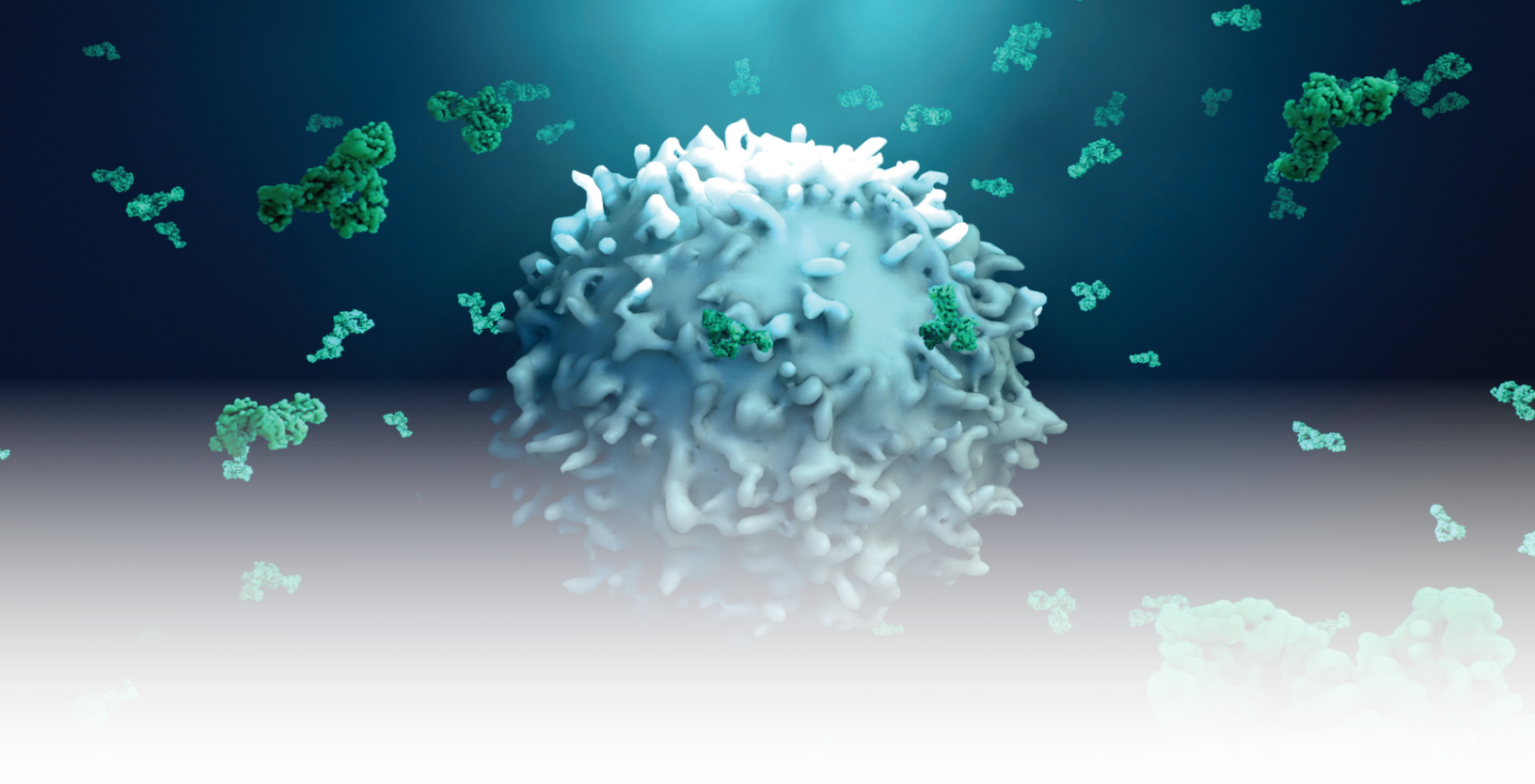


Versamune®: A New Generation of Cancer Immunotherapies

Despite recent progress in fighting cancer the sad reality is that it remains a leading cause of morbidity and mortality. Some of the most promising new treatments have emerged from the convergence of the oncology and immunology fields. These novel therapies that harness the power of the immune system to fight cancer are called **immunotherapies**. Cancer immunotherapies have significant potential to treat a broad range of cancers, and several have been approved by the FDA. To date, however, while progress has been made in developing new anti-cancer immunotherapeutic technologies and products, significant challenges limiting their clinical effectiveness remain.

On a basic immunological level, considerable hurdles impeding the ability of immunotherapy to harness the body's immune system most effectively persist. For example, approved checkpoint inhibitors have been demonstrated to be effective and for those patients who respond, the durability of their responses can be significant. Unfortunately, the rates of response reported are only in the range of 15-20%. Importantly, immune therapies, including checkpoint inhibitors, CAR-Ts and live-vector vaccines, remain burdened with significant systemic toxicities limiting their use either in the early-stage cancer setting or in combination with other approved anti-cancer treatments.

This paper provides an overview of cancer immunotherapy, describes some of the challenges to optimizing its potential, and introduces **Versamune®**, a proprietary T-cell activating platform engineered and developed to overcome some of these challenges in order to improve the treatment outcomes of patients with cancer.

What Is Cancer Immunotherapy?

Cancer immunotherapy is a form of cancer treatment that utilizes the power of the body's own immune system to recognize, attack and eliminate cancer. The ultimate goal of cancer immunotherapy is tumor eradication or, at least, regression.

The body's immune system is a complex biological network designed to defend against germs, other microscopic invaders, and cancer cells. Once the immune system recognizes an organism or cell as foreign or dangerous, it begins a series of complex reactions to identify, target and eliminate them. This is called mounting an immune response. Cancer immunotherapy takes advantage of the discovery that most cancer cells express unique proteins, also called tumor antigens, not normally expressed by healthy cells and thus can be recognized as abnormal and dangerous. Because the immune system is precise, it can target these dangerous cancer cells exclusively while sparing healthy cells. However, the challenge remains that cancer cells are often not perceived as dangerous or foreign, so the immune system becomes tolerant to them.

An ideal cancer immunotherapy should have the following attributes to maximize the opportunity for clinical effectiveness in patients. It should:

- Stimulate both tumor specific killer and helper T-cells within the body
- Activate, arm and expand large numbers of T-cells that recognize the tumor
- Alter or de-camouflage the tumor microenvironment (TME) to make the cancer enemy more visible or susceptible to attack by the immune system
- Generate immune memory, so if cancer cells return, the immune system is able to recognize and eliminate them
- Optimize safety and tolerability by limiting systemic inflammation and toxicity

As published in the June 2019 issue of The Journal of Immunology, a leading peer-reviewed journal in the field of immunology, Versamune® incorporates each of these attributes, leading to superior anti-tumor responses in pre-clinical studies. (Gandhapudi, et al., 2019). PDS Biotech's Versamune® technology platform is unique in its ability to successfully encompass the mechanistic attributes required to induce a safe and effective anti-cancer immune response.

How Does Cancer Immunotherapy Work?

An important function of the body's immune system is to scan for antigens not normally expressed in healthy tissue. Once an antigen has been identified as foreign, abnormal or dangerous, the antigen is presented to **T-cells**, a type of white blood cell effective at eliminating cancer cells and infectious agents (e.g. bacteria and viruses). The presentation of an antigen to T-cells is implemented primarily in the lymph nodes by specialized antigen presenting cells known as dendritic cells which are programmed specially to identify foreign antigens and to present them to T-cells. Unique proteins on the surface of dendritic cells, known as major histocompatibility complex (MHC) molecules, bind to the foreign antigen and display them on the cell surface for recognition by the appropriate T-cells. Then, once presented, a sub-population of T-cells known as the **CD8+ or killer T-cells**, are primed and respond to the specific foreign antigen by attacking and killing the cells containing the abnormal protein. Other T-cell sub-populations, such as CD4+ or helper T-cells, are also critical in regulating immune responses.

Cells communicate via chemical signaling. For an immune response to be triggered and to be effective, important immune signaling pathways must be activated to enable the body to induce messenger proteins known as cytokines and chemokines. Some of these cytokines and chemokines serve both to activate and expand T-cells and to arm the T-cells with the appropriate cancer-killing function.

An effective cancer immunotherapy must modulate these complex processes, enhancing activation and producing robust expansion of the critically important high-quality, tumor-specific T-cell populations, most notably CD8+ killer cells. As will be reviewed in more detail in the section below, the ability to promote the induction of therapeutic quantities of high-quality tumor-targeting CD8+ killer T-cells within a patient's own body has been a major limitation of cancer immunotherapy.

Production of adequate numbers of high-quality CD8+ killer T-cells alone, however, is insufficient to eradicate all cancer cells. One of the difficulties in treating cancer stems from the fact that cancer cells have the unique ability to suppress the immune system; they camouflage themselves or evade T-cell attack by activating immune mechanisms that suppress the ability of T-cells to detect or attack them. They accomplish this in part by increasing the population of immune suppressive cells, including cells known as regulatory T-cells (Treg) as well as other cell types, within the tumor microenvironment. An effective immunotherapy must overcome the tumor's immune suppressive mechanisms in order to successfully locate and attack the cancer cells.

Finally, cancers can be difficult to cure because they may recur even after successful initial treatment due to micro-metastatic (hidden) tumors disease that is not completely eradicated after treatment and that eventually expands. It is yet another task of the immune system to remain ever vigilant for recurrence, a vigilance mediated by memory T-cells which serve as the immune system's long-term memory. To be durable and effective over an extended period after treatment, and to minimize the likelihood of cancer recurrence, an immunotherapy should enhance this immune function as well.

What Have Been the Challenges of Developing Immunotherapies in Oncology?

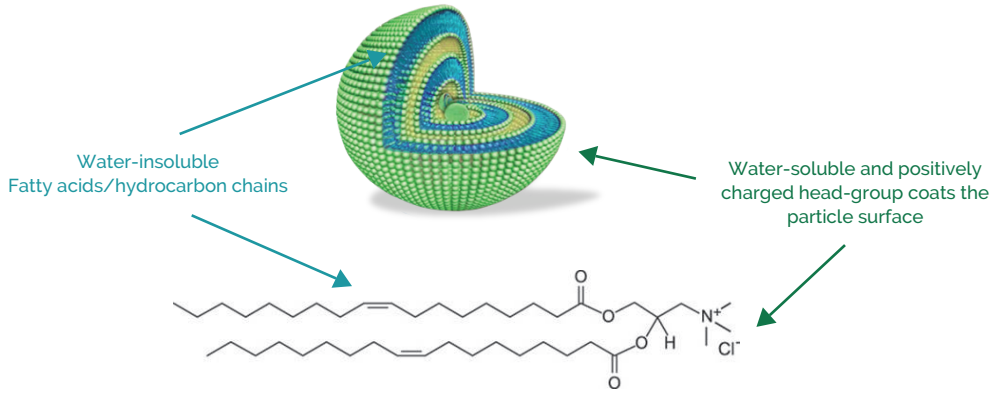
The inability to generate adequate quantities of unique, high-quality killer T-cells, to minimize systemic toxicities, to overcome the immune system's tolerance of the cancer, and to generate immunological memory, all limit the clinical effectiveness of immunotherapies.

On a fundamental biological or immunological level, one of the most daunting challenges confronting the development of effective immunotherapy is the development of a simple and easy to administer therapy that can promote the induction of highly potent, targeted, tumor-specific T-cells that can effectively treat cancer with minimal side effects. Suboptimal T-cell activation remains a key limitation of immunotherapies. Potential hurdles exist at all stages of the immunological process, including poor uptake of the antigen by the dendritic cells as well as inadequate processing and presentation of the tumor antigen.

Versamune®: Overcoming the Challenges of Cancer Immunotherapy

What is Versamune®?

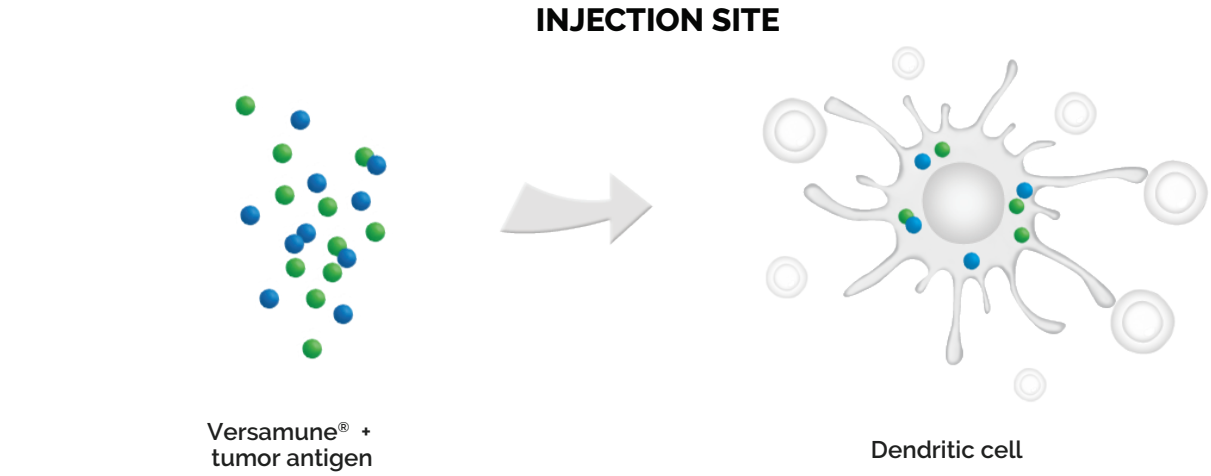
Versamune® is a proprietary T-cell activating platform designed to overcome the challenges of current immunotherapy in order to improve the treatment outcomes of patients with cancer. Versamune®-derived products are based on positively charged (cationic) and immune activating lipids that form spherical nanoparticles in aqueous media. These lipids include the R-enantiomer of 1,2-dioleoyl-e-trimethyl-ammonium-propane (R-DOTAP). Cationic lipids are positively charged molecules that have a water-soluble portion (head group) attached to a water insoluble tail. The water-soluble portion of the molecule has a positive charge and the water-insoluble portion is made up of hydrocarbon (also called fatty acid) chains. The nanoparticles, which are coated with a positive charge, are deliberately sized to mimic viruses, facilitating detection by the body's immune system and uptake by dendritic cells.

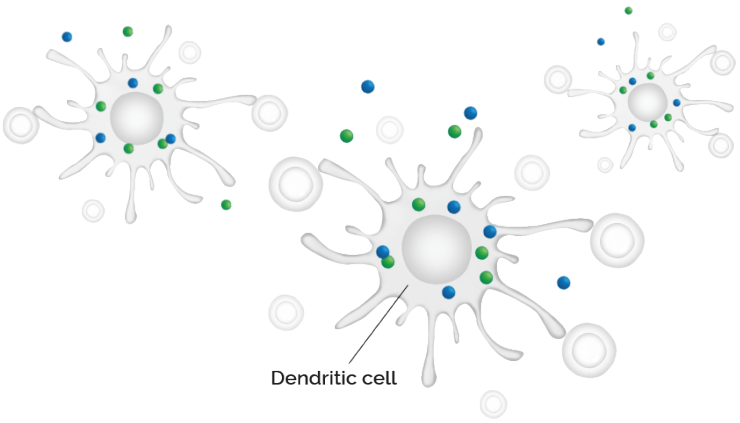


To treat a specific cancer, the unique or overexpressed antigen found on the surface of the cancer cells is manufactured, then mixed with the Versamune® nanoparticles to create a pharmaceutical product for simple subcutaneous injection.

Versamune® Promotes Dendritic Cell Uptake

One of the biggest challenges in developing a potent immunotherapy has been dendritic cell uptake. Versamune® is designed specifically to be taken up by dendritic cells in the skin. As noted, Versamune® nanoparticles are sized comparably to viruses normally taken up as part of the natural function of the dendritic cells, facilitating efficient uptake of the Versamune®-based immunotherapy. Studies evaluating the uptake of Versamune® nanoparticles by dendritic cells and epithelial cells, found almost exclusive uptake by the dendritic cells. Four hours following a single subcutaneous injection, about 80% of the dendritic cells in the draining lymph node were found to have taken up the Versamune®-based immunotherapy.



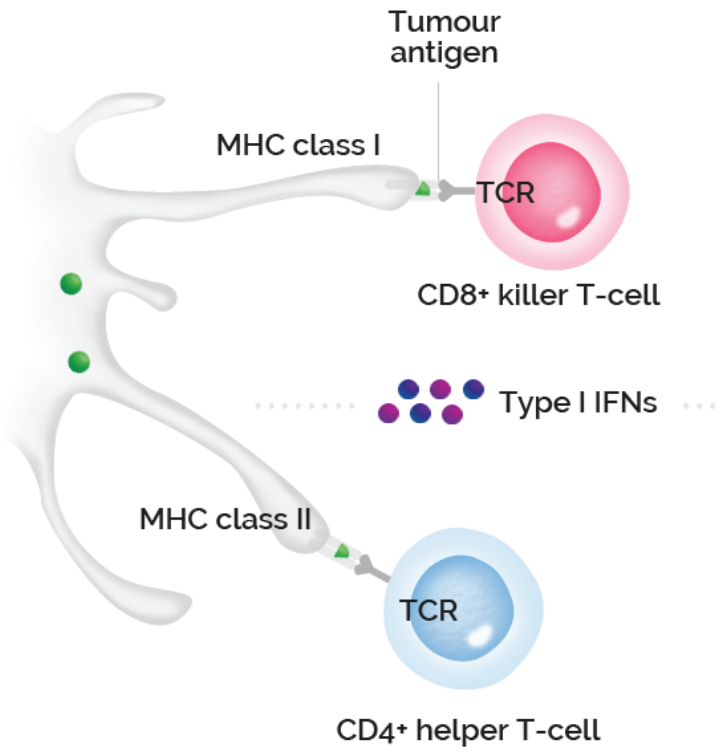


In addition, as the surface of Versamune® is positively charged and the body's cells are negatively charged, Versamune® tends to be quite "sticky" and serves as a depot at the injection site for several days following subcutaneous injection. Large numbers of dendritic cells thus have the opportunity to be recruited and to take up the nanoparticles.

Versamune® Promotes Efficient Antigen Processing and T-cell Presentation

When dendritic cells take up Versamune® nanoparticles they become activated, mature and begin recruiting additional dendritic cells. Once inside the dendritic cell, the tumor-associated antigen is released and processed into the requisite small peptides (pieces of protein) in the cell compartment known as the cytoplasm. An important advantage of Versamune® is its ability to fuse with and destabilize endosomes in the cytoplasm, promoting efficient entry of the antigen into the cell compartment where processing can take place. Processed antigen is turned into peptides that then utilize both the MHC class I and class II pathways. The MHC class I pathway is critical to programing CD8+ killer T-cells and the MHC class II pathway to programming CD4+ helper T-cells to recognize tumor antigens. When Versamune®-induced maturation occurs, the dendritic cells express costimulatory molecules on their surface, which facilitate the highly efficient uptake and presentation of antigens to the T-cells. This activity overcomes one of the most significant limitations of current immunotherapy development – the efficient priming of critical CD8+ killer T-cells against tumor antigens. Interestingly, Versamune® has been demonstrated to promote presentation of antigens to CD4+ helper cells as well.

Versamune® Demonstrates Effective Antigen Presentation to Both CD8+ Killer and CD4+ Helper T-cells

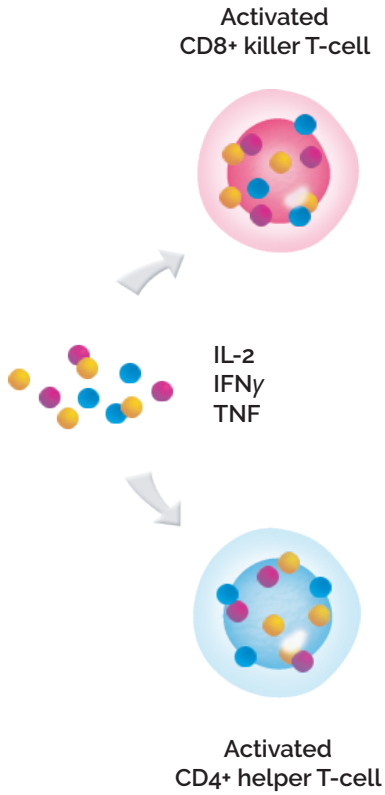


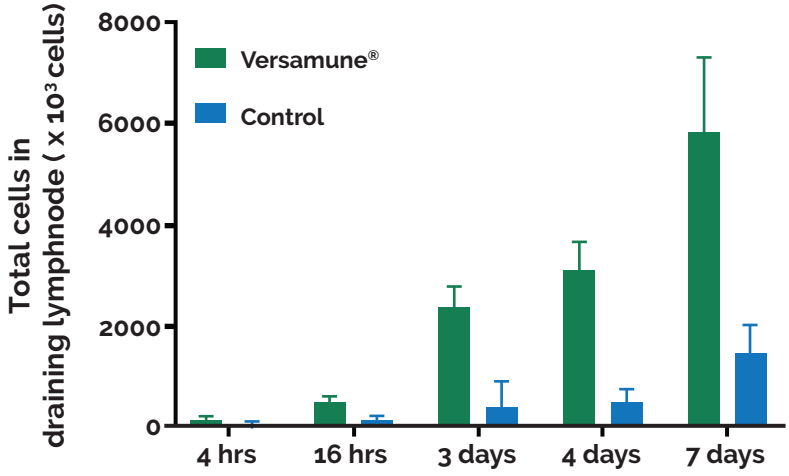
Versamune® Promotes Efficient Activation and Robust Expansion of High Quality Polyfunctional CD8+ Killer T-cells in Lymph Nodes

Ultimately mature dendritic cells migrate into lymph nodes, small glands located throughout the body containing white blood cells including T-cells, where much of the key immunological activity pertaining to the priming and expansion of T-cells takes place.

In the lymph nodes the dendritic cells present the tumor antigens to T-cells resulting in activation or priming of the T-cells to recognize the particular antigen expressed by the cancer. Importantly, Versamune® also upregulates type 1 interferon genes (type I IFN), which are responsible for critical immunological processes. This induces an important immunological protein called CD69 that facilitates interactions between the dendritic cell and T-cells in the lymph nodes. Upregulation of type I IFN signaling also induces multiple immune messengers called cytokines and chemokines that further signal T-cells to infiltrate into the lymph nodes. Powerful activators of CD8+ killer T-cells, such as CCL2 and CXCL10 are documented to be induced by Versamune® as well. As the Versamune®-induced production of chemokines appears to be restricted to the lymph nodes, the site of T-cell activation, it provides for both superior activation and expansion of CD8+ killer T-cells. Localization of these immune messengers within the lymph nodes and their limited presence in the blood circulation enhances the safety of the Versamune®-based immunotherapies. Thus, through the versality of its mechanisms of action, Versamune® safely promotes the efficient and robust expansion in-vivo of large numbers of highly potent (polyfunctional) CD8+ killer T-cells, both critical factors in developing a successful immunotherapy.

Versamune® Promotes Activation and Robust Expansion of Both Antigen-specific CD8+ Killer and CD4+ Helper T-cells

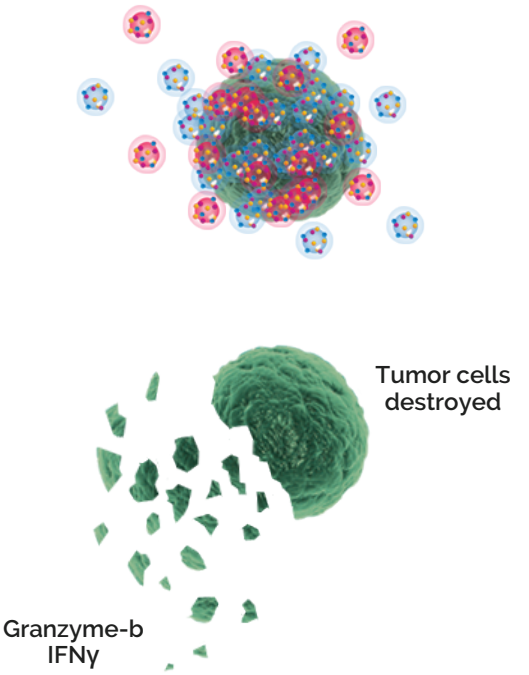


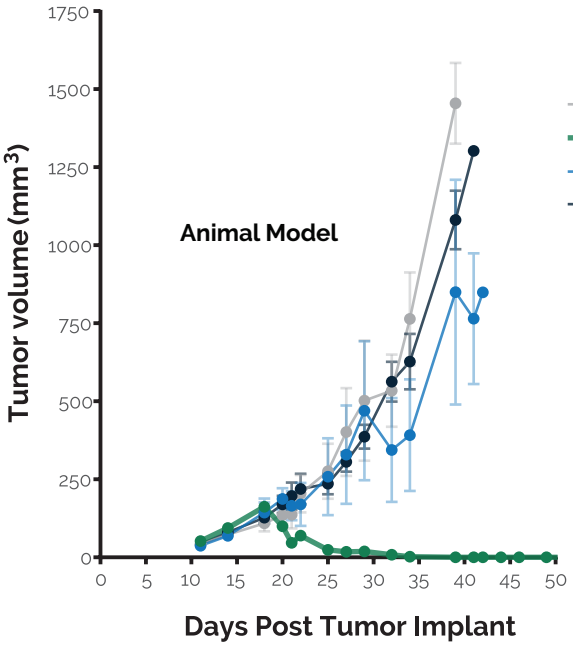


Versamune® Overcomes Immune Suppression

Regulatory T-cells (Treg) are a sub-population of white blood cells normally responsible for recognizing normal healthy cells and for preventing autoimmune disease. In cancer however, they are utilized by the cancer cells to evade immune detection. Versamune® results in significant alteration of the tumor microenvironment to reduce dramatically the Treg to killer CD8+ T-cell ratio making the tumors more susceptible to destruction by killer T-cells. Preclinical studies have demonstrated that lowering the Treg to CD8+ killer T-cell ratio with polyfunctional CD8+ killer and CD4+ helper T-cells promotes effective tumor lysis and regression.

Versamune® Makes Tumors More Susceptible to Destruction by Activated CD8+ Killer T-cells





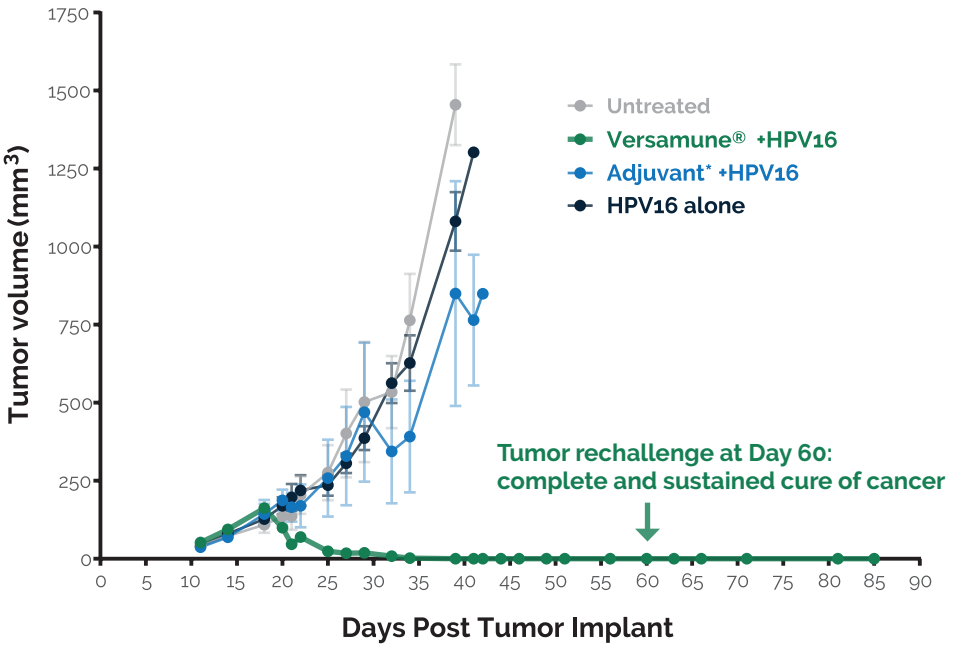
Tumor targeting CD8+ killer T-cells destroy tumors after a single treatment with Versamune® + HPV16

Overcoming a tumor's immune tolerance and minimizing its ability to evade detection is a significant goal of a successful cancer immunotherapy that together with potent T-cell induction may translate to enhanced tumor elimination.

Versamune® Induces Immune Memory

Memory T-cells allow the body to maintain tumor-recognizing and attacking T-cells for an extended period after treatment, with the ideal outcome of reducing cancer recurrence. Preliminary studies demonstrated that Versamune® protected mice who had experienced tumor regression against tumor reestablishment even when the mice were reinjected with the same tumor cells. This sustained protection was evidence of immune memory: persistence of antigen-specific T-cells to recognize tumor proteins associated with a particular cancer, as the animals were not protected against establishment of different tumors. Evidence of the potential for Versamune®-based immunotherapies to induce immune memory has also been demonstrated in a phase 1 clinical trial in humans.

Enhancing tumor-specific memory responses to monitor for and eradicate cancer cells well after initial treatment provides potential for significant clinical benefit by possibly reducing the incidence of tumor recurrence.

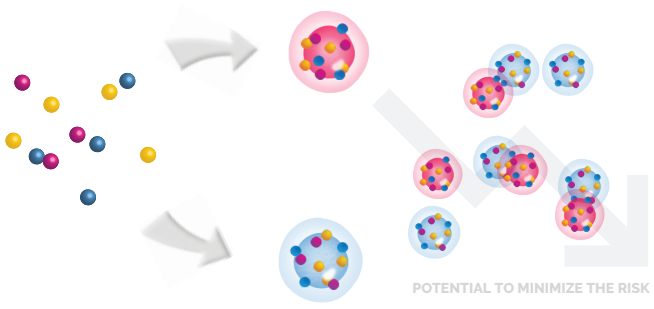


When cured mice are re-exposed to the same tumor, they remain free of cancer, confirming the presence of immune memory that recognizes the tumor when it reappears.

Versamune® Safety

Today, many cancer immunotherapies produce serious systemic autoimmune effects as well as inflammatory toxicities due to the increased presence and spikes of cytokines in the blood circulation. The mechanism of action of Versamune® as well as its design both contribute to the localization of cytokines in the lymph nodes and specific targeting of CD8+ killer T-cells to antigens in tumor tissue. Therefore the expectation is that Versamune®-based therapies will exhibit an improved and favorable safety profile compared to currently available treatments.

As noted, Versamune® is injected subcutaneously (under the skin) and its mechanisms of action are localized primarily in the lymph nodes. Further supporting these observations are data demonstrating that negligible levels of Versamune®-induced cytokines were detected in the blood of mice. Very low quantities of Versamune® were detected in the blood or in any organ outside of the lymph nodes.



The localized and sustained cytokine induction in the lymph nodes of Versamune® has the potential to

MINIMIZE THE RISK OF SYSTEMIC TOXICITY

Additionally , Versamune® is broken down (hydrolyzed) in the body into fatty acids and excreted, thus mitigating the potential for short- or long-term accumulation of the nanoparticles. These pre-clinical observations have been confirmed by early clinical data documenting that this localized and highly specific cascade of immune activity was associated with an absence of systemic toxicity at all doses tested. In a phase 1 clinical study designed to evaluate safety, all patients had transient swelling and redness at the injection site due to initiation of the immunological cascade at the injection site which cleared completely within 3-7 days. **No dose-limiting toxicities or long-term safety concerns were observed.**

In choosing and designing a Versamune®-based therapy, careful attention is paid to selecting specific, appropriate antigens because, as described above, Versamune® induces a strong T-cell response to the antigen. All of the antigens currently being evaluated in combination with Versamune® are present primarily in cancer cells which should therefore result in tumor-specific T-cell attack, thereby minimizing off-target toxicity and potential destruction of healthy cells and tissue.

Versamune® Potential as an Immunotherapy

Cancer Immunotherapy

The unique ability of Versamune® to modulate and enhance numerous critical steps required for an effective immune response and to be combined with targeted specific antigens found on tumor cells, offers several exciting opportunities to treat a variety of cancers. Further, its diverse mechanisms of action together with its favorable safety profile suggest therapeutic promise when used in combination with other treatment modalities or immunotherapies such as checkpoint inhibitors as well as in the single-agent monotherapy setting.

The current PDS Biotech pipeline of Versamune®-based therapies focuses on four key antigens associated with a broad variety of solid tumors that remain challenging to treat:

PRODUCT	INDICATION	COMBINATION	PC	P1	P2	P3	R	PARTNER(S)
Oncology								
PDS0101 (HPV16)	First line treatment of recurrent / metastatic head and neck cancer	KEYTRUDA®						MERCK
PDS0101 (HPV16)	Advanced HPV-associated malignancies	M7824 NHS-IL12						NIH NATIONAL CANCER INSTITUTE
PDS0101 (HPV16)	Stage IIb-IVa cervical cancer	Chemo-radiation						THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
PDS0102 (TARP)	Prostate and breast cancer	Immunotherapy						NIH NATIONAL CANCER INSTITUTE
PDS0103 (MUC-1)	Breast, colorectal, ovarian and NSCLC cancer	Immunotherapy						NIH NATIONAL CANCER INSTITUTE
PDS0104 (TRP2)	Melanoma	Immunotherapy						

PDS Biotech Funded Partner Co-Funded

Human Papillomavirus (HPV)-Related Cancers

Despite the successful introduction of HPV preventive vaccines, HPV-related cancers remain a significant component of the global cancer burden. HPV infection occurs in both men and women and is associated with head and neck (oropharyngeal), cervical, anal, vaginal, vulvar and penile cancers.

PDS0101 is PDS Biotech's lead Versamune®-based immunotherapy. PDS0101 combines Versamune® with a mixture of short proteins (peptides) derived from the cancer-causing HPV16 viral protein. HPV16 is the most pervasive and difficult to treat HPV amongst the 13 different high-risk, cancer-causing HPV types. In a preclinical study in the most widely utilized animal HPV-cancer tumor model, PDS0101 uniquely induced complete regression of the tumors after a single sub-cutaneous injection. These data prompted a phase 1 open-label, dose-escalation, proof of concept study of

PDS0101 in women with cervical intraepithelial neoplasia (CIN) infected with high-risk HPV types. The data demonstrated that PDS0101 was immunologically active at all three doses studied, confirmed induction of high levels of active HPV-specific CD8+ killer T-cells, and was associated with clinical regression of the cervical lesions that often occurred rapidly. These results suggest that PDS0101 activated the critical mechanisms in humans resulting in potent T-cells which target and effectively kill human HPV-positive cancer cells. All patients who experienced regression remained disease-free over the 2-year retrospective evaluation period, suggesting potential durability or memory of the immune response. The clinical data were presented at the 34th Annual Society for the Immunotherapy of Cancer Conference in November 2019 (Wood, et al., 2019).

Based on these encouraging preclinical and human data, PDS0101 is being studied in multiple phase 2 clinical studies in various HPV-related cancers.

PDS0102: T-cell receptor gamma Alternate Reading frame Protein (TARP)-Related Cancers

The TARP antigen is strongly associated with prostate and breast cancers. In the U.S. 450,000 patients are projected to be diagnosed with prostate or breast cancer this year. Approximately 90% of prostate cancers and 50% of breast cancers overexpress the TARP tumor antigen. In a human clinical study, the National Cancer Institute demonstrated that its proprietary TARP antigens were effectively recognized by the immune system in prostate cancer patients with PSA biochemical recurrence leading to a notable reduction in tumor growth rate. In preclinical studies, a dramatically enhanced TARP-specific killer T-cell response was observed when PDS-designed TARP antigens were combined with Versamune®. Preclinical development is ongoing.

PDS0103: Mucin-1 (MUC1)-Related Cancers

MUC1 is highly expressed in multiple solid tumor types and has been shown to be associated with drug resistance and poor disease prognosis. PDS Biotech is developing PDS0103, a Versamune®-based therapy in combination with novel, highly immunogenic, agonist epitopes of the MUC1 oncogenic C-terminal region to treat ovarian, breast, colorectal and lung cancers. In preclinical studies, similarly to PDS0102, a dramatically enhanced MUC1-specific killer T-cell response was observed when the novel antigens were combined with Versamune®. Preclinical development is ongoing.

PDS0104: Melanoma-Specific Antigens

The rates of melanoma have been rising rapidly over the past few decades and approximately 96,480 new melanomas will be diagnosed this year alone. More than 7,000 of these will prove fatal. PDS0104 combines Versamune® with various melanoma antigens including the Tyrosinase-related protein 2 (TRP2) which is highly expressed in melanoma. PDS0104 has been demonstrated in pre-clinical animal models of aggressive melanoma to have unique and significant anti-tumor activity as a monotherapy and has also demonstrated strong anti-tumor synergy in combination with checkpoint inhibitors. Preclinical development is ongoing.

Versamune® has demonstrated immunological compatibility with a wide array of tumor and pathogenic antigens. While PDS Biotech's current pipeline pairs Versamune® with four different tumor antigens, to address over 10 cancer types, more than 75 tumor antigens have been identified. The versatility of the platform suggests that Versamune® could work well with a wide range of identified tumor antigens and neoantigens. PDS Biotech is exploring the expansion of its Versamune®-based pipeline by pairing the technology with multiple tumor antigens to develop additional product candidates.

Versamune®: A Next Generation Cancer Immunotherapy with Exciting Promise

The field of cancer immunotherapy continues to make significant strides in the battle against cancer. Versamune® platform-derived products have several important potential immunotherapeutic advantages that may overcome many of the shortcomings of current immunotherapies and lead to new cancer treatments with strong efficacy and a highly favorable safety profile.

Versamune® appears to be unique in its ability to activate each of the critical steps required for effective immunotherapy. Most importantly Versamune®'s demonstrated ability to activate the critical immunological steps outlined below provide strong potential for superior efficacy in the treatment of several cancers:

- Induces an antigen-specific CD8+ killer and CD4+ helper T-cells response within the body
  - Promotes the uptake and processing of tumor antigens by dendritic cells
  - Promotes cross-presentation of these processed tumor antigens by the MHC class I and class II pathways to effectively stimulate both tumor-specific CD8+ killer and CD4+ helper T-cells, respectively
  - Triggers type I interferons within the lymph nodes associated with the 'foreign' and 'danger' signals of the immune system
- Activates, arms and expands large numbers of T-cells that recognize the tumor
- Alters the tumor microenvironment (TME) to "de-camouflage the tumor" to make the cancer more susceptible to attack by the primed T-cells
- Generates powerful immune memory, so if cancer cells return, the immune system is able to recognize and eliminate them
- Optimizes safety and tolerability by limiting systemic inflammation and toxicity

The above results in powerful anti-tumor CD8+ killer T-cell responses. Alteration of the tumor microenvironment allows for effective killing of the tumor by the active killer T-cells. Activation of Type I interferon signaling also promotes the induction of T-cell memory. Versamune®'s simple engineering, its ease of subcutaneous administration when admixed with proprietary antigens tailored to individual cancer types, its robust immunological activity and its favorable safety profile that avoids off-target systemic toxicities, make it an ideal platform to study in combination with other agents, where it may prove to be synergistic, and also as a monotherapy treatment.

In conclusion, the immunological mechanisms of action of the Versamune® platform result in a unique combination of potency and safety. Versamune®-based therapies offer tremendous promise for producing new treatments targeting a broad range of cancers. To this end, PDS Biotech looks forward to advancing the science of Versamune® to bring new and improved treatments to cancer patients.

References

- Gandhapudi, S., Ward, M., Bush, J., Bedu-Addo, F., Conn, G., & Woodward, J. (2019). Antigen priming with enantiospecific cationic lipid nanoparticles induces potent antitumor CTL responses through novel induction of a Type I IFN response. J Immunol, 202 (12): 3524-3536
- Wood, L., Edwards, L., Ferris, D., Nevadunsky, N., Isani, S., Conn, G., . . . Einstein, M. (2019, November 8). A Novel Enantio-Specific Cationic Lipid R-DOTAP + HPV16 E6 & E7 Antigens Induces Potent Antigen-Specific CD8+ T-Cell Responses In-Vivo in Subjects with CIN and High-Risk Human Papillomavirus Infection. Society for the Immunotherapy of Cancer (SITC) Annual Meeting, Oral Presentation (O17). National Harbor, MD.
- Smalley Rumfield, C.,Pellom, S.T., Morillon, Y.M. II, Schlom, J., Jochems, C. (2020) Immunomodulation to enhance the efficacy of an HPV therapeutic vaccine., Journal for ImmunoTherapy of Cancer 2020;8:e000612. doi:10.1136/jitc-2020-000612

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