THE MAKING OF LAMBIN

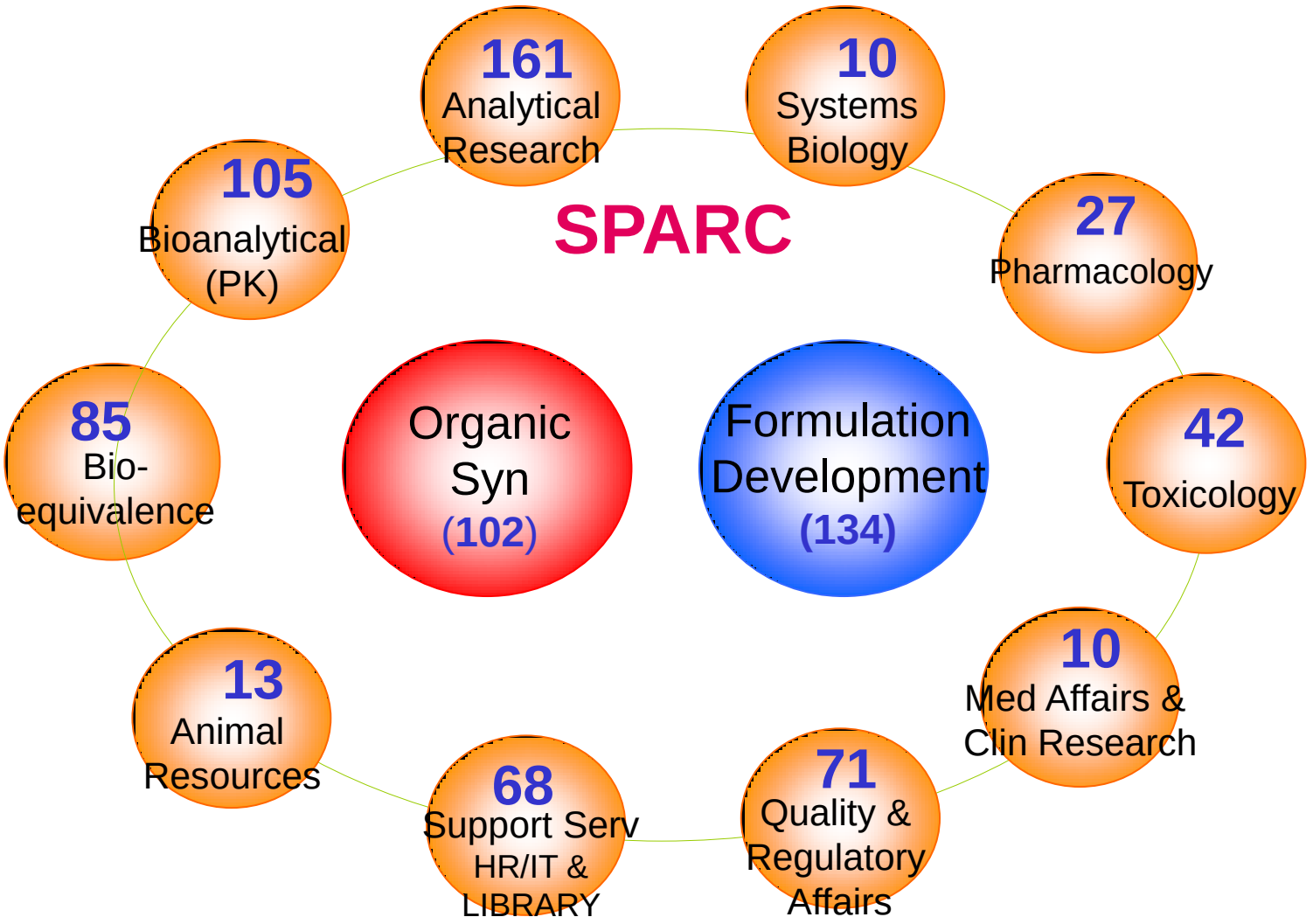
(Liposomal Amphotericin B for Injection)

N. Subramanian, M. Pharm., Ph. D.,
Formulation Development Department,
Sun Pharma Advanced Research Company Ltd (SPARC),
Tandalja, Vadodara

Sun Pharma Advanced Research Centre

- Inaugurated on December 14, 2004 by Dr APJ Abdul Kalam, the then Hon'ble President of India
- Current Strength of Scientists is over 800
- 3,50,000 Sq ft of Research Floor Space in 16-Acre Campus
- SPARC Mumbai, 100,000 sq ft & 147 Scientists

Sun Pharma Advanced Research Centre



Our Vision

Leadership through focused research

- **Development of high quality pharmaceutical products which matches the international standards by focused research using in-depth knowledge, skills and world class facilities for catering to Global markets including US and EU.**

QUALITY COMES FIRST

To achieve our vision….

- Set the standards very high
- Aim and achieve excellence in work
- Manufacture the products in world class facilities which are approved by USFDA, MHRA, etc.
- Keep stringent quality parameters to evaluate the products manufactured.

Accomplishments

- Patents Filed worldwide– 251; Granted- 83
- API Processes Developed – >180
- USDMF's/ CEP COS – Filed-157; Approved -90
- Products Developed so far – > 500
- US: filed-211; Approved – 120; (tentative approvals-11)

  EU: filed – 24 ; Approved – 12

  (Including Onco products like Docefrez, Oxaliplatin, Carboplatin, Irinotecan and Gemcitabine).
- Developing products for supplying more than 50 countries

Novel Drug Delivery Systems –Technology Platforms

Injectables

- Liposomal injections
- Nano particulate formulations
- Biodegradable Depot Injections.

Topical

- Dry Powder Inhalers (DPI)
- SMM Technology for Ophthalmic Formulations
- GFR Technology for Once a Day Ophthalmic formulations
- Microsphere topical delivery
- Transdermal delivery

Oral

- Gastro Retentive Innovative Device (GRID)
- Wrap Matrix System

Manufacturing facility

- Lyophilizers
- High Speed Homogenizers
- Spray Dryers
- High pressure Extruders
- High Pressure Homoginizers
- Rotary evaporators
- Air Jet Mills
- Transdermal patch coating machine
- Planetary mixer
- Ampoule/Vial filling machine
- Autoclave / DHS

Analytical facility

- High pressure liquid chromatography
- Liquid chromatography – Mass Spectroscopy
- Gas Chromatography
- X-ray diffractometer
- Nuclear magnetic resonance spectroscopy
- Cryo transmission electron microscope
- Scanning election microscope
- Particle size & Zeta potential analyser
- Freeze drying microscope
- Spray tech
- Universal Testing machine
- Viscometer

Technology based products marketed

- Liposomal Doxorubicin Hcl injection (LIPODOX)
- Liposomal Amphotericin B for injection (LAMBIN)
- Leuprolide acetate microsphere for injection (LEUPRIDE DEPOT)
- Octreotide acetate microsphere for injection (OCTREOTIDE DEPOT)
- Docetaxel for injection (DOCEFREZ)
- Tretenoin microsphere gel (SUPATRET)
- Cyclosporin microemulsion eye drops (CYCLOMUNE)

Technology based products

- Liposomal Doxorubicin Hcl injection (LIPODOX)
- Liposomal Amphotericin B for injection (LAMBIN)
- Leuprolide acetate microsphere for injection (LEUPRIDE DEPOT)
- Octreotide acetate microsphere for injection (OCTREOTIDE DEPOT)
- Docetaxel for injection (DOCEFREZ)
- Tretenoin microsphere gel (SUPATRET)
- Cyclosporin microemulsion eye drops (CYCLOMUNE)

Challenges in manufacturing a good liposomal product

- Manufacture a liposomal product which match point to point with that of the innovator product.
- Develop a product with same quality for all the markets including US and EU.
- In depth knowledge about the product and the process in order to manufacture liposomal product of consistent quality.
- Detailed characterization to understand the product and technology better.

Lambin

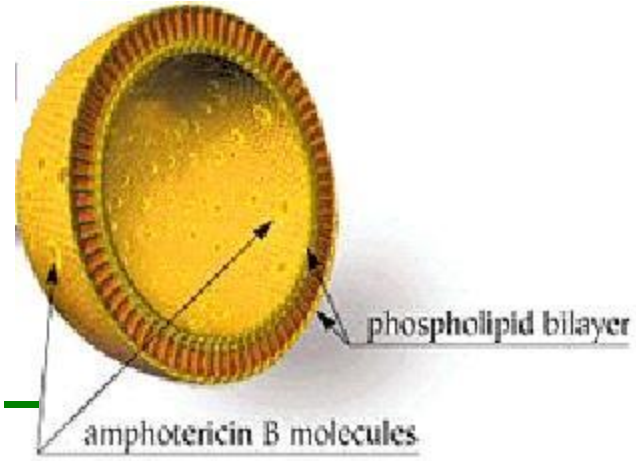
Liposomal Amphotericin B for injection

An accomplishment of 8 years of continuous, dedicated and uncompromising research involving a group of scientists at SPARC.

Total time spent by the scientists for the development of Lambin is around 1,50,000 hrs.

Lambin

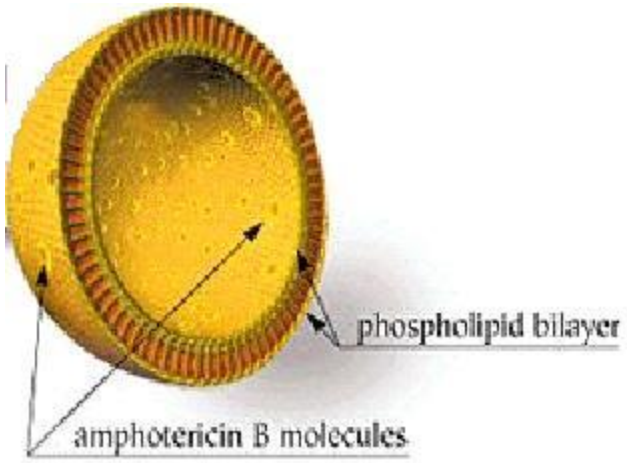
- Lambin Injection is a sterile, non-pyrogenic lyophilized liposomal formulation for intravenous infusion.



- Each vial contains
  50 mg of Amphotericin B – Drug,
  84 mg of Distearoyl phosphatidylglycerol – Anchoring lipid,
  213 mg Hydrogenated soy phosphatidylcholine – liposome forming lipid,
  52 mg Cholesterol – provides rigidity to liposome,
  0.64 mg alpha Tocopherol - antioxidant,
  900 mg Sucrose – Cyroprotectant and
  27 mg Disodium succinate hexahydrate - buffer.

Manufacturing of Lambin

- Drug lipid interaction for efficient encapsulation.
- Manufacturing of multilamellar liposome containing high amount of drug
- Size reduction into small unilamellar vesicles.
- Sterile filtration and stabilization by lyophilization.



Special Characterization studies of Lambin

- Particle size
- Zeta Potential
- Nano DSC study
- Potassium release study
- Cryo Transmission Electron Microscopic study
- Acute toxicity study in mice
- Repeated Dose toxicity in mice for 14 days
- Antifungal efficacy study in mice

Particle size comparison

The Particle size analysis performed using Malvern ZETASIZER NANO ZS.

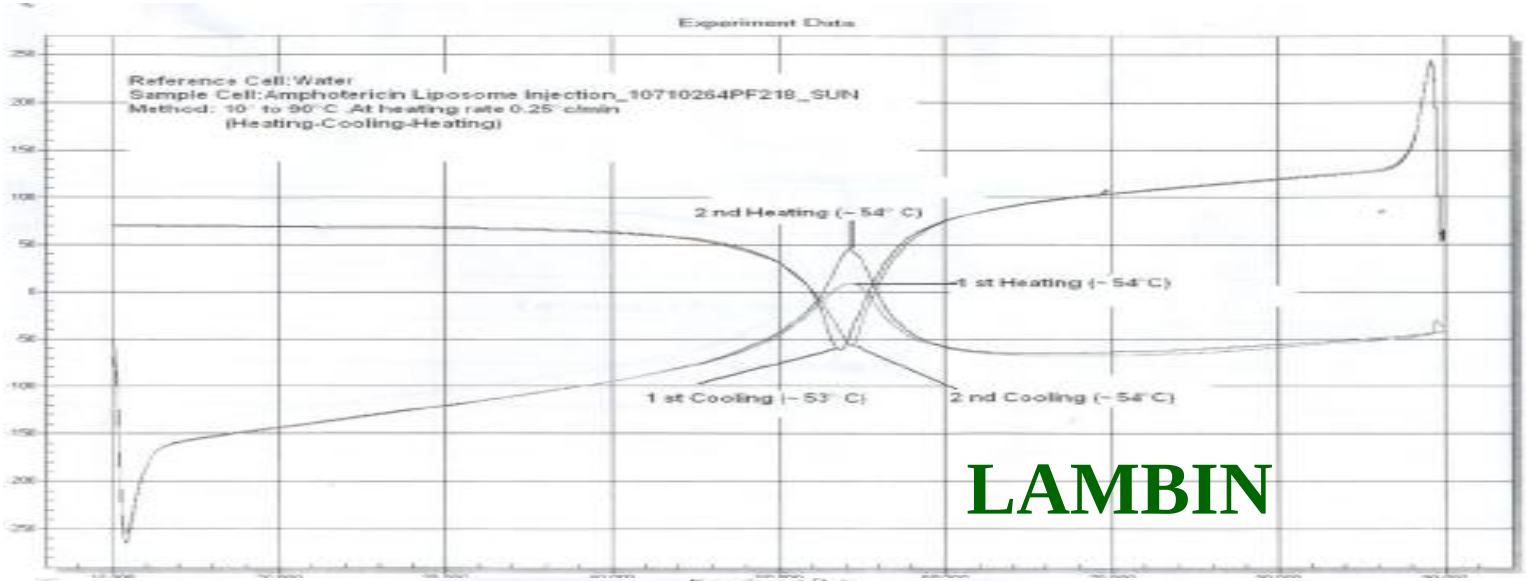
	Lambin	Innovator Product
Lot No.	JKJ2019	042546AD
Mean Particle size (in nm)	112.3	113.82

Zeta Potential Comparison

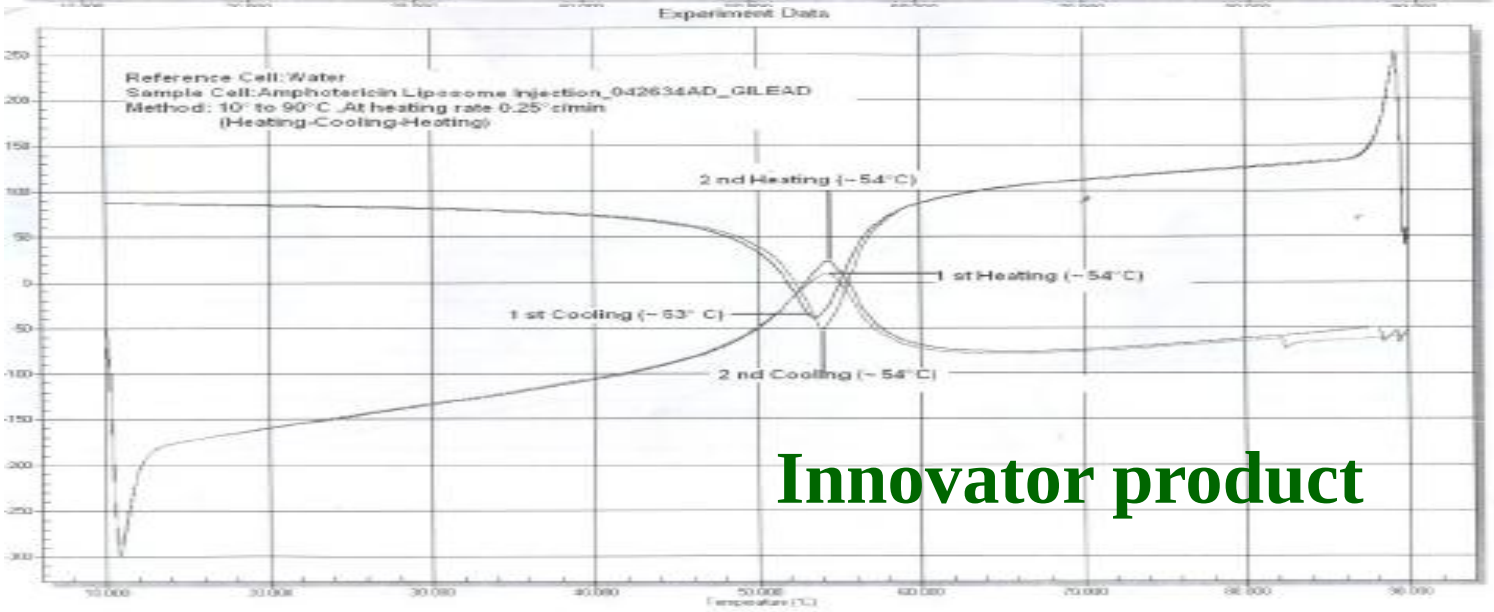
Zeta potential analysis performed using Malvern ZETASIZER NANO -ZS.

	Lambin	Innovator product
Lot No.	PF-S882-180	042546AD
Zeta Potential (in mV)	-41.68	-42.29

Comparative Nano DSC study

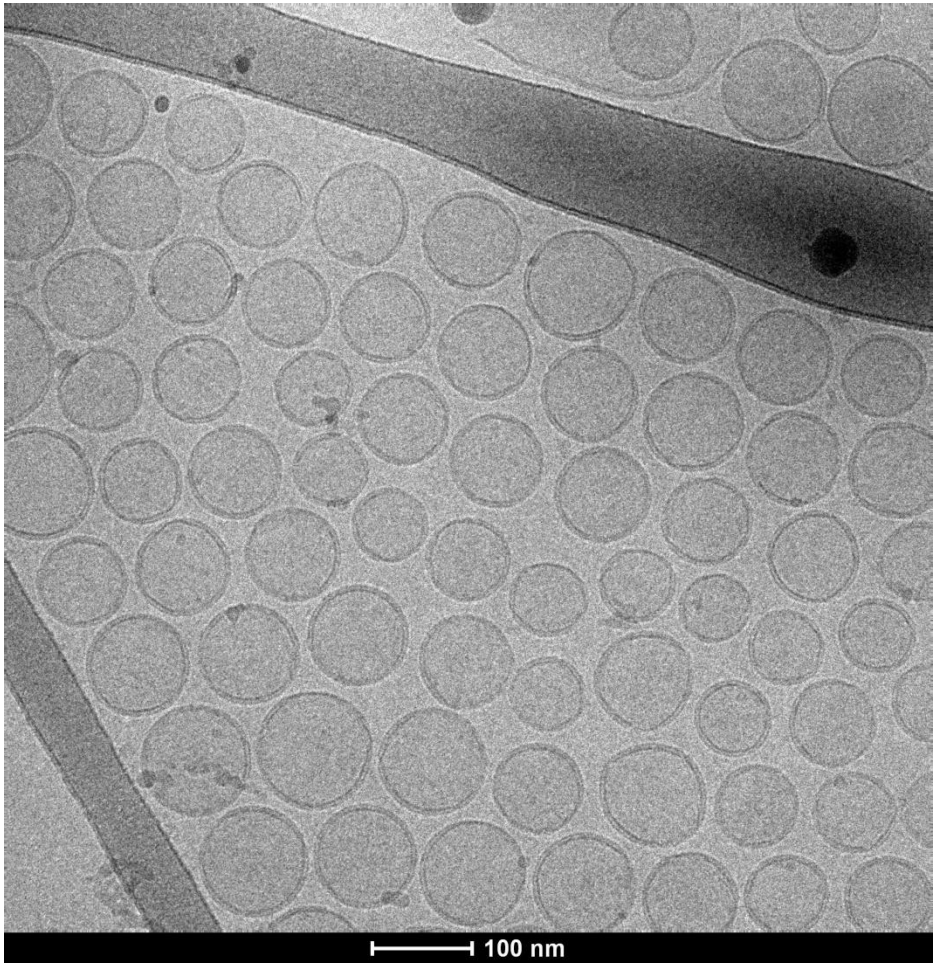


LAMBIN

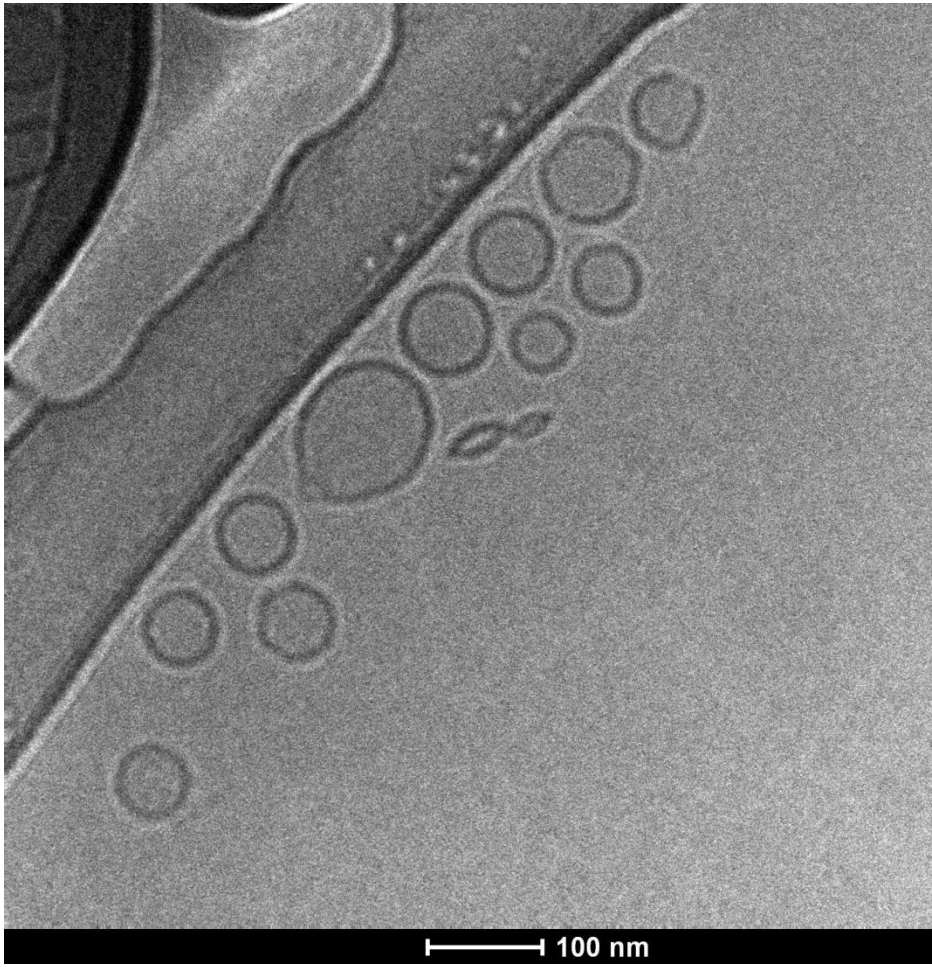


Innovator product

Cryo Transmission electron microscopy
Lambin Vs Innovator product



Lambin
Batch No. PF235



Innovator Product
Lot No. 042634AD

Potassium release study in Human Blood

	Lambin	Innovator Product
Lot No.	PF-S882-190	042634AD
K+ release (in ppm)	108.91	106.7

Sun's Lambin is equivalent to Innovator Product when tested for Potassium release in human blood, which shows that the Lambin is as safe as Innovator product when tested *in vitro*.

Comparative Acute Toxicity Study of Lambin and Innovator Product

	% Mortality	
Details	**Lambin**	**Innovator Product**
Lot No	**JK93280**	**042214CD**
15mg/kg Dose	**Not done**	**Not done**
30mg/kg Dose	**Not done**	**Not done**
60mg/kg Dose	**0%**	**0%**
100mg/kg Dose	**30%**	**40%**

Repeat Dose 14 Days Toxicity Studies in mice

Dose of 50mg/kg/day of Amphotericin B liposomes administered to CD-1 mice intravenously for 14 consecutive days.

Results of the study suggested that

- **No mortality was observed.**
- **No clinical signs were observed in any of the animals.**

Antifungal Efficacy study in mice

Group	Treatment	% Survival After Treatment						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
I	Saline Control	100	100	100	17	0	0	0
II	Placebo	100	67	67	0	0	0	0
III	Fungizone (Free AmpB)	100	100	83	17	17	0	0
IV	Liposomal AmpB (Innovator product)	100	100	100	67	50	33	33
V	Liposomal AmpB (Test) Lambin	100	100	100	83	67	67	50

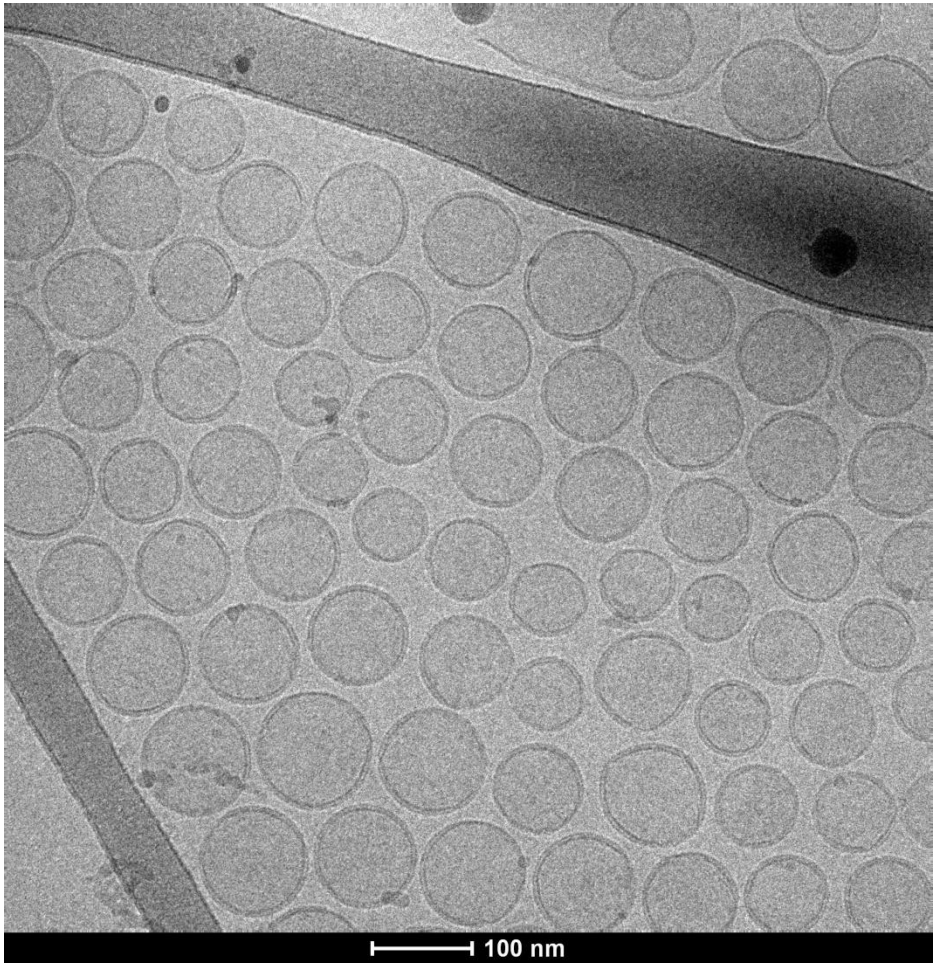
Antifungal activity of Sun's Lambin is equivalent to Innovator product when tested using Aspergillus model in mice

Lambin Vs Innovator product

- Similar physicochemical characteristics
- Similar safety and efficacy profile
- Lambin more affordable than Innovator product

Comparison of Lambin with few marketed Amphotericin products

Cryo Transmission electron microscopy
Lambin Vs "MLV with sonication" product



Lambin
Batch No. PF235

"MLV with sonication" product
Lot No. 10F08-008

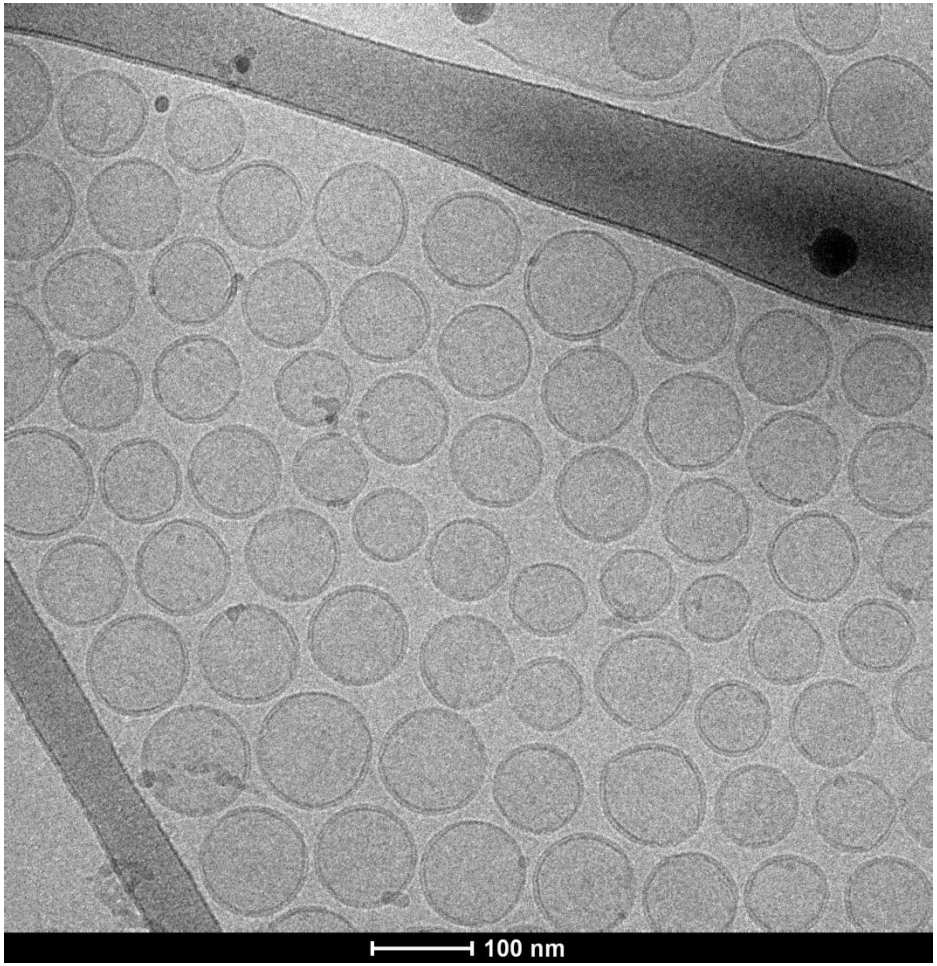
Acute Toxicity Studies - Lambin Vs "MLV with sonication" product

Details	% Mortality	
	Lambin	MLV with Sonication
Lot No	JK93280	25F08-002
15mg/kg Dose	Not done	40%
30mg/kg Dose	Not done	100%
60mg/kg Dose	0%	Not done
100mg/kg Dose	30%	Not done

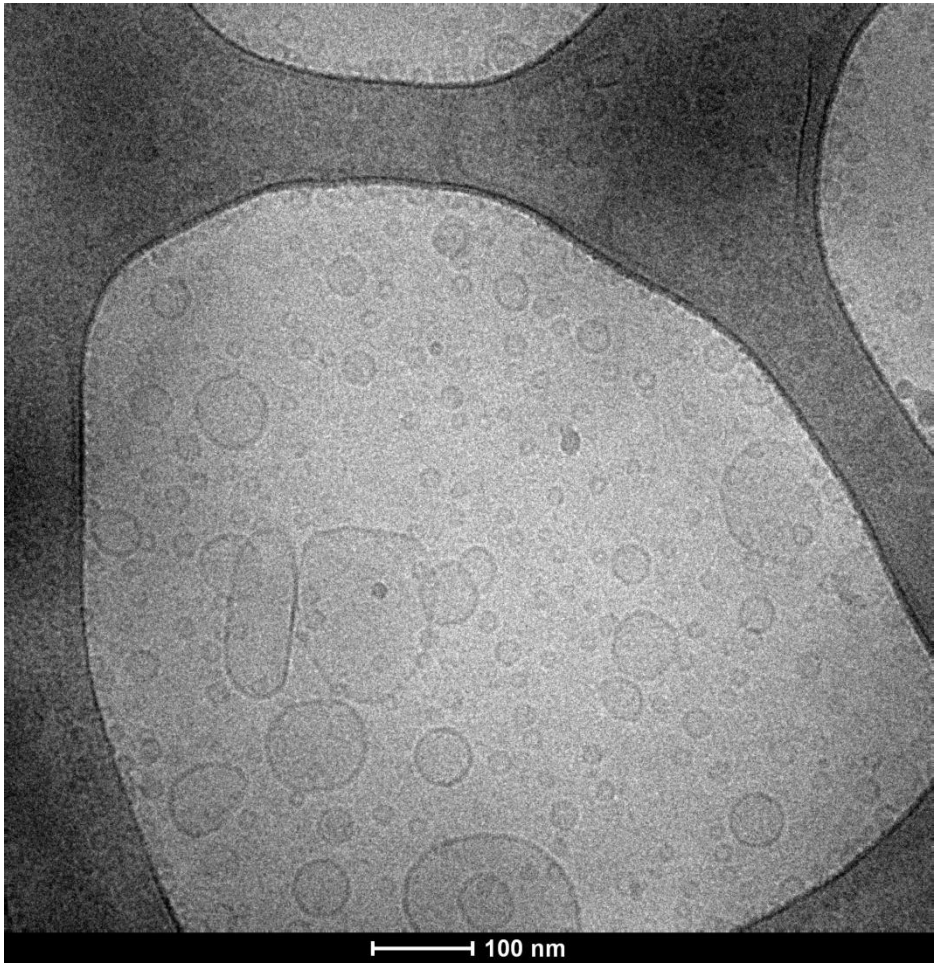
Lambin Vs "MLV with sonication" product

- Difficult, inconvenient handing procedures for "MLV with sonication" product (sonication).
- Higher lipid quantity used in "MLV with sonication" product compared to Lambin (1: 7 for Lambin and 1: 45 for "MLV with sonication" product).
- Particle size and shape of "MLV with sonication" product is highly variable.
- "MLV with sonication" product is highly toxic compared to Lambin.
- Possibility of liposomal aggregation is very high as the admixture is done in sodium chloride solution in MLV with sonication product.

Cryo Transmission electron microscopy-
Lambin Vs Nanosomal Product



Lambin
Batch No. PF235



Nanosomal Product
Lot No. K8605

Acute Toxicity Studies of few marketed liposomal Amphotericin products

Details	% Mortality	
	Lambin	Nanosomal product
Lot No	JK93280	K8605
15mg/kg Dose	Not done	Not done
30mg/kg Dose	Not done	100%
60mg/kg Dose	0%	100%
100mg/kg Dose	30%	Not done

Lambin Vs Nanosomal Product

- Highly variable in Particle size distribution in case of Nanosomal Product (13.5 to 531 nm) compared to Uniform PSD with respect to Lambin (38.7 to 258.6 nm) .
- No anchoring lipid present in Nanosomal Product to retain amphotericin B in liposomal bilayer.
- High toxicity in case of Nanosomal Product compared to Lambin.

Conclusion

Lambin
(An liposomal amphotericin B formulation)

- Lambin is prepared using highly pure raw materials.
- Lambin is prepared under rigorous quality assurance using state-of-the art cGMP facility.
- Lambin is evaluated for various quality control parameters to ensure its high quality.
- Lambin is evaluated in animals for safety.

Lambin is a liposomal system of high quality with high therapeutic safety for better patient compliance


Thank you !!!
30/10/2009 12:48