# *In vitro*, *ex vivo* and *in vivo* recombinant growth hormone synthesis, for gene therapy applications

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Recombinant growth hormone has been one of the first proteins to be synthesized via DNA recombinant techniques in the early eighties. It has also been one of the first to be used for studying animal models for gene therapy. This is due, besides to a real therapeutic need, also to its easy and sensitive detection by well-known immunoassay methods and to an evident phenotypic effect that can be observed and measured in several animal models (e.g. dwarf mice).

Retrovirally transduced primary human keratinocytes could secrete *in vitro* up to 7 µg hGH/$10^6$ cells /day, producing, via *ex-vivo* cutaneous grafting in immunodeficient dwarf mice, a weight gain of 0.060 g/animal/day (12-day assay), with circulating hGH serum levels of 0.2-0.3 ng/ml.

A similar *ex-vivo* strategy, when carried out with the use of mouse growth hormone (mGH) cDNA, presented a stable *in vitro* secretion of up to 11 µg mGH/$10^6$ cells /day (20 µg mGH/$10^6$ cells /day by clonal selection) and, thanks to an organotypic raft culture grafting, circulating mGH levels of up to 21 ng/ml. These, unfortunately, only for a very short time.

Considering the high discrepancy observed between *in vitro* and *in vivo* levels and sustainability, a comparative study was carried out by direct muscle injection of naked plasmid DNA followed by electroporation. Unexpectedly, circulating levels of 2-3 ng hGH/ml were obtained for at least 60 days, leading to a body weight gain of 0.042 g/mouse/day (0.174 g/mouse/day for the first 10 days) which represented ~33% increase to the initial weight. These data will be compared to those obtained by repetitive recombinant hGH injections in the same animal model.

*In vivo* recombinant protein generation, by direct plasmid DNA injection, in a way that is more similar to the natural synthetic mode, can be a future alternative to expensive high quality protein production, required for therapeutic use.