ImmunoTools IT-Box-139 Award 2012

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Bispecific antibody for glioblastoma immunotherapy

Bispecific antibodies are molecules that can bind to two different epitopes of antigens and can be used for cellular cancer therapy through retargeting of effector cells. Bispecific T-cell engaging antibodies have two arms, one arm specific for the target antigen and the other specific for the CD3 complex, thereby directing the cytotoxic T cells towards the target cell.

In my project, I have constructed a bispecific antibody with one arm specific for a cancer stem cell antigen and the other specific for the CD3 complex. Variable fragments of these two different genes have been linked with linker sequences to form a bispecific single chain variable fragment (bs-scFv). Our aim is to study the effectiveness of this bs-scFv *in vitro* and *in vivo* with glioblastoma tumor cell lines and also with primary cancer stem cells.

The bs-scFv was cloned in a bacterial expression system, and the protein was purified by Ni-affinity chromatography. The specific target binding properties of the antibody were checked in: a) glioma cell line U251 which were lentivirally transduced to overexpress the cancer stem cell marker, b) in primary cancer stem cells and also in 3) the CD3+ T cell line Jurkat. The specific cytotoxic properties of the antibody were checked *in vitro* in 24 and 48 hour assays by microscopy and FACS.
Currently we are testing the effect of the antibody to effectively recruit T cells and eliminate U251 tumors and primary cancer stem cell tumors in subcutaneous and orthotopic tumor models in immunodeficient mice. We have also submitted a patent application for this novel bispecific antibody construct.

Use of antibodies from ImmunoTools IT box 139:
For our studies, we would require many of the ImmunoTools antibodies, especially the T-cell specific ones. Since we are working on T-cell engaging antibodies, we constantly use the CD3, CD4 and CD8 antibodies in *in vitro* and *in vivo* studies. We are also working on the engraftment of human peripheral blood mononuclear cells (PBMCs) in immunodeficient mice. Such mouse models would be useful in studying the immune resonses *in vivo*. After the administration of the PBMCs, the mice are regularly analysed for the T-cell markers **CD3**, **CD4**, **CD8**, **CD45RA**, **CD45RO**, **CD62L** by FACS. To investigate whether the mice are repopulated with the various human leukocyte subpopulations, we need the **CD11b** antibody to identify the macrophage population, CD11c antibody to identify the dendritic cell population, CD14 antibody for the monocyte population and **CD19** antibody to discriminate the B cell population. Antibodies specific for the T cell activation markers **CD25** and **CD69**

are used regularly in our *in vitro* assays to study the T-cell recruiting and activating action of the bispecific antibody. We also use Annexin V to study the specific cytotoxicity of the target cells with our diabody. In several of these experiments, we use Control IgG1, IgG2A and IgG2B antibodies. In the future, we also plan to construct NK-cell engaging antibodies and we plan to use antibodies against the CD16 and CD56 markers for that purpose.