DESCRIPTION FOR THE GENERAL PUBLIC

Clostridium difficile is the cause of one of the most common hospital-acquired infections, which is antibiotic-associated diarrhea, which may develop pseudomembranous colitis and toxic megacolon, which in many cases leads to death of the patient. *Clostridium difficile* infection (CDI) is particularly dangerous to the elderly patients leading to the highest mortality rate. In Poland, the incidence of *C. difficile* bacterial infections is higher than the european average and is 76 cases per 10 000 hospital admissions. Mortality rate for patients diagnosed with pseudomembranous colitis, is 6-30%, and also maintain at a high level in patients who have not been previously diagnosed with pseudomembranous colitis. In the USA, the cost of treating one patient infected with *C. difficile* is 2 000-72 000$, and the cost of fight against diarrhea caused by *C. difficile* infection annually is around 1.1 billion dollars. It is worth noting that more and more patients are acquire the infection outside the medical care facilities.

The infection is usually caused as a result of antibiotic treatment, which disturbs the natural human intestinal microflora and creates favorable conditions for colonization of the gut. Treatment starts with discontinuation of antibiotic aministration that was applied before the infection, which usually helps in 23% of patients. If there is no improvement antibiotics in the form of metronidazole or vancomycin are used.

C. difficile has numerous virulence factors, which makes this pathogen difficult to control. Many research groups are working on new methods to combat this infection which would be an alternative to antibiotics. There is no commercially available vaccine to prevent infection. There is an ongoing phase III clinical trial of a vaccine based on the best known virulence components of *C. difficile* which are toxins. A and B toxins fragments as antigens evoke a systemic response which neutralizes the effect of toxins, but the induction of a local response in the gut is very poor. Protective effects of vaccines based on the toxins A and B have been proven in healthy volunteers. It was showed that the vaccine is safe and immunogenic, and that prevents relapse in people with recurrent CDI. However, there are reports of high variability of toxins produced by the bacteria, especially in the area of the strongest immune response excitation C-terminus of these proteins. It is not known whether the active production of anti-toxins antibodies will eliminate carriage and transmission of infection between patients. One of the tested solution is to use the immunogenic properties of the proteins constituting the surface layer of the pathogen as a vaccine components. One of the methods of preventing *C. difficile* infections may be specific antibodies directed against molecules involved in the adhesion process. Blocking of the binding sites on the surface of bacteria would prevent the adhesion of pathogens to the epithelial cells of the patient and thus stopping the infection development. There are many reported immunomodulatory components of the surface of *C. difficile* that can be used in vaccines. Some of them have conserved regions typical for many types of *C. difficile*.

The aim of the project is to find the locations of antibody binding to proteins on the cell surface of *Clostridium difficile.* Locating these regions will in the future allow us to design an effective vaccine. A specific epitope will then be coupled to a carrier protein in order to enhance the immunogenicity. The resulting conjugate will be tested in combination with novel nanoadjuvant *in vivo* and *in vitro*. Nanoadjuvant has antigen-protective properties and enhances the immune response in the vaccinated mice**. The result of the project will be well described formulation in the form of immunogenic conjugate and nanoadjuvant ready for further testing which may in the future result in the first Polish vaccine against *Clostridium difficile*.**

The tasks foreseen in the project include:

a) Screening for the bacterial proteins that are strongly bound by human antibodies using one of the techniques of epitope mapping.
b) Preparation of a conjugate of the synthetic peptide and carrier protein.
c) The combination of conjugate and nanoadjuvant.
d) Testing the biologic properties of the formulation *in vitro* (using cell lines).
e) Testing the protective properties of the formulation *in vivo* (using an animal model).