Overcoming Challenges in Manufacturing Scale-Up of Plasmonic Gold Nanorods: Bringing Photothermal Therapy to the Clinic.

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ABSTRACT

Siva Therapeutics is developing a simple, safe, and effective adjunct cancer treatment termed Targeted Hyperthermia™, which generates therapeutic heat emanating from within solid tumors using systemically injected precision gold nanorods and an infrared light engine – technology termed photothermal therapy. Heat has several beneficial effects for solid tumors, including selective induction of apoptosis in cancer cells, stimulation of the immune system, inactivation of cancer stem cells, and increased perfusion resulting in improved drug efficacy.

Targeted Hyperthermia provides precision heating of tumors with minimal collateral damage, using SivaRods™ polymer-coated gold nanorods and a SivaLum™ infrared light engine, and it promises to be a valuable adjunct to current drug therapies. While awareness of the therapeutic value of hyperthermia has been in the cancer community for many decades, implementing practical, safe, and cost effective hyperthermic therapies has been challenging. Nanotechnology has provided key tools for targeting heat to tumors, and photohermal therapy, in particular, has demonstrated efficacy, both in animal models, and now in the clinic [1].

A critical hurdle for photothermal therapy has been scaling up manufacture of nanoparticles to pilot batch size, while maintaining plasmonic properties and uniformity of the material. Siva has accomplished pilot scale manufacturing, and is currently undertaking full characterization of this material through a grant from the Nanotechnology Characterization Laboratory (https://ncl.cancer.gov/), which is supported by the National Cancer Institute, the FDA, and NIST. Additionally, Siva is developing a second generation LED-based infrared light engine with the ability to illuminate regions of ~10 cm in diameter with high intensity infrared light to excite nanorods that have concentrated in tumors. Together, these advances have made nanotechnology-enabled photothermal therapy more practical, safe, and cost-effective than was previously possible.

Keywords: cancer therapy, targeted hyperthermia, nanotechnology, solid tumors, gold nanorods, therapeutic heat, oncology, infrared light, photothermal therapy

1 GOLD NANORODS FOR PHOTOTHERMAL CANCER THERAPY

Gold nanorods are an excellent material for photothermal thereapy for a number of reasons. They posess longitudinal surface plasmon resonance (LSPR) which produces far more efficient photothermal conversion than the surface plasmon resonance (SPR) of spherical nanoparticles. Additionally, they can be tuned to a wide range of extinction wavelengths by varying their length-to-diameter ratio. This tunability enables the selection of optimal excitation wavelengths for various applications. For photothermal therapy, we identified 850nm as an optimal wavelength that incorporates good tissue penetration (up to 3 cm with a SivaLum infrared light engine), safety, low cost, and compatibility with nanorods having an aspect ratio of ~4.3 (see Figures 1 and 2.)

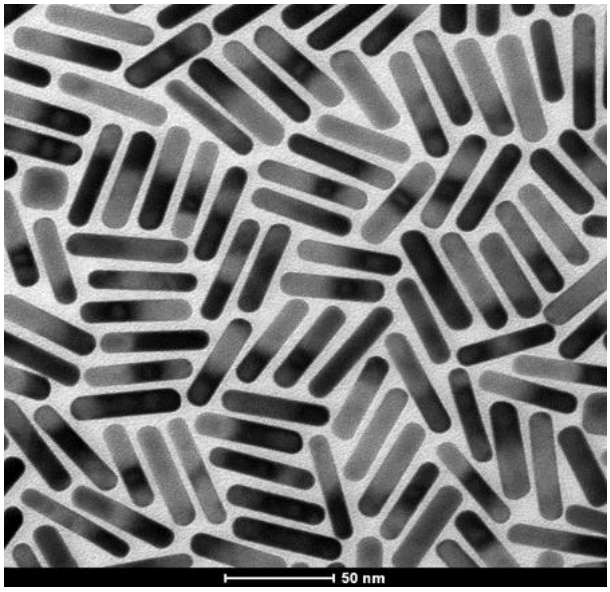


Figure 1: Electron micrograph of production batch of 12nm x 45nm SivaRod gold nanorods.

One of the limitations of nanorods as opposed to more tradtitional nanomaterials such as nanoshells, however, is that nanorods are more difficult to make at laboratory scale, and this challenge is compounded when the prospect of scaled up manufacturing is is addressed.

Practical, economical, simple scale-up of nanorod manufacturing is essential for bringing their use to market, and this hurdle has been a significant barrier to adoption. Together with our manufacturing partners at NanoHybrids, Inc., Austin, TX, we have successfully accomplished scaling of SivaRod production to 10 liter pilot batch size.

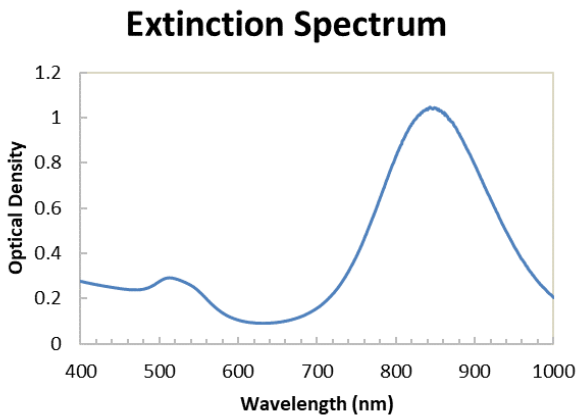


Figure 2: UV-VIS extinction spectrum of an OD1 sample of a production batch of 12nm x 45nm SivaRod gold nanorods.

Several important challenges had to be met in order to scale production of SivaRods, both in terms of batch size, and also in terms of manufacturing controls to ensure product uniformity.

For photothermal therapy, the nanorods are excited by an LED-based infrared light engine [2]. The light engine (Figure 3) has several advantages over more traditonal laser light sources.

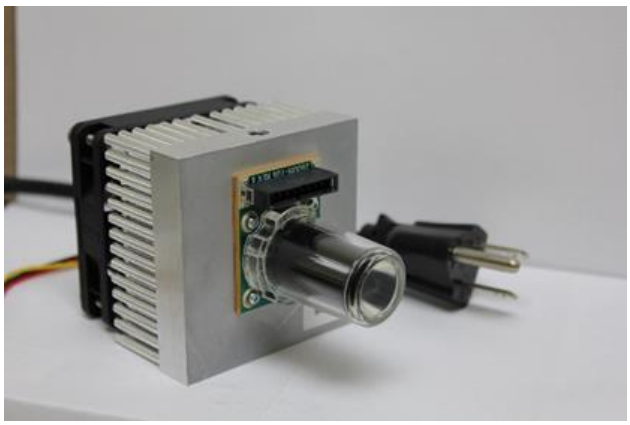


Figure 3: SivaLum LED infrared light engine.

First, the light engine is very simple and inexpensive, and it is easy to use. The LEDs are very powerful and efficient, delivering 1 watt/cm^2 over an area up to 10 cm in diameter. Second, the light engine is very safe, and requires minimal operator training. Third, the light engine design ia amenable with 'smart' feedback electronics that are being incorporated into the clinically amenable version.

2 PROOF OF CONCEPT DATA

Before undertaking manufacturing scale-up, it was critical to establish proof of concept of Targeted Hyperthermia photothermal cancer therapy in animal models.

We first measured tumor volume (Figure 4) and survival (Figure 5) in nude mice implanted with aggressive melanoma tumors. These studies were performed with Targeted Hyperthermia monotherapy.

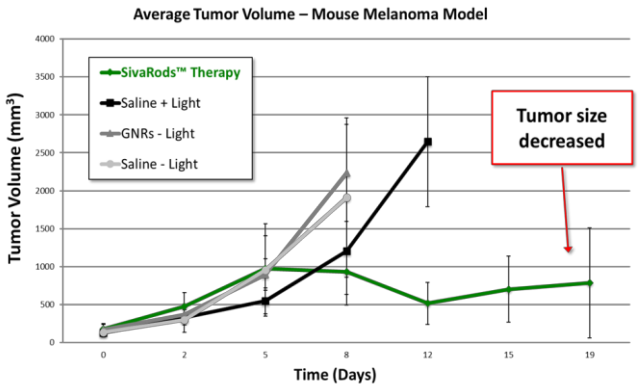


Figure 4: Tumor volume in nude mice implanted with B16F10 melanoma tumors, the green line represents animals treated with Targeted Hyperthermia.

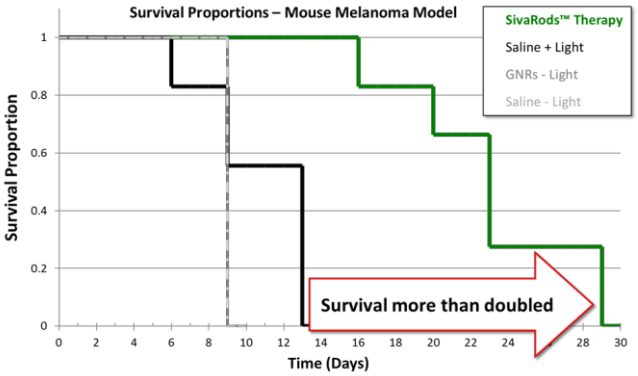


Figure 5: Survival of nude mice implanted with B16F10 melanoma tumors, the green line represents animals treated with Targeted Hyperthermia.

As Figures 4 and 5 show, tumor volume decreased dramatically and survival more than doubled with Targeted Hyperthermia monotherapy.

We next looked at Targeted Hyperthermia combined with the melanoma drug Zelboraf. Figure 6 shows the results of one such study; reduction of tumor mass and dramatically increased survival are evident – untreated animals were all sacrificed by Day 25. Fifty percent of the animals in the combination treatment group were termed 'durable cures' by the contract lab which performed the study.

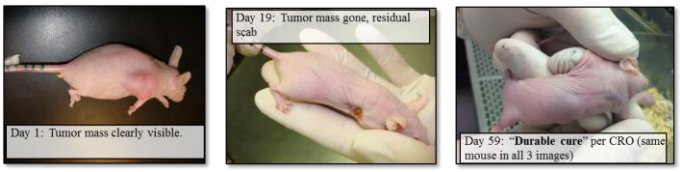


Figure 6: Nude mice implanted with A2058 tumors. Day 1, immediately after treatment, Day 19, and Day 59 images are shown; same mouse in all 3 images.

3 NANOROD MANUFACTURING – PRACTICAL ASPECTS

Synthesizing a laboratory batch of nanorods of a few tens of microliters and producing a 10 liter pilot nbatch of material are very different undertakings. What works at microscale often does not work, or is not practical, at larger scale. We outline here some of the critical issues.

3.1 Simplicity and Cost of Manufacturing

Manufacturing simplicity and cost are intimately involved. While laborious, complex operations may be used in the laboratory, they are typically neither practical nor cost-effective for large-scale manufacturing.

Current SivaRod manufacturing procedures have accomplished these objectives in a number of ways, including:

a) All reactions are at room temperature and pressure;

b) All reactions are in conventional stirred vessels, no exotic equipment or systems are necessary;

c) Centrifugation steps have been eliminated and replaced with technologies such as tangential flow filtration;

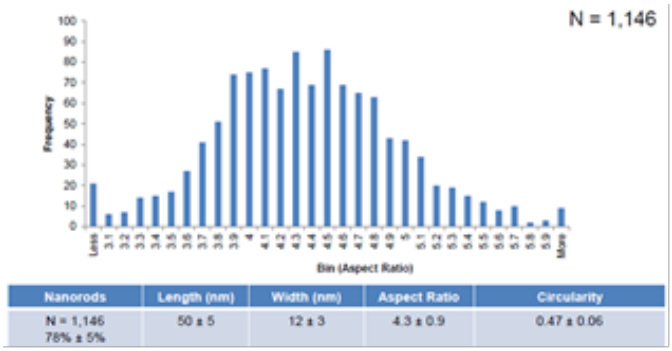
d) All chemical contaminants (e.g. CTAB) are removed (to below the level of detection) as part of the manufacturing process; and

e) Larger batch size brings economy of scale, since QA/QC costs are fixed independent of batch size.

It is worth noting that the cost of metallic gold is almost negligible in this process, since a projected human dose will contain about 1 gram of gold, which costs roughly $43.00 at current gold prices.

3.2 Uniform Nanorod Size and Aspect Ratio

A major challenge with increased scale of manufacturing is to maintain uniformity of nanorod size and aspect ratio. Small, research-scale batches of matierial are notorious variable in terms of rod geometry, yield, contaminants and many other characteristics.



Nanorods	Length (nm)	Width (nm)	Aspect Ratio	Circularity
N = 1,146 78% ± 5%	50 ± 5	12 ± 3	4.3 ± 0.9	0.47 ± 0.06

Figure 7: Size histogram of a production batch sample of SivaRods gold nanorods. Data from the NCL program.

As manufacturing scale has increased, we have concomitantly introduced a range of manufacturing processeses, systems, and controls which enable the production of large batches of material with >78% of the nanorods at the target size and aspect ratio (Fig 7).

This result has been achieved with continuous, incremental improvements in manufacturing process and control. A key improvement has been the incorporation of multiple steps of tangential flow filtration (TFF) at several points in the manufacturing process. The development of economical TFF equipment has been an important advance for nanomanufacturing in general and for this process.

3.3 Sterility, Endotoxins, and Contaminants

In addition to producing material of uniform particle size and shape, sterility and contaminants have long been challenges for production of nanomaterials intended for therapeutic use.

Sterility can be challenging, because many naomaterials are not stable at typical heat sterilization temperatures and pressures. Other methods, such as ethylene oxide and ionizing radiation may have application, but may also introduce unwanted effects on the nanomaterials. Establishing and maintaining sterility from the start of the manufacturing process, by using validated sterile and endotoxin-free materials, is therefore very important. Maintaining a sterile and endotoxin-free environment is also essential.

Similarly, chemical contaminants, notably cetyltrimethylammonium bromide (CTAB) for nanorod production must be controlled and mitigated. Dialysis, filtration, and chemical substitution steps are typically used to remove such contaminants. No protocol will work for all applications, and – at least in our case – extensive testing was required to identify optimum methods for addressing these issues.

The resulting production material is sterile, has endotoxin levels below allowable limits, and contains no detectable CTAB or other chemical contaminants; this is am important aspect of our scaled manufacturing.

3.4 Cytotoxicity and Safety

Several different cytotoxicity tests were performed, both with- and without infrared light excitation. Figure 8 shows the negative results of a typical LDH cytotoxicity assay; MTT assays yielded simlar results. A variety of assays including hemolysis, complement activation, platelet aggregation, and plasma coagulation was also performed, all with negative results.

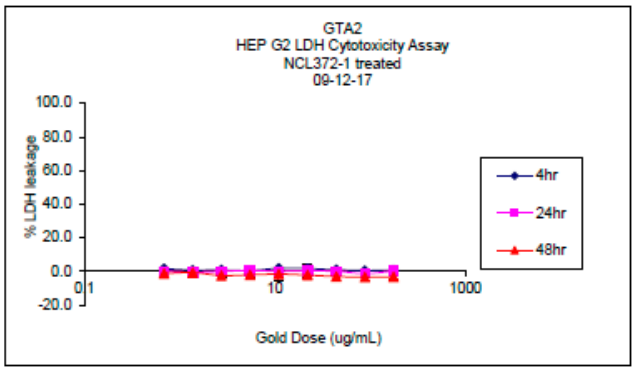


Figure 8: Results of a LDH cytotoxicity assay in HEPG2 cells.

These results establish a strong in vitro safety profile, and in vivo safety studies are currently underway. Completed studies in which treated animals were carried to 100 days in excellent health suggest that the in vivo safety studies will have positive results.

4 TRANSLATION TO THE CLINIC

The promise of nanotherapies for cancer and other diseases has long been on the horizon, but bringing many of these into the clinic in a safe, practical, economical way has been challenging [3]. A key barrier to wider adoption of nanotherapies has been the challenge of scaling their manufacturing to commercial levels, and establishing sterile, endotoxin-free, contaminant-free, safe material at the same time.

Because of this, we have focused on practical scaling of manufacturing over the pase several years. Strong proof of concept was in place, but without practical, scaled-up manufacturing, clinical translation could not take place.

It is important to note that the manufacturing improvements which resulted in our current methods were serially incremental in nature – there was no single 'magic step' that was discovered. Rather, an extensive series on small, continuous improvements in virtually every area of manufacturing was necessary

As greater numbers of naotechnology-based therapies near the clinic, practical manufacturing of these materials will become increasingly important. Resources such as the Nanotechnology Characterization Laboratory are at the forefront of defining the criteria necessary for success in this field.

Figure 9 shows a production sample of a projected human dose of SivaRods gold nanorods. Even a few years ago, a batch of material this size and of this quality would have been impossible, but today we are reducing it to practice.

Figure 9: A projected human dose of SivaRods – 30ml of and OD100 dispersion for injection.

ACKNOWLEDGEMENTS

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