SELECTION OF RECOMBINANT ANTIBODIES FOR DIAGNOSIS AND TREATMENT OF NATURALLY OCCURING CANINE LYMPHOMA - Abstract

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Lymphomas are a heterogeneous group of malignancies, characterized by malignant lymphoid cell proliferation, representing 83% of all canine hematopoietic tumors. Canine lymphoma requires prompt chemotherapy but cure is rarely achieved. Within this context, there is a pressing need to develop novel therapeutic strategies. Monoclonal antibodies (mAbs) against antigens on cancer cells offer an alternative tumor-selective treatment approach. However, most mAbs are not sufficiently potent to be therapeutically active on their own. Antibody-drug-conjugates (ADCs) seem to be the solution to overcome the major problems related to cytotoxic drugs and mAbs when used on their own. As a result, pharmaceuticals companies and academia are intensely focused in the development of highly specific and potent ADCs. Over the past decade, we have been showing the great potential of rabbit derived single domain antibodies (sdAbs) for therapeutic applications. Therefore, we are exploring the properties of sdAbs to develop improved ADCs and to contribute to a translational of new cancer treatments for canine lymphomas.

Currently a canine lymphoma biobank was already constructed from lymph nodes collected from dogs followed at FMV Teaching Hospital. Three rabbits were immunized with primary cells obtained from the canine lymphoma biobank. The immune response was monitored by serum analysis (ELISA and FACS). After the final boost, rabbits were sacrificed and spleen and bone marrow were harvest for total RNA isolation and cDNA synthesis. cDNA was used to construct sdAb VH and VL libraries. The results obtained showed that all rabbits presented a selective and specific immune response against primary canine lymphoma cells and against CLBL-1, a canine lymphoma B-cell line used as control. To validate potential targets for immunotherapy recognized by antibodies presented in rabbit serum, experiments were also performed by SDS-PAGE and Western Blot. Binding activity of the polyclonal sera against total protein extracts from lymphoma primary cells, CLBL-1 and PBMC (control) were analyzed and potential receptors were identified. Results showed also a high CD20 expression in canine lymphoma primary cells and CLBL-1, validating this receptor as a potential cancer target and the presence of a significant anti-CD20 antibody titer in rabbit polyclonal sera. To be able to select for highly specific ADCs, a functional cell phage display selection was performed to identify antibodies against lymphoma-associated antigens. In parallel, we are also testing a library of pharmacologically active compounds on lymphoma cell lines to choose the most potent drug to conjugate with our lead antibody candidates.

With this combinatorial strategy we expect to develop an alternative therapeutically approach by developing a selective and effective new ADC anti-cancer drug for treatment of B-cell malignancies. Moreover, due to their characteristics the selected antibody fragments can also be explored as biomarkers in the diagnosis field of canine lymphoma.

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