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Complement factor B and protein C2 point mutants, a method to enhance the activity of anti-neoplastic antibodies, pharmaceutical composition containing the point mutants and the use thereof.

Product description

Following the introduction of antibodies capable of recognizing cancer cell determinants into standard anticancer therapy, a significant increase in patient survival times and a reduction in tumor progression rates were observed.

Rituximab, an agent recognizing CD20 molecules, was used in the treatment of leukemias and lymphomas as the prototype therapeutic anti-neoplastic antibody.

Along with a number of other antibodies authorized for clinical use, rituximab efficiently kills neoplastic cells with the complicity of the human immune system. The binding of the antibody to the tumor cell surface activates the complement system, i.e. an enzymatic cascade within the blood serum; the activated components of the complement system are then incorporated into the membranes of target cells. The final outcome of complement activation consists in generation of pores for subsequent enzymatic lysis of the target cell. In addition, complement-coated cancer cells are easier to absorb by other immune cells in the course of phagocytosis.

Despite the revolutionary impact of anti-neoplastic antibodies on cancer therapy, not all patients are capable of achieving clinical response; in addition, treatment resistance may develop in some of the patients. One of the reasons behind the resistance to therapeutic agents exerting their action with the complicity of the complement system consists in the complement system inhibitors being overexpressed on the surfaces of cancer cells and the depletion of the complement protein pool due to antibodymediated activation, resulting in insufficient quantities of these proteins being available with subsequent drug doses.

The invention relates to point mutants of human proteins forming the complement system convertases C3 and C5, namely complement factor B and protein C2 proteins, for use in enhancing the cytotoxic activity of antibodies in the treatment of cancer.

A solution is proposed as part of this invention consisting of anti-neoplastic antibodies being supplemented by mutated human proteins. Said proteins constitute parts of enzyme (convertase) complexes responsible for the proteolysis of complement protein C3 and C5 to their respective active fragments C3b and C5b. Example proteins of this type include the B factor which is a building block within the structures of convertases of the alternative complement pathway and the C2 protein which plays a similar role in the classic complement pathway. Relevant mutations within these proteins

facilitate counteraction of complement inhibitors, thus enhancing the activity and half-life of enzymatic complexes for optimum production of C3b and C5b fragments for a maximum cytotoxic effect.

When added to CD20-positive lymphoma cells and specific anti-CD20 antibodies within human serum in an *in vitro* test, the mutants of complement protein C2 and factor B afforded a complete lysis of cells moderately susceptible to anti-CD20 antibodies. In addition, protein C2 mutants led to a statistically significant increase in the apoptotic rate of cells with poor susceptibility to anti-CD20 antibodies. Importantly, it is not possible to enhance the cytotoxic effect in cell lines characterized by moderate or low susceptibility to anti-CD20 antibodies by increasing the anti-CD20 antibody levels without the addition of mutant proteins. Moreover, *in vitro* studies showed that the addition of mutant complement proteins C2 to serum samples collected from patients treated with rituximab prior to each infusion of the drug (administered in 4-week intervals) facilitates effective use of residual drug remaining after previous administration and induce its cytotoxic effect without the need of drug replenishment.

Keywords

Complement system, anti-CD20 antibodies, factor B, complement protein C2, complement inhibitors

Legal status of the product

Patent Office of the Republic of Poland, patent application

Complement factor B and protein C2 point mutants, a method to enhance the activity of anti-neoplastic antibodies, pharmaceutical composition containing the point mutants and the use thereof.

Application year: 2021

Patent no.: Pat.237868

Patentee:

Medical University of Gdańsk

International application (PCT):

Complement factor B and protein C2 point mutants, a method to enhance the activity of anti-neoplastic antibodies, pharmaceutical composition containing the point mutants and the use thereof.

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Complement factor B and protein C2 point mutants, a method to enhance the activity of anti-neoplastic antibodies, pharmaceutical composition containing the point mutants and the use thereof.

US:

Application year: 2020

Patent no.: US17045058

Patentee:

Medical University of Gdańsk

EU:

Application year: 2020

Patent no.:

EP199999726774.3

Patentee:

Medical University of Gdańsk

Product proposed

The invention relates to point mutants of human proteins forming the complement system convertases C3 and C5, namely complement factor B and protein C2 proteins, for use in enhancing the cytotoxic activity of antibodies in the treatment of cancer.

Research funding obtained to date

The project has been financed from the:

- HARMONIA grant program of the National Science Center
- SONATA BIS 4 grant program of the National Science Center
- PRELUDNIUM 15 grant program of the National Science Center

Market and competition analysis

Inhibition of convertases is one of the main mechanisms responsible for the resistance of cancer cells to the complement system-based therapies. The search for model anti-tumor resistance mechanisms consists in silencing the expression of complement inhibitors by means of transfection with siRNA constructs of SNA constructs or in the use of bispecific antibodies capable of recognizing the

antigenic determinants of tumor cells while simultaneously blocking the complement inhibitors (e.g. CD55).

A disadvantage and limitation of the former solution consists in the necessity to accurately deliver the siRNA constructs into the tumor cells due to the widespread expression of complement inhibitors within the human body. The latter approach is limited to simultaneous inhibition of only one complement inhibitor as determined by antibody specificity while several different types of inhibitors are produced or captured from the environment by the cancer cells.

Advantages of the proposed product

The invention consists in the use of potentially pathogenic proteins as immunotherapy supplements for significant enhancement of the cytotoxic activity of immunotherapeutic agents. Supplementation with appropriately mutated complement protein Cd and factor B enhances the cytotoxic activity of anti-neoplastic antibodies exerted via the complement system while simultaneously replenishing the pool of consumable components of the complement system required to sustain the cytotoxic potential of the human serum.