

Sustained Release Multivesicular Liposomal (DepoFoil®) Formulation of IFN- α_{2b} that provides Sustained Plasma IFN- α_{2b} levels for Seven Days

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(formerly SkyePharma Inc.)

Objectives

The objective of our work was to develop a sustained-release DepoFoil® formulation of IFN- α_{2b} that provides:

- High IFN- α_{2b} encapsulation efficiency (>85%)
- No loss of IFN- α_{2b} bioactivity during manufacturing
- Maintenance of efficacious serum IFN- α_{2b} levels in rats for 7 days
- Minimal post-administration release and low serum Cmax/Cmin ratio
- Long term storage stability at 2-8°C

Methodology

Formulation Manufacture

IFN- α_{2b} was encapsulated within DepoFoil particles using a high-efficiency double emulsification process that is applicable for use with both small molecules and biomolecules. To manufacture DepoIFN- α_{2b} particles, an aqueous IFN- α_{2b} solution was emulsified in an immiscible lipid solution to produce a W/O emulsion. The W/O emulsion was then emulsified with a second aqueous solution to produce a W/O/W particle suspension. The organic solvent was evaporated from the particles to produce multi-chambered DepoIFN- α_{2b} particles (Figs 1a,b). The particle suspensions were then washed and stored in normal saline. After manufacture, DepoFoil formulations were characterized with respect to:

- IFN- α_{2b} encapsulation efficiency by RP-HPLC
- Structural integrity of the encapsulated IFN- α_{2b} by SDS-PAGE (Invitrogen Corp)
- Bioactivity of the encapsulated IFN- α_{2b} by cytopathic effect assay (CPE, PBL Biomedical Laboratories)
- Particle size distribution by laser light diffraction analysis (Horiba LA-910)

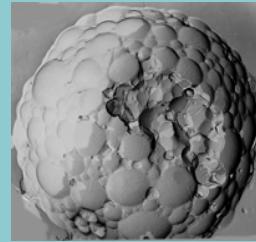


Fig 1a. Electron micrograph of DepoFoil particle

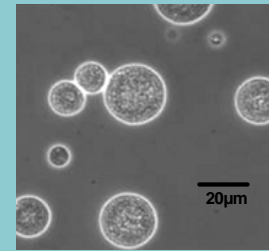


Fig 1b. Phase contrast micrograph of DepoFoil particles

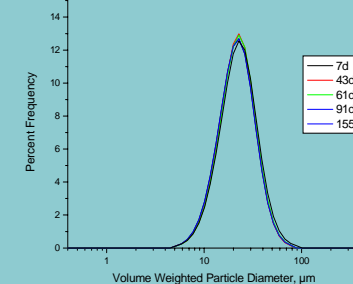


Fig 2. Particle size distribution of DepoIFN- α_{2b} suspension

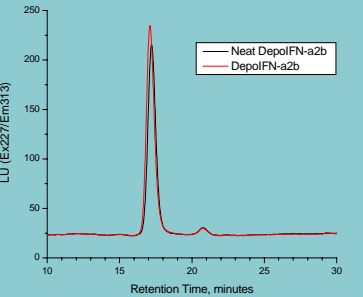


Fig 3. RP-HPLC chromatogram of encapsulated vs neat IFN- α_{2b}

DepoIFN- α_{2b} Pharmacokinetics and Biocompatibility

DepoIFN- α_{2b} formulations were evaluated in a rat model as follows: DepoIFN- α_{2b} was administered by subcutaneous (SC) injection in the rat hindlimb. Blood was collected via the saphenous vein, and serum, isolated by centrifugation from the blood samples, was analyzed by ELISA (Bender MedSystems). The injection site was evaluated visually to determine biocompatibility of the DepoIFN- α_{2b} formulations.

Long Term Storage Stability (2-8°C)

DepoIFN- α_{2b} formulations stored at 2-8°C were evaluated at intervals up to 6 months to determine their physical and chemical stability. Physical stability was determined using microscopy, particle size distribution and analysis of IFN- α_{2b} released from the particles (RP-HPLC). Chemical stability of the encapsulated IFN- α_{2b} was determined using RP-HPLC and SDS-PAGE analysis.

Results

Formulation Characteristics

IFN- α_{2b} was encapsulated in DepoFoil particles with 86% efficiency. Less than 1% of the IFN- α_{2b} was free in the normal saline storage solution. DepoIFN- α_{2b} particles were spherical (Fig 1b) and had monomodal particle size distribution (20µm median diameter, Fig 2). The encapsulated IFN- α_{2b} was shown by RP-HPLC and SDS-PAGE to have retained its chemical integrity (Figs 3, 4). The bioactivity (IU/mg) for encapsulated IFN- α_{2b} was unchanged as measured by the cytopathic inhibition assay.

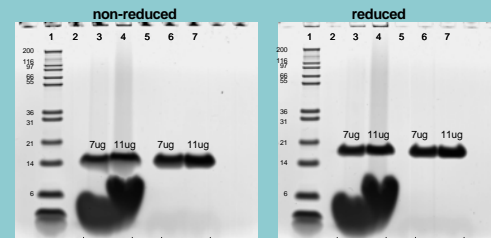


Fig 4. SDS-PAGE analysis of DepoIFN- α_{2b} following 6M storage at 2-8°C (Coomassie stain)

DepoIFN- α_{2b} Pharmacokinetics and Biocompatibility

Rat plasma pharmacokinetics were determined for five replicate batches of the DepoIFN- α_{2b} formulation (Fig 5). Following SC administration, only a small fraction of the encapsulated IFN- α_{2b} was released from the particles. Thereafter, DepoIFN- α_{2b} formulations released IFN- α_{2b} in a near zero-order fashion for up to 7d. The Cmax occurred between 4-5 days. Accumulation of AUC was near linear for up to 7d (Fig 6). CPE analysis of IFN- α_{2b} -containing rat serum samples showed that the IFN- α_{2b} released from DepoIFN- α_{2b} particles retained ~100% bioactivity.

DepoIFN- α_{2b} formulations were well tolerated. There were no indications of irritation or erythema at the site of injection during the 7 day study period, nor at necropsy. Furthermore, the subcutaneous DepoIFN- α_{2b} depots were not visually detectable at the site after 10 days.

Long Term Storage Stability

DepoIFN- α_{2b} formulations were chemically and physically stable during 2-8°C storage for the duration of the monitoring period (6M). There was no detectable change in total IFN- α_{2b} content nor particle size distribution of the DepoIFN- α_{2b} formulations (Figs 7, 2). There was no detectable leakage of IFN- α_{2b} from the formulations during storage. Unencapsulated (free) IFN- α_{2b} never exceeded 1% during 6 months of storage.

SDS-PAGE analysis of samples stored for 6M showed that the encapsulated IFN- α_{2b} was unchanged relative to an IFN- α_{2b} solution control. IFN- α_{2b} oligomerization was not detectable by Coomassie staining (Fig 4, LOD ~1.8%). Silver staining of overloaded samples was required to visualize oligomers, and the extent of oligomerization in DepoIFN- α_{2b} stored for 6M was indistinguishable from unencapsulated IFN- α_{2b} solution (Fig 8).

Conclusions

- IFN- α_{2b} was encapsulated with 86% efficiency into DepoFoil particles. The particle suspensions were monomodal, and the median particle size was 20µm. The suspensions contained less than 1% free IFN- α_{2b} .
- DepoIFN- α_{2b} delivered bioactive IFN- α_{2b} in a near-zero-order fashion for up to 7d in a rat subcutaneous model. DepoIFN- α_{2b} was biocompatible in the rat.
- DepoFoil-encapsulated IFN- α_{2b} retained its chemical integrity and bioactivity for at least 6 months during 2-8°C storage.
- DepoIFN- α_{2b} was physically stable during 2-8°C storage. There was no change in particle size distribution nor percent free IFN- α_{2b} during 6 months storage.
- DepoFoil encapsulation represents an attractive solution for the delivery of small molecules and biologics.

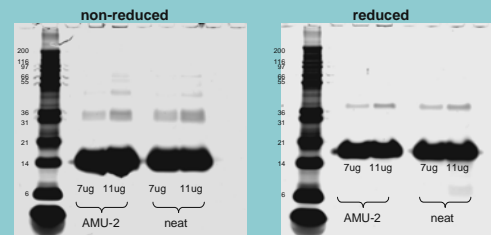


Fig 8. SDS-PAGE analysis of DepoIFN- α_{2b} following 6M storage at 2-8°C (Silver stain)

DepoFoil formulations offer:

- Efficient encapsulation of small molecules and biologics
- Precedence in two marketed products
- A well tolerated SC depot
- Near zero-order pharmacokinetics
- Long term stability

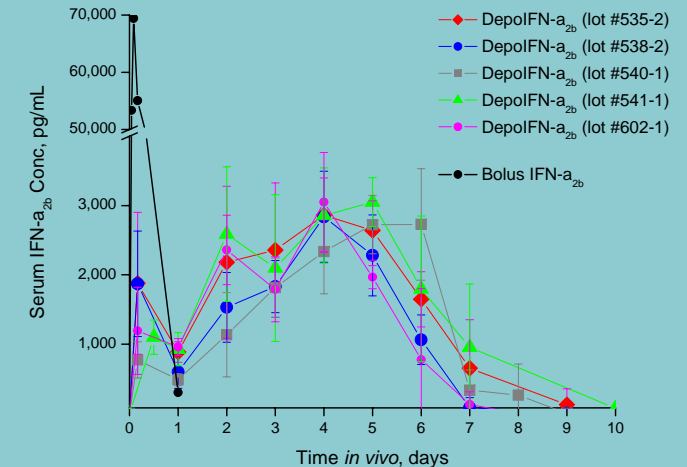


Fig 5. Rat serum IFN- α_{2b} levels following administration of DepoIFN- α_{2b} vs bolus IFN- α_{2b}

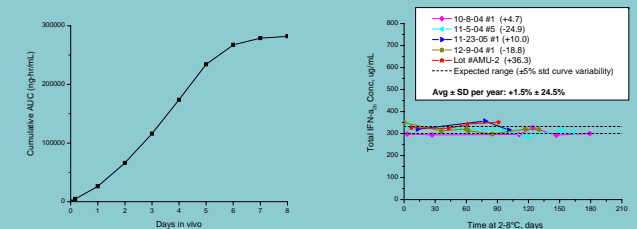


Fig 6. Cumulative AUC for IFN- α_{2b} in rat serum following DepoIFN- α_{2b} administration

Fig 7. RP-HPLC analysis of DepoIFN- α_{2b} during 6M storage at 2-8°C

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DepoFoil encapsulation represents an attractive solution for the delivery of small molecules and biologics. DepoFoil formulations offer:

- Efficient encapsulation of small molecules and biologics
- Precedence in two marketed products (DepoCyt™ and DepoDur™)
- A well tolerated SC depot
- Near zero-order pharmacokinetics
- Long term stability