

## PRODUCTION OF INTERFERON-B (IFN- B) IN *E. COLI* AND STRATEGIES FOR IMPROVING ITS BIOAVAILABILITY AND PHARMACOLOGICAL PROFILE

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IFN- $\beta$  is the main therapeutic agent which convincingly reduces multiple sclerosis relapse rate and retards disability. IFN- $\beta$ , expressed both in mammalian and bacterial cells, is used in therapy. However, even if costs and yield in *E. coli* are very advantageous, its production is extremely difficult, due to its toxicity and extreme insolubility. Bio-Ker set up a thermo-inducible expression system with optimized codon usage, which allows the expression of a fusion protein reaching a yield up to 2mg/l. Biological activity of the purified protein was evaluated by measuring inhibition of cell multiplication, and phosphorylation of Stat1 in HeLa cells.

Principal issues related to IFN- $\beta$  are protein aggregation, with loss of bioavailability up to 70%, and high immunogenicity, with production of neutralizing Ab which interrupt therapy. In order to ameliorate the bio-similar product, Bio-Ker produced some site-specific muteins, according to molecular modelling design. Muteins have been evaluated for *in vitro* and *in vivo* pharmacological profiles.

Moreover, IFN- $\beta$  naturally exhibits a relatively short serum half-life, and we are planning to obtain a long lasting IFN- $\beta$  mutein by conjugation to polymers which will entrap the protein in pharmaceutical controlled release devices to increase their bioavailability and to improve their pharmacological profile.

Pharmacological, pharmacokinetic and pharmacodynamic data will be discussed.