

NOVEL G-CSF MUTANT FOR CONJUGATION

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Human Granulocyte-Colony Stimulating Factor (h-G-CSF), encoded by a single gene located on chromosome 17, is a glycoprotein hormone that stimulates the proliferation and differentiation of neutropoietic progenitor cells to granulocytes and functionally activates the mature neutrophil.

The natural human glycoprotein exists in two forms, a 174- and 180- aminoacid long protein with a molecular weight of 19,600 Dalton. The more-abundant and more-active 174-amino acid form has been used in the development of pharmaceutical products by recombinant DNA (rDNA) technology.

The recombinant form of h-G-CSF is used in oncology and hematology with certain cancer patients to accelerate recovery from neutropenia after chemotherapy, allowing higher-intensity treatment regimens.

This form synthesized in an E.coli expression system is marketed as Filgrastim (Neupogen®) and the structure differs slightly from the native glycoprotein.

We developed a plasmid construct that allowed successful expression of a non-glycosylated methionyl extended variant of G-CSF fusion protein in *Escherichia coli*, biosimilar to Filgrastim. This protein was extracted and purified from cellular inclusion bodies and refolded into the biologically active form to show colony stimulating activity.

Afterwards, we decided to produce several mutant proteins by site-directed mutagenesis in order to introduce residues able to selectively bind conjugated polymers. The *in vitro* bioactivity assay based on proliferation of murine myeloblastic cell line NFS-60 and on receptor binding assay showed the same biological activity as Filgrastim. We are planning to conjugate the most promising mutant proteins by an enzymic technology already used in Bio-Ker using PEG (polyethylene glycol) as a vector, in order to increase serum half-life.