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Prodrug Nano-Squalene Bioconjugate Drug Products

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1. Introduction

In 2019, Professor Couvreur and I formed a development stage company, Squal Pharma, based on the unique amphiphilic prodrug platform technology developed by Prof. Couvreur called Squal Nano Pro that forms squalene-based nanoparticles via covalent linkages to a variety of compounds yielding new chemical entities.

2. Materials and Methods

Squalene, a biocompatible lipid, is conjugated to high potential drugs (e.g., pain, anticancer, spinal cord injury, burns and wound healing, anti-infectives, siRNA) yielding novel pharmaceuticals. Squalene-drug conjugates spontaneously self-assemble as highly coiled, compact nanoparticles in water without surfactant and polymer, for efficient manufacturability.

3. Results and Discussion

NSBs display extended blood circulation times, improved bioactivity, and reduced toxicity in multiple preclinical models. This technology platform has several applications with high drug loading that is supported by robust in-vitro and in-vivo data in animal models for a variety of indications.

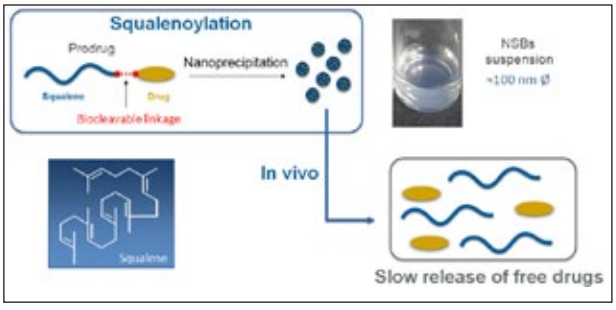

Figure 1 *Formation of nanosqualene bioconjugates (NSBs)*

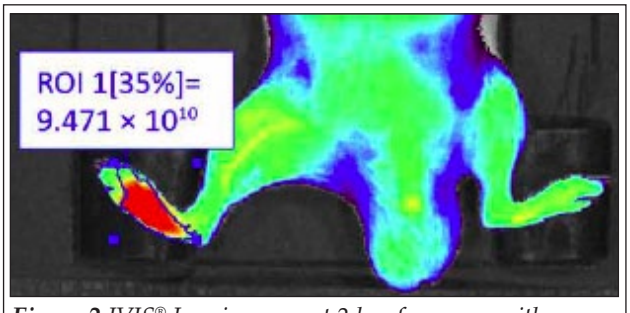

Figure 2 *IVIS® Lumina scan at 2 hr of a mouse with an inflamed right rear paw after intravenous administration of fluorescent LENK-SQ-Am NPs.*

A. Pain – Squal Lenk

The endogenous neuropeptide Leucine enkephalin (LENK) is conjugated to squalene. SQUAL – LENK formed with Squal NanoPro technology is an efficient approach to use the currently unusable LENK to act as an analgesic drug via peripheral opioid receptors (no addiction potential).

Self-assembly into NSB's; Slow release of peptide; C_{max} at 45 min; Degraded over 10 hrs; Analgesic effect only in inflamed tissues; Exclusively acts on peripheral opioid receptors; Longer duration than morphine

B. Oncology – Squal Dox/Squal Gem

Robust *in vitro* and animal studies show pharmacokinetics and tissue distribution of SQUAL – DOXORUBICIN with increased plasma and tumor concentration, decreased cardiac concentration/ cardiac toxicity vs. free doxorubicin. 57% drug payload; 3-fold faster uptake. Free doxorubicin is not efficacious in human pancreatic cancer, an orphan indication. SQ-Dox NAs -- high efficacy in human pancreatic and murine lung xenograft models as shown below.

Following IV injections of Nano SQ-DOX: -95% tumor growth inhibition (pancreatic) vs 29% (free drug) -90% tumor growth inhibition (lung) vs 3% (free drug)-Less toxicity; No weight loss (lung) vs

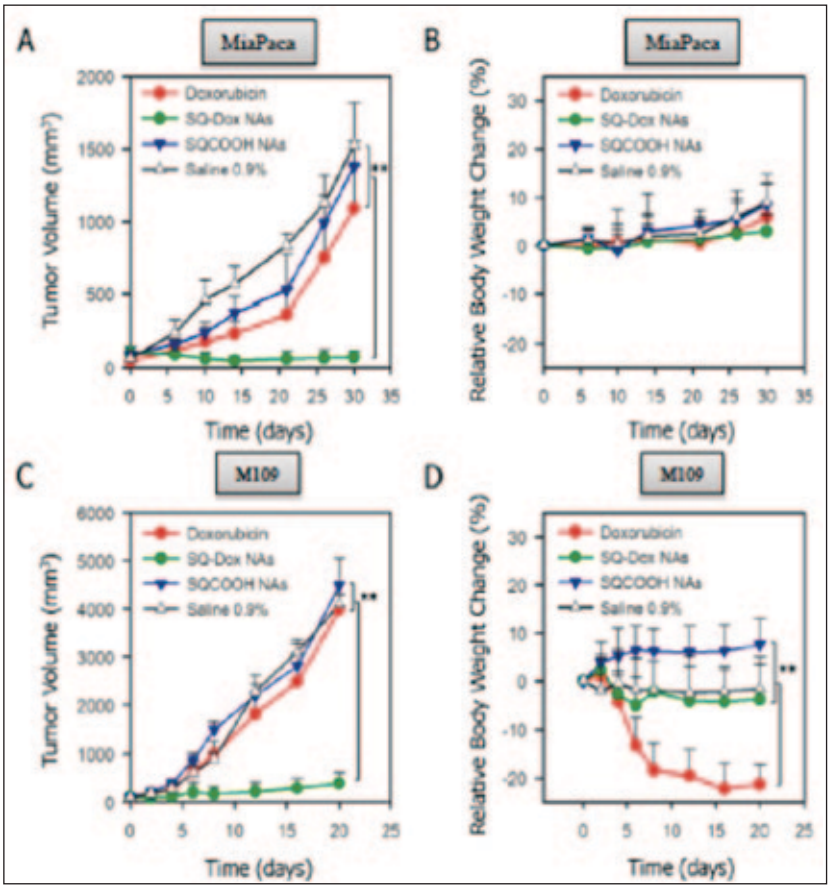

Figure 3 *Tumor volume and body weight changes in transfected with a human pancreatic cell line (MiaPaca) and a mouse lung tumor (M109).*

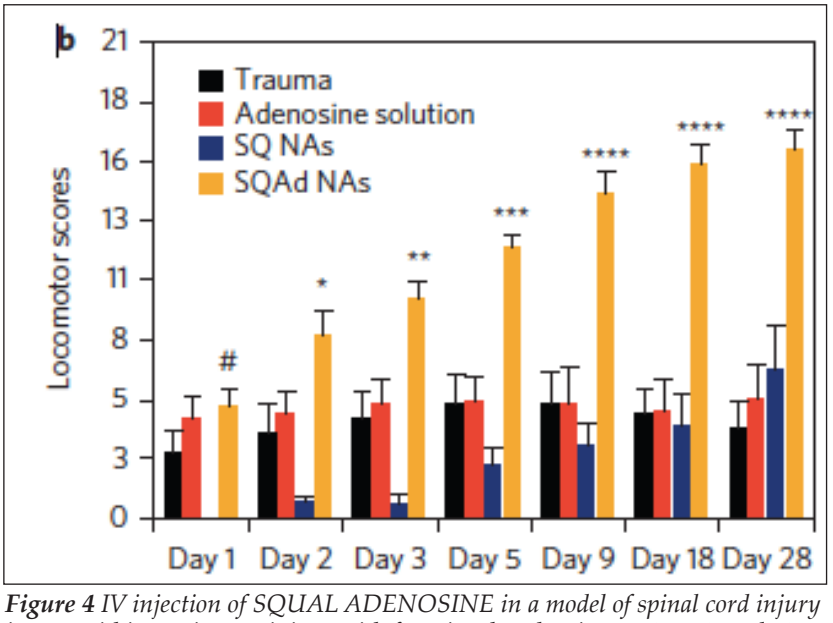

Figure 4 *IV injection of SQUAL ADENOSINE in a model of spinal cord injury in rats within 5 min post injury with functional evaluations at 24, 48, and 72 h post-injury to up to 28 days (n=10) comparing Trauma control, adenosine solution, SQ Nano Assemblies with no drug and SQAd NAs (SQ Adenosine NAs).*

20% loss (free drug) -The presence of squalene leads to selective extravasation in tumors and indirect active targeting of cancer cells

Squalene Itself Enhances Drug Delivery into Cancer Cells

Like all squalenoylated bioconjugates, SQ-DOX & SQ-GEMCITABINE, insert into LDL, a significant difference from other nanoparticle delivery systems. LDL endocytic uptake can be highly increased in rapidly growing malignant cancer cells. Selective delivery of anticancer drugs to rapidly growing cancer cells can be achieved by taking advantage of their high receptor-mediated uptake of low-density lipoproteins (LDLs). Both *in vitro* and in vivo data demonstrate that such an interaction leads to the preferential accumulation of SQ-Gem and SQ-Dox in cancer cells (MDA-MB-231) with high LDL receptor expression.

C. Spinal Cord Injury -Squal Ad

All animals treated with Adenosine-squalene particles (SQAd NAs) showed a complete recovery of their hindlimbs after 72 hours.

D. Wound-Burns-Cosmetic -Squal Vit C

Vitamin C is well known for collagen biosynthesis, however its instability limits its application. Currently it is difficult to deliver to the dermis in optimal dosages. Vitamin C is hydrophilic, there is a marked interest to find methods of efficient transepidermal delivery of the stable active compound.

SQUAL – Vitamin C significantly upregulates Collagen III leading to a significant increase of dermal thickness in skin explants. Topical SQUAL-Vit C offers the potential for the treatment of burns, wounds and photo-aging.

4. Conclusions

Squalene, a natural and well-tolerated biocompatible triterpene that forms novel prodrugs: Squal NanoPro. It is easily bioconjugated to many drugs (e.g., anticancer, antibiotics, pain, spinal cord injury, wound healing etc.) yielding new chemical entities. Squalene provides unique delivery potential. NSBs spontaneously self-assemble in water as nanoparticles with high encapsulation efficiency (>50%). They exhibit excellent, reliable manufacturability (i.e., easy scale-up). There is no dose dumping. The show improved activity, reduced toxicity and avoid drug resistance.

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