Advancements in combinatorial chemistry and high-throughput screening have culminated in the production of volumes of chemical libraries for pharmaceutical and biotechnology companies looking to expand product pipelines. However, a major hurdle in the development of new chemical entities from these libraries lies in the intrinsic physiochemical properties of the compounds themselves. Poor aqueous solubility continues to relegate potentially useful drugs to the sidelines as formulation techniques for intravenous and oral delivery lag behind the ability to produce and screen the libraries.

Medical chemistry techniques can be utilised to alter the solubility of a drug by creating different salt forms or prodrugs. However, the pharmaceutical activity may be less potent, they may be slower acting due to the necessity of conversion to the active form, and these strategies may be very costly with long optimisation times.

Several strategies have been employed to formulate active pharmaceutical ingredients (APIs) in nanoparticle drug delivery vehicles to impart aqueous solubility and also have the added benefits of improved circulation time, minimised exposure of the API outside the target area, and have triggered release of the API payload at the target area. Moreover, their multi-functionality permits the incorporation of cell-targeting groups, diagnostic agents, and a multitude of drugs in a single delivery system.

Polymer micelles are particularly attractive due to their ability to deliver hydrophobic therapeutic agents; allowing for systemic delivery of compounds that are completely insoluble without a delivery vehicle. The foundation of Intezyne’s technology is based on the IVECT™ method for the production of stabilised polymer micelles.

"IN CONTRAST TO SIMPLE BLOCK COPOLYMER MICELLES, THE IVECT™ METHOD UTILISES A MULTI-BLOCK COPOLYMER DESIGN, WHERE EACH SEGMENT (OR BLOCK) OF THE CARRIER HAS BEEN TAILORED TO ADDRESS THE MOST CRITICAL ISSUES FACING TARGETED DRUG DELIVERY."

Here, Adam Carie, PhD, Senior Scientist, Preclinical Development, Jonathan Rios-Doria, PhD, Manager, Preclinical Studies, Habib Skaff, PhD, Chief Executive Officer, and Kevin Sill, PhD, Chief Science Officer, all of Intezyne, explain the IVECT™ method which, in contrast to simple block copolymer micelles, is a multi-block copolymer system, where each segment of the carrier is tailored to the specific application. In vitro and in vivo data from studies of IT-141, an IVECT™ formulation of the irinotecan metabolite, SN-38, for the treatment of cancer, are also presented.
Simple diblock copolymer micelles have been and are still being employed for drug delivery applications, but these systems often lack biocompatibility, post-administration stability, and effective strategies to target active cell targeting groups to their surface. Despite these drawbacks, block copolymers and polymer micelles still offer a number of chemically tunable features, such as high drug loading capacities and the ability to encapsulate a variety of therapeutic classes, which are not readily accessible using other technologies (for example, polymer-drug conjugates, dendrimers and liposomes).

In contrast to simple block copolymer micelles, the IVECT™ method utilises a multi-block copolymer design, where each segment (or block) of the carrier has been tailored to address the most critical issues facing targeted drug delivery. This modular and versatile drug delivery platform can be chemically manipulated to accommodate a wide range of drugs, improve post-administration stability, vary the micelle size, control the release of the therapeutic and target specific diseased cells.

Using this modular approach, Intezyne has created a cross-platform drug delivery system that has been carefully tuned to encapsulate and deliver small-molecule drugs, oligopeptides and proteins, DNA/RNA, and contrast agents for medical imaging, using only two separate polymers.

Each segment of the IVECT™ system has been constructed from biodegradable and/or biocompatible building blocks, increasing the likelihood of achieving regulatory approval. Figure 1 shows a schematic of the IVECT™ system components, with hydrophobic amino acid (AA) blocks (yellow) designed to form the encapsulation core where hydrophobic small molecules (red spheres) are stably housed until the micelle is degraded. Stabilising AA blocks (green) keep the micelle intact once the formulation is dissolved in the bloodstream. The hydrophilic poly(ethylene glycol) (PEG) block (blue) forms a protective corona around the micelle, giving the delivery system stealth-like properties to avoid protein opsonisation and the reticulo-endothelial system (RES). Additionally, the distal termini of the PEG blocks can be modified with peptide targeting groups that can actively target specific cells and potentially facilitate receptor-mediated endocytosis. Each block of the polymer has been tailored in order to fine-tune the size of the micelle for optimal delivery characteristics, as well as for optimal drug loading.

Initial development efforts for the IVECT™ method are focused on encapsulating chemo-therapeutics for the treatment of cancer. One such new formulation utilising the IVECT™ Method is IT-141. The active pharmaceutical agent of IT-141 is SN-38, a topoisomerase I inhibitor, and the active metabolite of Pfizer’s Camptosar (irinotecan).

While SN-38 has exhibited excellent in vitro efficacy, its poor solubility in water (10 \(\mu\)g/mL) has thus far limited its clinical use. Using a proprietary triblock copolymer, Intezyne has successfully encapsulated SN-38 in the IVECT™ DDP at loadings of up to 15 % (w/w) of the final formulation.

This formulation, IT-141, is freely soluble in water at concentrations up to 200 mg/mL. At this concentration of IT-141, SN-38 is effectively dissolved in water at 30 mg/mL, resulting in solubility that is more than a thousand times higher than free SN-38. Furthermore, SN-38 possesses a lactone ring that is unstable a physiological pH, rendering it inactive as an anti-cancer agent. Encapsulation of SN-38 within the micelle core shields SN-38 from the bloodstream thus inhibiting degradation of the active compound.

One of the unique aspects of polymer micelles is that they can effectively bypass renal clearance, and avoid the reticulo-endothelial system and subsequent uptake by the liver due to their inherent physical dimensions. In addition, the micelle’s particle size of approximately 50-150 nm allows for preferential accumulation in a solid tumour based upon the enhanced permeation and retention effect (EPR).\(^1\)

Since solid tumours grow much more rapidly than their healthy counterparts, the blood vessels supplying the tumour are often ill-defined and possess a large number of pores, typically in the range of 200 nm. When introduced into this porous section of blood vessels, particles of 20-200 nm can readily enter into the tumour environment. However, these same particles have a difficult time diffusing from the tumour environment back into the bloodstream through another pore, and are essentially trapped in the tumour environment. Due to the design of the IVECT™ platform, IT-141 also possesses this advantageous particle size. Measurements made by dynamic light scattering (DLS) show IT-141’s particle size to be roughly 130 nm with a standard deviation of ±10 nm. Thus, the EPR effect allows for tumour targeting based solely upon particle size.

Herein lies a significant advantage of IVECT™ over of traditional polymer micelles. If the micelle is rapidly diluted to below its critical micelle concentration (CMC, or the concentration above which micelle formation is favourable), as occurs after a therapeutic is injected into a patient, it will degrade into a mixture of polymer and drug, and will not be able to utilise the EPR effect. Notably, IT-141 has shown micelle stability of up to 24 hours at biologically relevant concentrations in plasma, as characterised by \(\text{in vitro}\) DLS experiments. This greatly enhanced micelle stability is a result of the IVECT™ stabilisation block and translates to a longer circulation time, allowing the delivery vehicle a greater chance of localising at the site of disease.

**IN VIVO EFFICACY OF IT-141**

Initial efficacy studies focused on the comparison between Camptosar and IT-141 in human colorectal cancer using a HT-29 mice xenograft study. The study was performed with
equimolar doses of both Camptosar and IVECT-encapsulated SN-38, then compared with a control group consisting of the IVECT™ polymer alone. As shown in Figure 2, IT-141 exhibits tumour inhibition of 107% (53% regression) opposed to 21% inhibition by Camptosar. Furthermore, only 25% of the tumours treated with Camptosar responded to treatment, while IT-141 elicited a response in 100% of the tumours. All mice remained healthy with stable body weight throughout the study.

Furthermore, a study was performed to explore the dose response of IT-141 on HT-29 tumour xenografts in nude mice and is represented in Figure 3. Doses of 5, 15, 30, and 45 mg of SN-38 were administered per kg of animal body mass (mg/kg). Animals were dosed by tail vein injection on day zero, four, and eight. While the 5 mg/kg dose exhibited no statistical deviation from the control, inhibition of 61% was observed for the 15 mg/kg group. Higher doses of 30 mg/kg exhibited tumour inhibition of 108%, with 111% inhibition for the 45 mg/kg dosage group. These results correspond to an average tumour regression of 61% and 88% respectively. In addition, four out of seven mice treated with 45 mg/kg of IT-141 had no discernible tumour on day twenty of the study. Again, all mice remained healthy with stable body weight throughout the study and 100% of the mice responded to the treatment.

**PHARMACOKINETIC AND BIODISTRIBUTION PROFILE**

Pharmacokinetic and biodistribution data was generated from both healthy CD-1 mice and mice with HT-29 xenografts. IT-141 was administered by a fast IV bolus into the tail vein and plasma and organs were collected by cardiac puncture at times of 5 and 15 minutes, 1, 4, 12, 24, 48, and 72 hours with three mice utilised for each time point. Figure 4 shows the SN-38 concentration in plasma collected from HT-29 tumour-bearing mice over 72 hours.

Analysis of the plasma concentration versus time curve resulted in the following pharmacokinetic parameters: an area under the curve of 28.0 (hours x μg SN-38 per mL plasma) overall half-life of 4.1 hours, and a terminal half-life of 15.6 hours. This data exhibits marked improvements over Camptosar, which has an area under the curve of 3.2 and a terminal half-life of 3.5 hours.

**SAFETY AND TOLERABILITY**

The maximum tolerated dose (MTD) was determined for a single tail vein injection of IT-141 in both HT-29 tumour-bearing mice and healthy CD-1 mice. The mice’s body weight and overall health were monitored for seven days after injection. Mice that were administered with higher doses of IT-141 exhibited gastro-intestinal toxicity (swollen and/or necrotic large intestine and reduced spleen size), a routinely observed adverse side effect of camptothecin derivatives. Based upon survival rates and overall mouse health after one week, the single dose MTD was determined to be 60 mg/kg and the multiple dose MTD (3xQ4D) was determined to be 45 mg/kg in HT-29 tumour-bearing animals.

To further understand the potential toxicity from IT-141, histopathology was performed on HT-29 tumour bearing mice following three administrations of IT-141. Once tumour xenografts reached approximately 100 mm³ the mice were randomised into groups of 6-8 mice per group and treated with saline, IT-141 at 60 mg/kg, or IVECT™ polymer control (empty micelles). The sample was administered by a fast IV bolus into the tail vein once every fourth day for a total of three treatments. Vital organs and tumour tissues were collected on...
day 18 of the study for histological processing by haematoxylin and eosin staining. Pathological analysis revealed the major toxicity of treatment to be neutropenia as determined by the presence of extramedullary hematopoiesis in the spleen. This was expected, as neutropenia is a known dose-limiting toxicity for SN-38. Slight liver toxicity was seen in some treatment groups, as evident by oval progenitor cell proliferation in the liver samples. No drug-related toxicities were observed in the heart, lungs, kidney or brain.

**CONCLUSION**

IT-141 represents an important advancement in the treatment of colorectal cancer by enlarging the therapeutic window, reducing toxicity, and greatly increasing efficacy when compared with current chemotherapeutic options.

Likewise, Intezyne has employed the IVECT™ method to encapsulate over 20 distinct chemotherapeutics, including taxanes, anthracyclines, topoisomerase II inhibitors, and kinase inhibitors, among others. Ultimately, the goal of Intezyne is to treat cancer better by enlarging the therapeutic window of cancer drugs by increasing the local drug concentration in the tumour environment and decreasing the toxicities associated by minimizing the exposure of the free drug to the rest of the body.

**REFERENCE:**


**Figure 4:** Pharmacokinetic Profile of SN-38 from IT-141 and Camptosar.