Continuous Delivery of Interferon-α2a Via Subcutaneous Depots

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Abstract

Purpose: To identify depot formulations for weekly to monthly delivery of immuno-modulatory, anti-viral and anti-proliferative interferon α2a

Methods: DURECT Depot formulations were prepared as suspensions of interferon in a mixture of a pharmaceutically-acceptable solvent, biodegradable polymer, and other excipients. Upon subcutaneous injection, the depot forms at the injection site. Release of interferon begins immediately upon administration and continues for a period of time. Concurrently, the excipients degrade, eliminating the need for removal. Different excipient combinations were screened for optimal sustained delivery of interferon. Formulations were studied both *in vitro* and *in vivo*, and evaluated based on physical and chemical stability, injectability and PK and PD

Results: Pre-formulation solubility and stability experiments showed that interferon α2a could be suspended in depot vehicles as a lyophilized powder with sucrose serving as a stabilizing and bulking agent. Suspension formulations were selected on the basis of controlled release over 28 days *in vitro*, and stability of unreleased protein. PK studies of these formulation in immune-suppressed rats demonstrated release of < 10% of initial interferon α2a content in the first day post dose, followed by controlled release over one month. Results from anti-viral and anti-proliferation assays confirmed the sustained delivery of interferon. A primate PK study demonstrated the ability of the depot to deliver interferon over 2 to 4 weeks

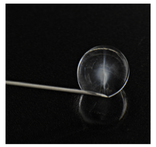
Conclusions: DURECT depot formulations can deliver active interferon from 2 to 4 weeks by stabilizing protein and controlling its release

Interferon Subtypes and Therapeutic Applications

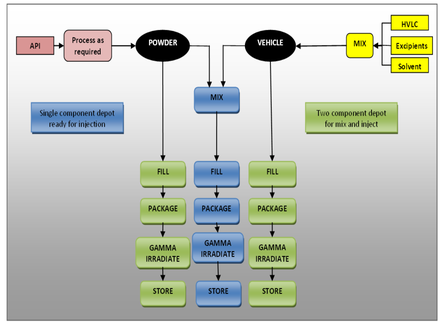
- Interferons (IFNs) – Proteins synthesized by eukaryotic cells in response to viral infection; they are anti-viral and immuno-modulatory
- Several different types of therapeutic IFNs – α, β, γ
- IFN α_{2a} - Hepatitis C
- IFN α_{2b} - Hairy Cell Leukemia, Malignant Melanoma, AIDS-related Sarcoma, Chronic Hepatitis B / C
- IFN β - Multiple Sclerosis
- IFN γ - Malignant Osteopetrosis

DURECT Depot Injectable Formulations

- Parenteral depots for small molecules, peptides and proteins
- Delivery over days to months
- Several mechanisms to control release
- Ability to modulate initial release
- Injectable through fine needles
- High drug payload / low injection volume
- Biodegradable and biocompatible
- Formulations
  - Stabilized API powder suspended in a liquid vehicle (viscosity ~ 100cP)
- Preparation
  - Lyophilize / spray dry API powder, then homogenize with vehicle



Manufacturing CLOUD™ Depot Formulations



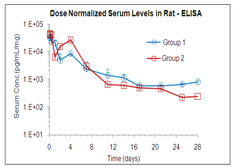
Rat PK of DURECT Depot Formulations

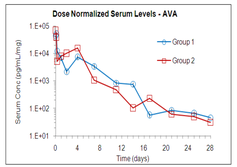
Formulations		
Drug / Excipient	**Vehicle**	**Group**
1mg dose of Ifn-α2a + protein stabilizer 1, 20mg/mL suspension 50μL dose volume	CLOUD Vehicle 1	1
1mg dose of Ifn-α2a + protein stabilizer 2, 20mg/mL suspension 50μL dose volume	CLOUD Vehicle 1	2

Protein powders were obtained by spray drying

- 1mg of Ifn-α2a was dosed to each rat in each group (N=6)
- The stabilizers protect the protein from degradation in the depot itself throughout the duration of delivery, but also confer some control over release from the formulation
- Rats were immune suppressed with cyclosporine and methyl-prednisolone
- We included an immune normal group as a comparator
- Dosing injections required a few seconds, using 25Ga needles

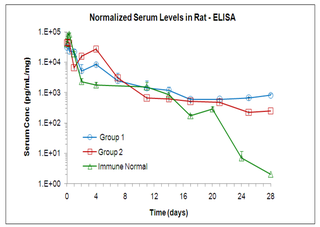
DURECT Depot Interferon α2a Rat PK





- (Left) As measured by ELISA, serum levels produced by both formulations reached high levels within 6 hours of dosing. These levels were roughly maintained out to day 4, after which they declined gradually ~ 40-fold out to day 11, beyond which time they were steady
- Group average C_{Max}/C_{SS} ratios were 45 and 109, and BA_{28d} was 25±4% and 40±6%, respectively
- (Right) The anti-viral activity (AVA) of pooled serum samples from each group was measured and serum levels estimated from the observed activity. The ELISA and AVA profiles differed in some details: The latter declined from day 4 to day 20, reaching a steady state value about 10-fold lower than the former

DURECT Depot Interferon α2a Rat PK

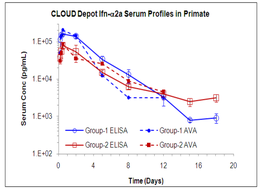


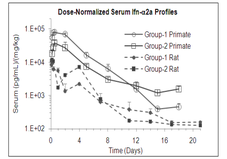
- Serum profiles similar to those observed in groups 1 and 2 were obtained in immune normal rats, except that steady state was achieved early and then levels fell strongly beyond day 14, presumably due to an immune response mounted in the rats

DURECT Depot Interferon α2a Primate PK

- The primate PK study utilized the same formulations as in the rat study and was conducted in Cynomolgus monkeys (N=4)
- However, protein loading in both formulations was doubled to 40mg/mL
- Each animal received a 50μL/kg injection, via a 25Ga needle, for a dose of 2mg/kg
- For safety reasons, the monkeys were not immune suppressed (potential to shed hepatitis B virus)

DURECT Depot Interferon α2a Primate PK





- (*Left*) Average serum profiles determined by ELISA and Anti-viral Assay (AVA). Serum samples from individual animals in each treatment group were analyzed by ELISA. Pooled serum samples from each treatment group were analyzed by AVA
- Up to day 12, formulation 1 produced substantially higher serum levels than did formulation 2, but with steeper decline from days 3-12, and lower apparent steady state values established at day 15. C_{Max}/C_{SS} ratio was 188 and 28, and *BA* through day 22 was 35 and 100%, respectively (Wills & Soike, J Ifn Res **8**, 1988, 427; Wills *et al*, J Ifn Res **4**,1984, 399)
- (*Right*) Dose-normalized serum profiles up to day 20 were qualitatively similar for primates and immune-suppressed rats, but levels were ~ 10x higher in primate, and input into the systemic circulation was slower

SC Delivery of Native Ifn-α2a: Conclusions

- The goal of SC delivery of native IFN via DURECT depots is to achieve therapeutic protein levels in man for a duration of at least 28 days from a single injection
- Based on antiviral activity and serum levels required for systemic efficacy, we hypothesized that a lower limit of effective concentrations in serum is 100 pg/mL
- We have devised two depot formulations, injectable through 23-25Ga needles, that exceeded this threshold in immune-suppressed rats for the desired duration, with 25-40% absolute bioavailability, and peak/steady state ratios in the range 40-100
- These formulations were subsequently studied in immune-normal primates, in which the serum levels obtained exceeded the efficacy threshold for 3 weeks. Beyond that time, circulation of neutralizing antibodies reduced serum levels below the limit of detection
- The Ifn-α2a content of these formulations ranged from 20-40mg/mL, such that only 50μL injection was required for monthly delivery in rats
- In both rats and primates, injection site tissue reactions were as mild as those of commercial depot products
- Thus, the DURECT depot platform is well suited for continuous delivery of proteins at least up to 20kDa molecular weight