Evaluation of tumor vasculature normalisation on effectiveness of the therapeutic melanoma vaccines in mice models.

Melanoma in a phase of dissemination is still an incurable disease. Despite recent breakthroughs in targeting melanoma genes mutations, or controlling own anticancer defense mechanisms (immunotherapy) did not manage to eliminate melanoma. Although therapeutic cancer vaccines effectively activate defense cells against the tumor, the tumor cells may inactivate immune cells. This is due to the ability of the tumor, considered today as perfectly functioning organ to "turn off" the human defense mechanisms. This occurs as a result of the setting intra-tumor hypoxia that activates a chain of events, which include inter alia pathological blood vessel formation (neo-angiogenesis). The vessels are leaky, twisted, let in all kinds of cells, and do not provide adequate supply of oxygen, which exacerbates hypoxia. The tumor forms specific microenvironment. The tumor cells destroy armed immune cells, what paralyzes anti-tumor defense. Tumors grow further, metastasize and finally lead to the death of the patient. Particularly resistant to any kind of therapy is the fraction of tumor cells referred to as cancer stem cells (CSC), or initiating cancer cells, which drive tumor progression.

The main objective of the planned project is a combination of specific activation of the anti-melanoma immune responses with therapeutic melanoma vaccines constructed on the basis of CSC, with agents normalizing tumor hypoxia (normoxia) in a melanoma mouse model. The secondary aim is to understand the molecular mechanisms underlying the interaction between tumor and a host, and the role of CSC and vasculature play in the process of modeling the tumor microenvironment. The specific objectives are to compare the results of melanoma vaccine mono-therapy with results of the drug elevating the concentration of oxygen in the tumor (normoxia) and the combination of both therapies. In addition, the mechanisms leading to the elimination of tumor cells with particular reference to CSC will be analyzed. The tumor oxygen concentration, "repair" of pathological vessels, the expression on tumor and defense cells molecules that kill immune cells will be monitored. For analysis "the gel tumors" will be used as a model. The certain gel substances at 4 degrees C are in liquid form, while at 37°C they gel. The melanoma vaccine cells or wild-type melanoma cells will be dispensed in liquid form of the gel substance and injected to mice under the skin, where they will form a gel tumors. They in turn will be infiltrated by immune cells, which are then isolated from the gel nodules and assessed. This will allow analyzes of mechanisms involved in activation of the immune system and elimination of tumor cells. In order to assess the cellular composition of the tumor microenvironment, the wild-type melanoma cells will be injected subcutaneously (without the gel). After a time, when they reach the right size, they will be removed, fixed, cut into slices, stained and evaluated under a microscope.

Assembly of these data will help to design new, more effective biological therapies and active small molecules. We have more and more data indicating that cancer can resist treatment with one drug and it is necessary to combine several together, especially with distinct mechanisms of action. We expect, therefore, that the planned research project will provide us with the basic knowledge, which in the future will be applied in practice.

The project will be implemented in cooperation with Prof. C. Kieda and Prof. JM. Lehn (France). Prof. LM Lehn is the inventor of a drug normalizing hypoxia. Prof. Kieda and Prof. Lehn are co-inventors of new derivatives of the basic drug.