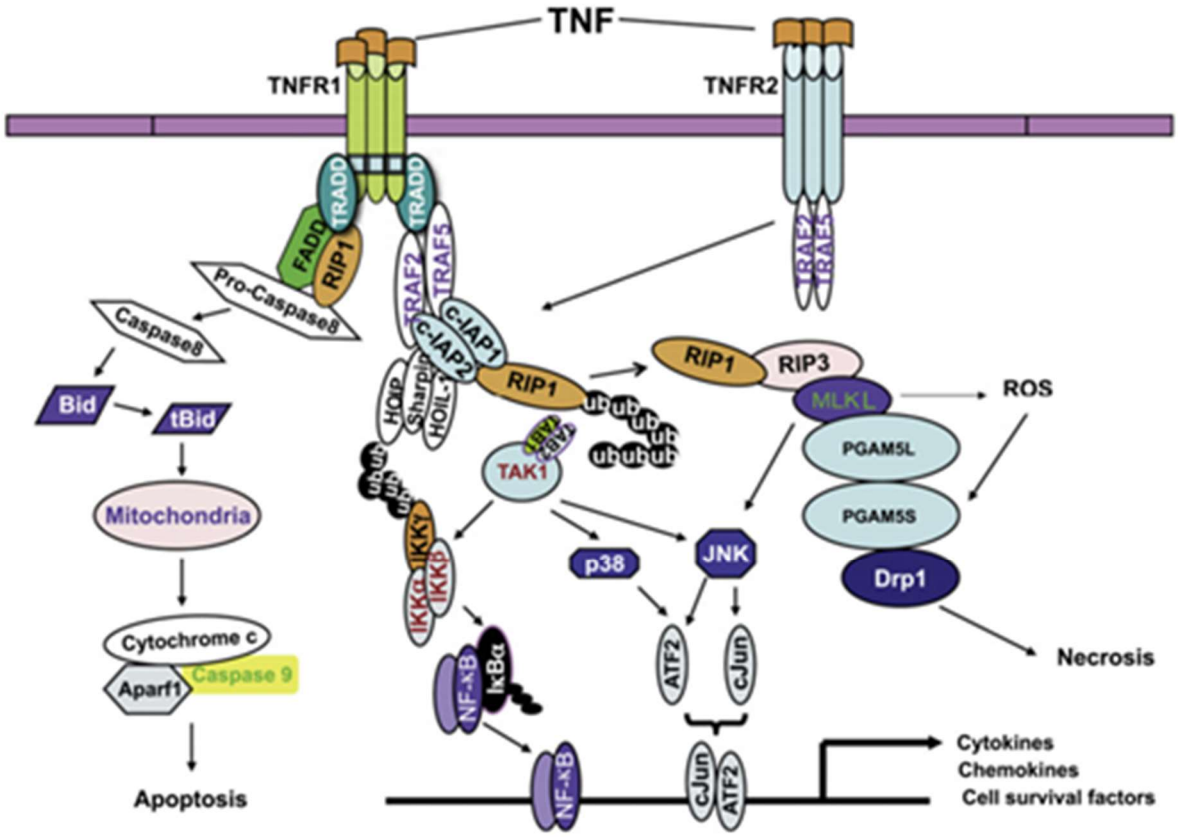
In the tumor microenvironment, in addition to the bound complexes of Equation 1, membrane associated TNF-α (tmTNF-α) can also be shielded by sTNF-Rs. The removal of sTNF-Rs conceptually uncovers the mTNF-α function to enhance intratumoral myeloid cell depletion of immunosuppressive myeloid-derived suppressor cells (MDSC) through induction of reactive oxygen species (ROS). [58-60]. This is followed by subsequent anti-tumor activity of sTNF-α especially in high TACE expressing tumors. tmTNF-α is converted to free soluble TNF-α by TACE (TNF-alpha Converting Enzyme) a member of the disintegrin metalloproteinase family. In some tumors, high levels of TACE in the tumor microenvironment results in the cleavage of tmTNF-α to free sTNF-α. Although this leads to a decreased tmTNF-α activity with respect to M2 phenotype depletion, elevated levels of free TNF-α promotes replenishment of anti-tumor macrophages through monocyte recruitment to the tumor microenvironment. Elevated levels of sTNF-α promote the differentiation of the recruited bone marrow derived monocytes to anti-tumor M1 myeloid phenotypes. Additionally, TNF-α blocks the activity of neutrophil-like cells, the source of IL-13 in the tumor microenvironment [61]. IL-13 is known to promote monocyte differentiation to the protumor M2 phenotype. Overall, the increase in TNF-α activity is followed by a decrease in the numbers of pro-tumor M2 cell types with concurrent increases in anti-tumor M1 anti-tumor macrophages within the tumor microenvironment. The expectation is that there is initiation of the immune response upon removal of sTNF-Rs through tmTNF-α activation as well as free sTNF-α [59,60,62]. The depletion of myeloid derived suppressor cells (MDSC) in the tumor through TNF-R depletion and sTNF-α activation is likely to be more important for tumor control than the potential depletion of M2 mediated by tmTNF-α [63]. Of relevance is that the anti-tumor effects of sTNF-α are transiently induced by the removal of the sTNF-R inhibitors in Immunicom's approach, which adds an additional level of safety. Thus, there are several mechanisms whereby TNF-α contributes to the enhanced anti-tumor immune activity. Aside from the theoretical considerations, we have shown that anti-tumor effects indeed occur upon removal of sTNF-Rs as observed in preclinical studies in dogs.





TNF-α's Apoptosis Mechanism of Action

While it may seem at odds that TNF-α mediates both destructive and protective processes, its potent activity clearly needs to be highly regulated to avoid healthy tissue destruction and systemic poisoning when TNF-α is released due to infection, insult or disease. Left unregulated, TNF-α's potent inflammatory process and pro-necrotic/apoptotic activity could easily lead to the



uncontrolled destruction of healthy tissues surrounding the site of infection or insult. Therefore, TNF-α's additional activation of protective processes makes sense to avoid an uncontrolled response. This is especially true when considered in light of the extracellular regulation of TNF-α and the intricate intracellular regulation of cellular apoptosis. It is now understood that intracellular regulation of apoptosis is largely controlled by the Bcl-2 family of proteins. [17], [18] This family contains pro-survival members (Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A1 – each containing a BH3 binding domain) and pro-apoptotic members which are split between the Bax group and the BH3 group (Bim, Bad, Bid, Bik, Bmf, Puma, Noxa and Hrk). The pro-apoptotic BH3 members (normally in a non-active state), in the face of cytotoxic signaling, become activated and serve to block the function of the pro-survival members allowing two other proteins, Bax and Bak to form oligomers in the mitochondrial and cellular membranes that permeabilizes the membranes and destabilizes cellular function. In this permeable state, other apoptotic proteins such as cytochrome c and the caspase cascade ultimately mediate cell destruction.

Intracellularly this complex family of proteins is directly impacted by cell signaling agents like TNF-α. In this case, TNF-α's apoptotic pathway ultimately leads to activation of Bid (part of the BH3 group) to tBid by way of caspase 8, a part of the caspase cascade that ultimately drives cellular

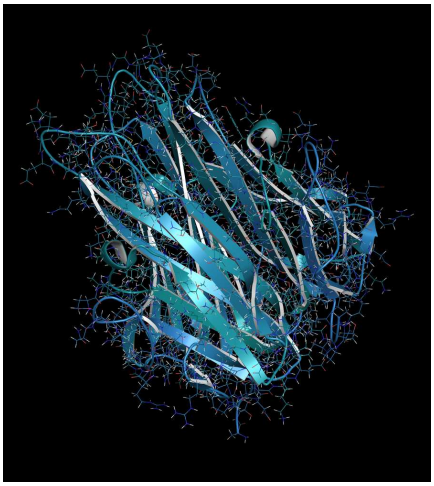
demise. How this complex family of proteins responds to different varieties of external cytotoxic stimuli and different cytokine stimuli (both at the site of inflammation and in surrounding tissues) is beyond the scope of this document. While Immunicom endeavors to more fully understand how TNF-α mediates the process of apoptosis, it is clear that TNF-α can activate Bcl-2 family members directly responsible for promoting apoptotic activity. It is also is now accepted that the dual role of TNF ligands (TNF, TRAIL, FasL, both pro-apoptotic and anti-apoptotic) can be selectively shifted by chemotherapy towards apoptosis induction in tumor cells by blocking the anti-apoptotic pathway (NFkB by Bortezomib (Velcade)) or by blocking inhibitors of apoptotic proteins (e.g. XIAP) with Smac mimetics. Therefore, Immunicom's approach to reduce sequestration of TNF-α (described below) at the local cancer tumor site by removing soluble TNF-α receptors (and therefore increase its local activity) is fully supported by known biological functions.

The History of Soluble Tumor Necrosis Factor Receptors and Their Impact on Cancer

The potential role of TNF receptors in cancer go back as far as studies in the 1970's when "blocking factors" were found in the blood of cancer patients that suppressed the immune response. [19]–[21] In the 1990's, these "blocking factors" were finally identified in the serum and ultrafiltrate of cancer patients as the soluble receptors of TNF-α (sTNF-Rs). [22]–[24] In 1991, Aderka found sTNF-R levels in the serum of cancer patients were significantly higher than non-cancer patients and correlated with disease stage. [25] More recent research has confirmed that sTNF-Rs enhance tumor development and persistence in vivo. [26] While sTNF-Rs normally serve to protect against excessive TNF levels in vivo (neutralizing the potentially lethal concentrations of TNF-α released by activated immune cells), too many sTNF-Rs act to down-regulate the processes stimulated by TNF-α. Most cancers at specific stages of development trigger an inflammatory response from local tissues (inflammation is often the first indication of cancer). This inflammatory response, driven by TNF-α is suppressed when sTNF-R concentrations are abnormally elevated, as often found in cancer. [27] Beyond external effects, cancer cells can also be induced into apoptosis via endogenously stimulated TNF-α production in response to Smac mimetics [28] and even some standard chemotherapeutic agents like Paclitaxel that stimulate TNF-α release. Therefore, the removal of sTNF-Rs not only has the potential to induce therapeutic affects directly, but could improve existing therapies that stimulate endogenous TNF-α production or localized release of TNF-α from recruited immune cells.

TNF-α is a homotrimeric cytokine capable of binding two distinct membrane receptors, TNFR-1 and TNF-R2. As each TNF-α molecule has the capability to bind three receptor molecules, and because receptors in addition can homodimerize and/or homotrimerize, large cellular membrane complexes are formed upon TNF binding, trapping TNF-α with high effective affinity, based on avidity effects. A well-known mechanism of regulation of membrane expression of TNF-α receptors is proteolytic shedding by metalloproteinases of the ADAM family, leading to the soluble extracellular domains of these receptor molecules, still capable of binding TNF-α, albeit with quite low affinity. Rising circulating levels of these soluble receptors, however, as observed in various diseases including cancer, result in effective TNF-α sequestration lowering the TNF-α levels available for membrane receptor binding, thereby reducing subsequent induction of the respective intracellular signaling (in fact, some activation of immune cell function is driven by membrane bound TNF upon binding to its cognate membrane receptors (particularly for TNFR-2)). Accordingly, in cancer patients, only after removal of soluble TNF-α receptors from the blood by apheresis can endogenously produce TNF-α be fully available for cellular signal induction,

leading to its well defined anti-tumoral effects. It is this sequestration process that Immunicom's LW-02 device effects by reducing the amount of circulating sTNF-Rs.



TNF-α cytokine protein 3D rendering

Targeting the Soluble Receptors of TNF-α to Enhance Immune Response to Cancer

The over-production and shedding sTNF-R1 and sTNF-R2 is recognized as a negative prognostic indicator for human breast, melanoma, colorectal and bone sarcomas and has been shown to correlate negatively with patient survival. [29][30] This protective effect, that is easily measurable systemically, is modifiable by changing the ratio of bound to soluble TNF-R's systemically. Immunicom's LW-02 device rapidly shifts the ratio of sTNF-Rs to help pull local sTNF-Rs away from the area of inflammation, in this case cancer tumors. As intracellular pools of sTNF-Rs are reduced, local TNF availability increases as more free TNF is made available for cellular signaling. Dissociation constant values for sTNF binding to TNF-R positive cells have been determined mainly under conditions of reduced membrane fluidity (0 degrees Celsius; thereby allowing no formation of large TNF/TNF-R clusters and subsequent internalization), and typical values are in the order of 100 pM. [31] Typical values for the proinflammatory response of a cell (activation of NF-kB) are in the low pM range. TNF concentrations necessary for induction of the apoptotic program are significantly higher, however, corresponding well with the dissociation constant values from cellular binding studies. Accordingly, significant NF-kB-mediated responses can be induced when only a few cellular membrane receptors have bound the ligand, whereas apoptosis induction needs high numbers of ligated receptors. The difference in these two cellular responses is quite well understood on the basis of mathematical models. [32] Using TNF derivatives capable of binding only single receptor molecules, or only two receptors, rather than three receptor molecules bound by wild type TNF [33] the group of Scheurich has determined that the dissociation constant for binding of single, soluble TNF receptors to TNF-α is low and is in the order of 5 nM (personal communication, unpublished data).

In addition to induction of inflammatory processes and intracellular apoptotic pathways, TNF-α induces cellular immune action through T-helper cells that contribute to tumor killing through activated killer T cells. This transient activating effect occurs even in the presence of high concentrations of T-suppressor cells, particularly when the TNF-α concentration fluctuates rapidly. Our highly effective column induces this effect by providing a rapid, transient reduction of sTNF-R serum concentrations through high efficiency capture that should effectively increase local TNF-α levels. The induced, rapid, transient increase in TNF-α levels should avoid down-regulation of killer T-cell activity, as seen with steady state infusions of TNF-α. [19] How much and how long reduction in the intracellular pool of sTNF-Rs is required, how quickly this pool recovers and how much free TNF is needed to elicit an immune response are questions being addressed in this and future proposed studies.

Subtractive Apheresis as a Cancer Treatment Regimen

Apheresis, unlike hemodialysis, separates the patient's blood into its cellular and humoral fractions in order to remove a particular fraction from the patient's blood. This allows for the removal of specific component, including "blocking factors", without significantly impacting or losing critical

cellular components. Initial studies in the 70's and 80's using non-selective plasma filtering showed positive responses in 47 of 162 cancer patients treated, but required large plasma exchanges and did not attempt to distinguish the "blocking factors". Ultrapheresis soon replaced the undifferentiated method, with semi-selective filtering to only remove low molecular weight proteins (<150kDa), which included these "blocking factors". [34]–[36] And while this method showed promise in reducing tumor size and generally improving quality-of-life (QoL), the method also required large amounts of fluid exchange and occasionally caused tumor lysis syndrome (TLS).

In these early studies, autopsies of patients that died from TLS revealed massive immune-mediated tumor necrosis, providing strong support that removal of specific blocking factors therapeutically could lead to effective immune activation (if only it could be done safely). Subsequent improvements to the technology, termed adsorptive apheresis, used affinity chromatography to selectively remove unwanted inhibitors. For example, Staphylococcus Protein A Immunoadsorption was used to remove IgG and "circulating immune complexes" [37][38] to treat myeloma and solid breast, melanoma, lung and thyroid tumors. Enhanced lymphatic response and activation of complement was observed, leading to partial remission in approximately 28% of patients. Subsequent development of a polyclonal rabbit antibody targeting primarily sTNF-R's (but also IL-2) by Lentz (Oncosorb Column) was first described in the treatment of 9 patients with metastatic cancer. A 12-treatment course resulted in significant reductions in sTNF-R blood levels and produced clinical symptoms of local tumor lysis evidenced by local inflammation, subsequent tumor necrosis and a reduction in tumor size in most patients (including complete resolution of tumor in several subjects). [39] In a study of 15 breast cancer patients with metastatic disease, Oncosorb treatment resulted in tumor size reductions of 50 to 75% with all patients having improved Karnosky Performance Status after therapy.

While promising, reports of leaching of potentially dangerous amounts of column materials into the patient's bloodstream, variability in removal of sTNF-R's, sometimes short-lived effects and development of complications like tumor lysis syndrome raised doubt that whole body Immunopheresis™ was a practical therapeutic approach. This led to alternative approaches to utilizing TNF's immune stimulating activity while avoiding its high toxicity and resulted in studies using a technique called isolated limb perfusion (ILP). Both ILP and a similar method, isolated limb infusion (ILI), have been practiced for decades with varying degrees of success. In a recent review by Deroose et al [40], 167 ILP's were performed between 1991 and 2004 combining TNF-α perfusion with melphalan for treating in-transit melanoma metastasis. With an overall response (OR) rate of 89% and a CR rate of 61%, it is clear that TNF, when used appropriately and under controlled application, can affect a significant positive therapeutic response. It was noted that high dose ILP (up to 4mg per limb) produced more positive results (in combination with melphalan) than lower dose TNF. These high doses of TNF likely drive local concentrations of TNF higher than would naturally occur at the site of the IT melanoma. And by isolating the limb, the systemic pool of sTNF-R's is not available to sequester the high concentration of TNF. This provides strong evidence that Immunicom's approach to stimulating a local TNF response by reducing systemic levels of sTNF-R's will help to drive local TNF delivery by the patient's body that would not be possible without reduced sTNF-R levels.

Despite the success of TNF based ILP, ILP is not a practical solution for metastatic disease outside of the peripheral vasculature. This is clearly shown by the fact that there was no correlation

between TNF dose in ILP and overall survival, indicating that the therapeutic effect was localized to the limb being treated, as would be expected. [40] But what ILP and previous whole-body apheresis approaches have indicated is that modulation of TNF using apheresis approaches can induce a therapeutically effective immune response, if only it could be controllable and predictable.

The Path to an Immunopheresis™ Cancer Therapy

Immunicom has resolved the primary technical problems of past apheresis approaches (discussed above) that have prevented its widespread use as a therapeutic treatment for cancer. By utilizing a recombinant, single chain form of TNF-α (sc-TNF-α) in combination with and optimal bead resin, and an optimized manufacturing process for the column, Immunicom has resolved the issues of capture specificity, capture ligand leaching and capacity.

Combined with the Spectra Optia's high efficiency for plasma exchange procedures, its ability to optimize conditions based upon patient factors like total blood volume, hematocrit and animal weight, and its capability for blood flow rates of 10 to 100 mL/min with a secondary plasma device (TPE-SPD), we can now effectively control the entire therapeutic process in a safe, measurable manner. This apheresis control is very similar to the "prescriptive" dialytic approaches employed with hemodialysis, based on patient urea kinetic modeling. We believe that, along with our technical product developments described below, this combination Immunopheresis™ system can effectively induce an immune response that safely induces tumor lysis.

Based on data from preclinical studies in dogs, Immunicom has obtained evidence for TNF-α induction during therapeutic apheresis as observed by elevations of soluble receptors from baseline levels. Canines with naturally occurring cancers have been studied as a preferred comparative oncology model, as canines are also the smallest vertebrate on which Immunopheresis™ can be safely performed.

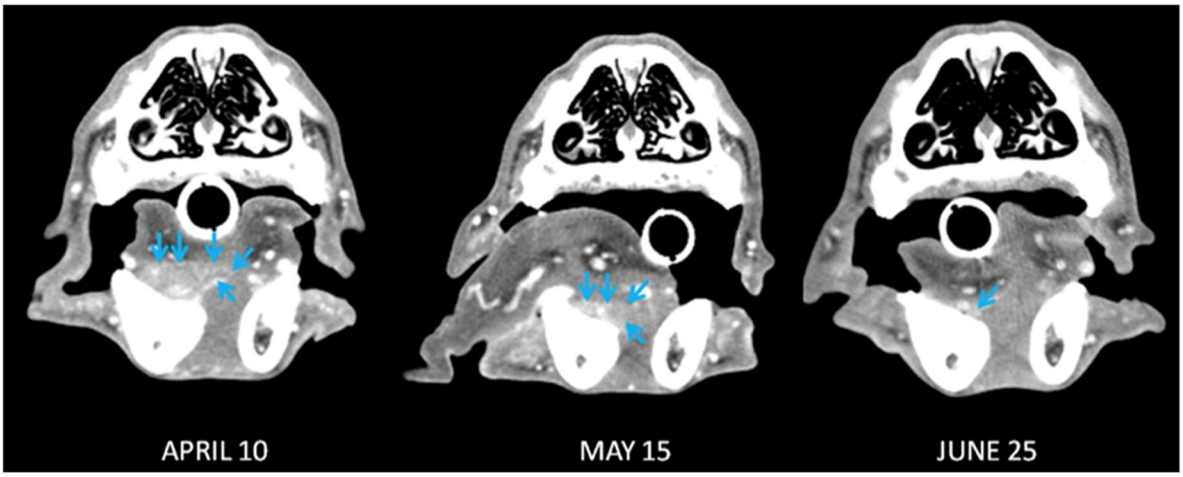
ADVANTAGES OF COMPARATIVE ONCOLOGY	
Clinical	**Commercial**
Cancers occur naturally	*Preferred approach by FDA and EMA*
Strong genetic similarities to humans (same type of cancers with same cause)	***Revenue opportunity in human and potentially veterinary markets***
Produce a normal immune response similar to humans	***Decreases time-to-market***
Metastasis (spreading of cancer) similar to humans	*Provides real-world use experience through veterinary market*
Similar response to radiation, chemotherapy, etc.	*Improves commercialization and human market adoption*
Recurrence/Resistance similar to humans	***Improves safety in human studies***
Shorter time required to study treatments	***Lowers cost of human studies***

	Part A	Part B
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)
Mean/Median Survival/ (Range) *in days* **	~ 81 /~110 *(31 - 359)*	~ 132/~164 *(54 – 433)* ↑

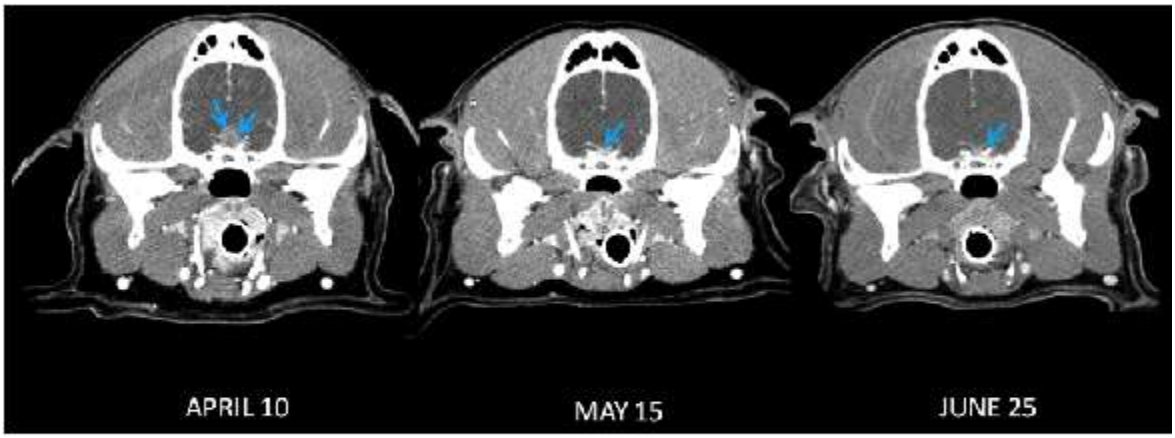
**Dogs studied were deemed to have aggressive disease that would have likely required euthanasia within 4 to 6 weeks of diagnosis to prevent undue pain and suffering. The Tx prolonged survival with excellent QoL during and immediately post treatment (in contrast to chemotherapy). Part B data generated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

The LW02 device has been shown to reduce or halt the growth of metastatic tumors in approximately half of the 16 evaluable animals studied to date (~50% Best Overall Response). Quality of Life (QoL) was maintained throughout the course of therapy for all animals. The treatment was shown to be generally safe and well tolerated, with no dog being withdrawn from therapy due to therapy related adverse events. One canine was completely cured of multiple tumors, including a pituitary mass, over the course of 18 treatments. (**Figure 3**) We thus believe that there is a strong rationale supporting the development of Immunicom's Immunopheresis™ affinity chromatography technology as a subtractive immunotherapeutic approach for treating cancers.

FIGURE 3



Pituitary mass in brain, pre-treatment, 11mm (April); Pituitary mass no longer evident, post-treatment (June)



A) Pituitary mass in brain, 11mm; **B) & C)** pituitary mass no longer evident

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