phosphorex

microspheres, nanoparticles & drug delivery

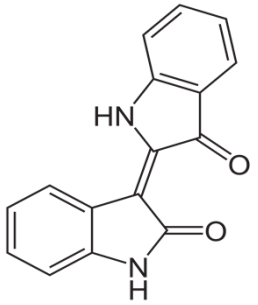
A Novel Indirubin Nanosuspension for Potential Treatment of Glioblastoma

Bin Wu, Theresa Logan, Paul Boucher, Shuren Shen and Nicholas Boylan

Phosphorex, Inc.

Background

- Indirubin was originally isolated from the leaves of the Chinese herb, Isatis tinctoria L.[1]
- In the clinical setting, it has been shown to be effective in the treatment of chronic myelocytic leukemia (CML) and was approved for CML indication in China.[2]
- Indirubin has various pharmacological effects, including antitumor and anti-inflammatory activities.[3]
- Indirubin is a potent inhibitor of cyclin-dependent kinases (CDKs) showing a strong affinity for CDKs (IC50s in the 50-100 nM range) in kinase selectivity study, thereby exerting potent cytotoxicity on some tumor cells, including glioblastoma (GBM).[4-5]
- Indirubin was also reported to induce pro-apoptotic genes, interfere with mitochondrial function, and STAT signaling leading to cell death.[6-7]
- Indirubin is a potent inhibitor (IC50s in the 5-50 nM range) of glycogen synthase kinase-3β (GSK-3β).[8]
- Indirubin also inhibits angiogenesis in endothelial cells.[6]



Indirubin

1. Hoessel, R., *et al. Nat. Cell Biol.* **1,** 60-67 (1999)
2. Zhijian Xiao, et al, *Leuk Lymphoma*, 2002, Sep; 43(9): 1763-8
3. Hideo Suzuki, et al, *World J Gastroenterol*. 2013, **19** (17): 2718–2722 (2013).
4. Marko, D., et al, *Br. J. Cancer*, 2001 Jan., 84(2): 283-9.
5. Williams, Shanté P., et al, *Cancer Research, 2011,* **71** (16): 5374–5380.
6. Zhang, Xiaoli, et al, *Intl J. Cancer, 2011,* **129** (10): 2502–2511.
7. Sangkil Nam, et al, Proc. Natl. Acad. Sci. USA, 2005 Apr 26; 102(17): 5998–6003.
8. S. Leclerc, et al, *J. Biol. Chem.* 2001 Jan 5; 276(10: 251-60.

Indirubin: Potential Therapy for GBM

Research[1] showed:

- When human glioblastoma cells were transplanted into one brain hemisphere of mice, indirubin-treated animals survived significantly longer than control and showed no migration of tumor cells to the opposite hemisphere.
- Indirubin reduced the migration of tumor cells by 40% in treated animals versus control.
- Treated tumors showed a lower density of blood vessels, and new blood-vessel growth was reduced in intracranial tumors.
- Indirubin reduced endothelial-cell migration by approximately 50%. (Endothelial cell migration is required for tumor angiogenesis.)

1. Williams, Shanté P., et al, *Cancer Research, 2011,* **71** (16): 5374–5380.

Indirubin: Poor Solubility Hampers Pharmaceutical Development

- Indirubin has extremely low solubility (~ µg/ml) and permeability.
- Low solubility limits its bioavailability, efficacy and delivery.
- In research where indirubin needs to be dosed in animals, an organic solvent such as DMSO is typically used, which may cause cytotoxicity and other adverse effects.
- Due to its insolubility, indirubin cannot be formulated for administrative routes other than oral formulations.
- Indirubin derivatives such as 6-bromoindirubin-3'-oxime (BIO) have been developed to address the solubility issue, however, the solubility increase has been marginal.
- GBM is a difficult-to-treat cancer, and it is desired that indirubin be delivered parentally to ensure high efficacy. Therefore, there is a need to develop an injectable formulation of indirubin for the treatment of GBM.

Nanosizing of Poorly-Soluble Compounds Increases Solubility

- Reducing particle size of insoluble APIs to increase their solubility is a commonly accepted formulation strategy.[1]

- Processing insoluble drugs into nanocrystal particles increases surface area and thereby leads to faster dissolution.[2]

- In addition to enhancing solubility/bioavailability, nanosuspension may also have other benefits:[2-4]
  - Rapid onset of action
  - Reduced food effect (fed/fast ratio) and more consistent dosing
  - Reduced toxicity
  - Opportunity for higher dosing
  - Longer circulating in case of IV dosing
  - Alternative dosage forms and administration routes

- Abraxane, nanoparticle albumin-bound paclitaxel, provides a good example of how nanoparticle formulation can help improve therapeutic effect and reduce toxicity.[5]

1. M. R. Gigliobianco, et al, *Pharmaceutics,* 2018, Sep; 10(3): 134
2. Colombo M., et al, Int. J. Pharm. 2017;521:156–166.
3. Wang T., et al, *In. J. Pharm.* 2018;546:10–19.
4. Mitri K., et al, *Int. J. Pharm.* 2011;420:141–146.
5. Miele, E., et al, *Int. J. Nanomedicine,* 2009, 4, 99-105

Experimental: Preparation and Characterization of Indirubin Nanosuspension

- Nanosuspension of indirubin was prepared using a bead mill.[1,2] Briefly, indirubin (obtained from Aldlabs, Woburn, MA) was added to tubes containing a Poloxamer-188 (Sigma-Aldrich, St. Louis, MO) solution and zirconium oxide beads (diameter, 0.12 mm; Next Advance, Troy, NY). The mixture was processed using a Bullet Blender (Next Advance). The resulting nanocrystalline indirubin was collected in sterile water and purified by filtration to form an indirubin nanosuspension.

- Particle size and size distribution of the nanosuspension were determined on a Malvern Zetasizer.

- The contents of indirubin in the nanosuspension was determined using an HPLC system (Shimadzu Corp, Kyoto, Japan) and a Zorbax 300SB-C18 column, 5 µm, 250 × 4.6 mm (Agilent, Santa Clara, CA), with 45% acetonitrile in water at 1 mL/min and ultraviolet detection at 292 nm.

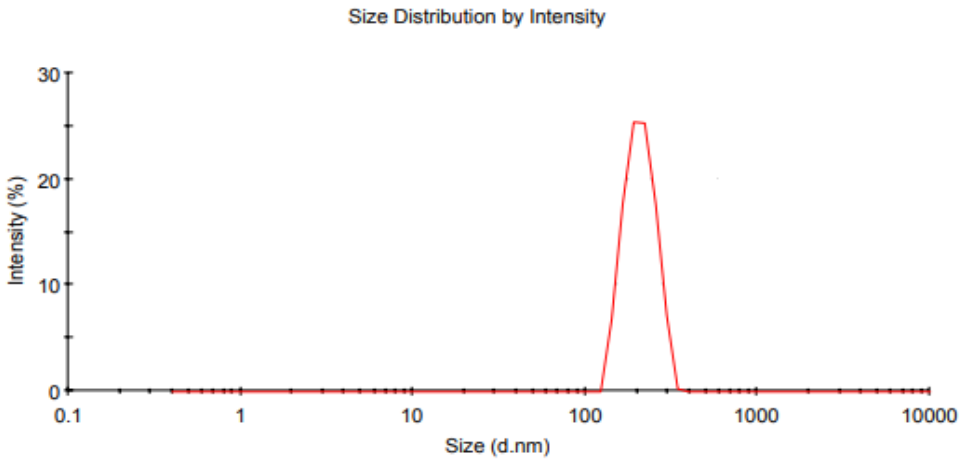
- Solubility was measured by stirring indirubin or its nanosuspension in a PBS solution containing 0.2% Tween-20 at 37°C for 1 hour, followed by HPLC measurement as described above.

1. B. Wu, US Patent 10,675,359
2. B. Wu, US Patent 10,039,829

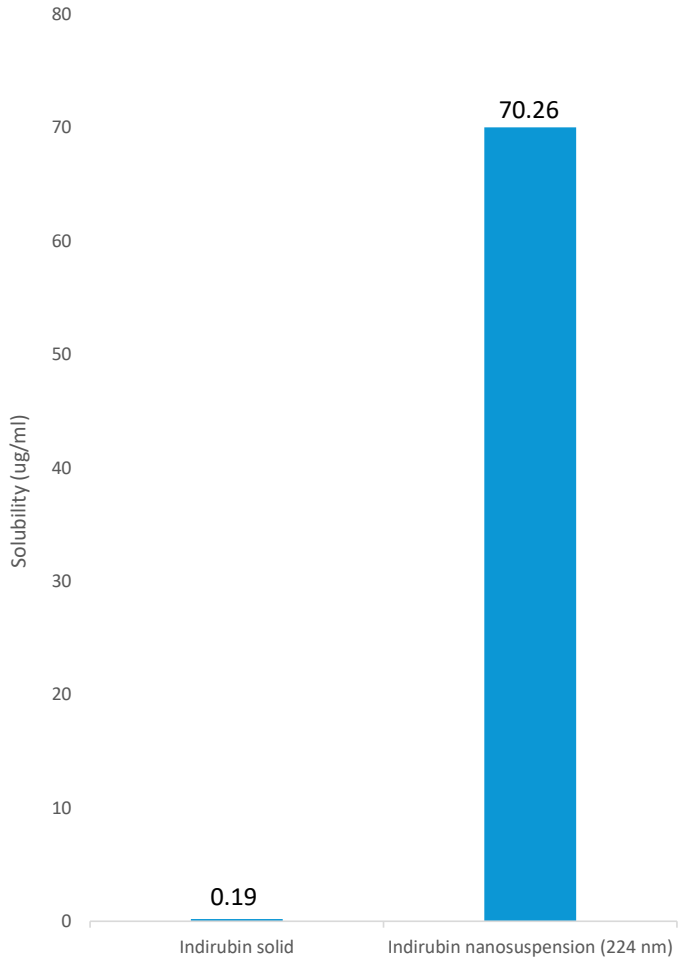
Experimental: In Vivo Glioblastoma Study

- 20 mice were injected with human U251 glioma cells in the flank. Once established tumors were allowed to grow to a size of 100-150 mm^3 before treatment began.
- The control group of mice (n=10) were injected with an aqueous solution of Poloxamer-188.
- The treatment group of mice (n=10) were treated with 200 mg/kg of the indirubin nanosuspension via intratumoral injection.
- Animals in treatment groups were injected every other day for a total of 12 injections.
- Bodyweight and tumor volume were measured every other day.
- Once treatment was complete, animals were monitored for tumor volume and bodyweight until 51 days from the start of the study had passed in order to assess the long-term effects of treatment.

Characterization of Indirubin Nanosuspension



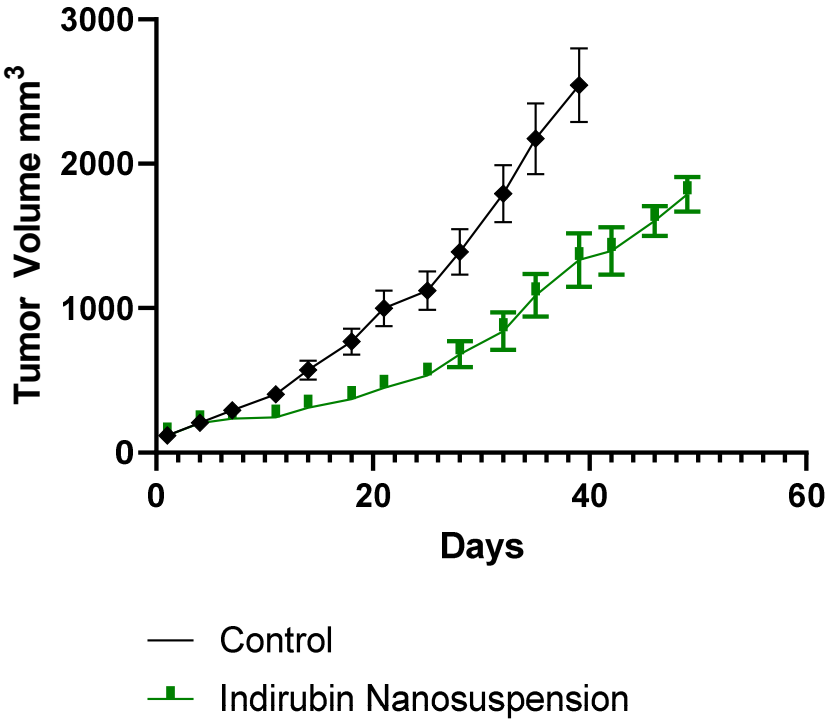
Indirubin Nanosuspension (Sample ID)	Mean Diameter (nm)	Standard Deviation (nm)
1	209.2	41.6
2	209.3	42.4
3	224.1	88.9

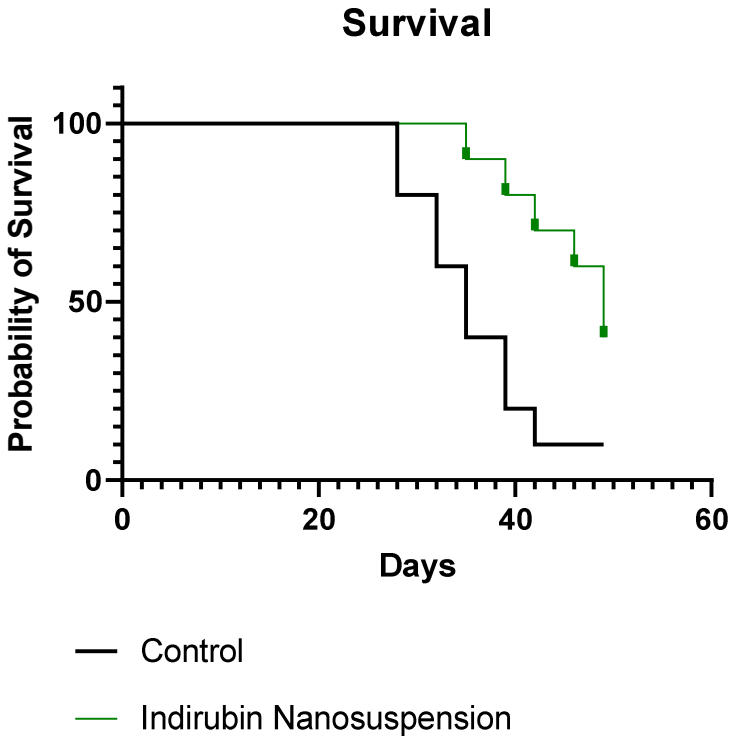


Solubility is increased from 0.2 µg/ml to 70 µg/ml, a 365-fold increase

phosphorex
microspheres, nanoparticles & drug delivery

Indirubin Nanosuspension Suppressed Tumor Growth and Increased Survival in Mouse GBM Model





Summary

- Indirubin, a potent CDK and GSK-3 inhibitor with low solubility, was successfully processed into a nanosuspension form.
- Indirubin nanosuspension has a mean particle size of 200-225 nm.
- The indirubin nanosuspension was found to be 365 times more soluble than indirubin itself.
- Indirubin nanosuspension was injected intratumorally to the mice inoculated with human U251 glioma cells and showed significant suppression on tumor growth:
  - At the end of treatment, the mean tumor size of the treatment group was approximately 50% of the mean tumor size for the control group.
  - After the end of treatment, tumor growth continued to slow down, with the mean time to reach the tumor size endpoint of the study (2000 mm^3) being approximately 40% longer for the treatment group than the control (48 vs 34 days).
  - Throughout the study no individual animal in the treatment group had a bodyweight reduction of >95%, indicating that the treatment produced little systemic toxicity.

Conclusion and Forward-Looking

- It was demonstrated that the nanosuspension could be formulated into an injectable dosage form without the need for solvent like DMSO, making it possible to formulate indirubin into a parental therapeutic.
- When injected into an animal glioblastoma model, the nanosuspension inhibited tumor growth.
- The nanosuspension may be combined with chemo-, radio-, or immuno-check point therapy to further enhance the efficacy on GBM.
- The indirubin nanosuspension may be useful for treating other difficult-to-treat diseases that are associated with CDK or GSK-3, including breast cancer, pancreatic cancer, psoriasis, and Alzheimer's disease.