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A Novel Method of Developing a High-Affinity Monoclonal Antibodies to Capture Native or Pegylated Human Granulocyte Colony-Stimulating Factor (G-CSF)

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ABSTRACT

G-CSF (granulocyte-colony stimulating factor) is a haematopoietic growth factor which usually used as a drug with chemotherapy to boost white blood cell regeneration. In current study, an association of the augmented expression of G-CSF has been revealed in many advanced tumors and/or poor prognosis in some clinical cases. Immunohistochemical analysis and immunoassay to determine the protein expression level of G-CSF in advanced-stage or poorly differentiated adenocarcinoma become essential to monitor patients who are receiving or are to receive medication. Thus, the detection of human G-CSF level becomes very important in the patients under medication.

In this study, by using our proprietary techniques, we've developed a panel of high-affinity monoclonal antibodies which were selected by native G-CSF and Pegylated G-CSF in vitro. The Kd of the monoclonal G-CSF antibodies is ranged from 50 picomolar to 1 micromolar. Higher affinity to pegylated human G-CSF and relatively low affinity to native human G-CSF were observed when spleenocytes were challenged by the same dosage of two different format of proteins. We've selected the highest affinity antibody as a capture reagent, detected the lowest levels of native human G-CSF at 2.1 pg/ml and pegylated G-CSF at 4.8 pg/ml by recovery assays. The linear range of G-CSF detection is 5 pg/ml-15 ng/ml in both proteins.

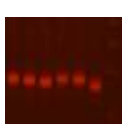
In conclusion, we have developed a novel technique to produce a panel of high affinity antibodies. Additionally, we discovered the different performance of the native G-CSF and pegylated G-CSF in vivo. Our observation provided contradictory concerns in protein drug discovery: half-life enhancement or minimum immunogenicity.

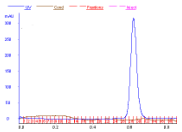
MATERIALS AND METHODS

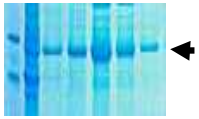
1: G-CSF recombinant protein construction/purification and Pegylation

Human G-CSF (175 aa/19kDa)

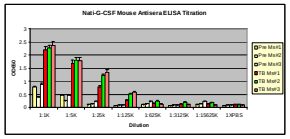
maplgptgpl pqsflllkcle qmrkvqadgt alqetlcath qlchpeelvl lghalgipqp 61 plsscssqal qlmgclrqlh sglfiyqgll qalagispel aptldtlqld ttdfainiwq 121 qmedlgmapa vpptqgtmpa ftsafqrrag gvlvasnlqs flelayralr hfakp

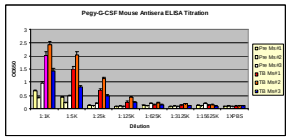




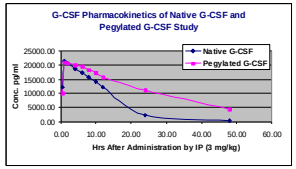


2: Antibody development and characterization





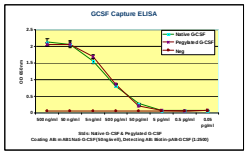
3: Pharmacokinetic studies of Nati-G-CSF and Pegy-G-CSF on two group of animals (5x/group)



4: G-CSF ELISA Capture Assays

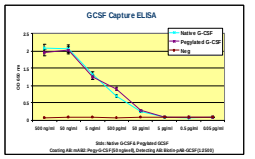
Sample1: Coating with mAB1: anti-Nati-GCSF (2 ug/ml @25ul/well)

	Native GCSF	Pegylated GCSF	Neg
500ng/ml	2.125	2.098	0.098
50ng/ml	2.065	2.065	0.096
5ng/ml	1.592	1.686	0.098
500 pg/ml	0.809	0.886	0.095
50pg/ml	0.282	0.228	0.093
5pg/ml	0.095	0.092	0.093
0.5pg/ml	0.082	0.093	0.094
0.05pg/ml	0.070	0.095	0.072



Sample 2: Coating with mAB2: anti-Pegy-G-CSF (2 ug/ml @ 25ul/well)

	Native G-CSF	Pegylated G-CSF	Neg
500 ng/ml	2.0909	1.9531	0.0754
50 ng/ml	2.0638	2.0198	0.0777
5 ng/ml	1.3275	1.2463	0.08
500 pg/ml	0.6892	0.9077	0.0751
50 pg/ml	0.251	0.2807	0.0822
5 pg/ml	0.0817	0.0923	0.0794
0.5 pg/ml	0.0724	0.0805	0.0867
0.05 pg/ml	0.0864	0.0848	0.0969



CONCLUSIONS

The technique of generating high affinity antibody may become an attractive strategy for the therapy of carcinomas. In this study, we demonstrated that the native G-CSF and Pegylated G-CSF performed differently in vivo. The half-life of pegylated form is much longer than the native form (25 hr vs 10 hr). The follow-up study with the same animals were conducted to generate different antibodies varying from its applications. We obtained a panel of antibodies to establish quantitative immunoassays to detect two forms of G-CSF. Our results indicated that the different antibodies may be related to the different form of the proteins and/or lead us to a new area for the recombinant protein therapy: half-life extended by pegylation may lead an immunogenic enhancement due to the prolong exposure of the protein to its immune system.

REFERENCES

1: A Cancer Journal for Clinicians: new developments in cancer, November 27, 2007, 1542-4863 http://caonline.amcancersoc.org

2: Morstyn G, Dexter T, Foote M. Physician Desk Reference (2008) Filgrastim (r-metHuG-CSF) in clinical practice, 44th Edition (3) 51-71

3: Ab[Max] Human G-CSF Immunoassay Kit (AbboMax, Cat# 700-101)

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