Immunotherapy based on monoclonal antibodies (mAbs) targeting cancer cells is now developed as a valid approach to treat cancer. Suppressive mechanisms in immune system are normal for maintaining immune tolerance to self-antigens. However, these suppressive regulatory pathways are also considered as one of the main reasons for the failure of cancer immunotherapies because they induce peripheral tolerance of tumor-specific immune responses and allow tumor growth. Regulatory T cells have been revealed as the most important population of immune suppressors, and their depletion has been reported to enhance antitumor immune responses. CTLA-4 was reported as a critical target for regulatory T cell function when blockade of CTLA-4 mediated signals has been suggested as a possible strategy to treat cancers. The first anti-CTLA-4 human mAb, ipilimumab, was approved in 2011 by the FDA for use in metastatic melanoma. Success for ipilimumab was reported in a large phase III clinical trial involving patients with metastatic melanoma, who had undergone previous therapies.

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**Abstract**

Immune checkpoint blockade therapy is now a mainstay of cancer therapy. Among the most promising targets explored are cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), and their respective ligands. In this study, we designed and characterized the anti-CTLA-4 mAb 3D3 to explore its capacity to enhance immune responses against tumors. We evaluated the efficacy of 3D3 against several tumor models and established the safety and immunomodulatory profiles of the mAb in vivo and in vitro.

**Material and Methods**

In vivo experiments: mice were obtained from Charles River (France). Animals were orthotopically or subcutaneously injected with syngenic tumor cell lines. They received repeated intraperitoneal injections of antibody directed against CTLA-4. During the course of the experiment, animals were terminated under anesthesia when they displayed significant signs of physiological changes. Animal housing and experimental procedures were performed according to the French and European Regulations and HRC Guide for the Care and Use of Laboratory Animals. Animal facility is authorized by the French authorities (Agreement N° B21231011EA). All procedures using animals were submitted to the Animal Care and Use Committee of Oncodesign (Orsay) and agreed by French authorities (CMREE agreement N° 91) [1, 2, 3].

Immune cell detection in mice tissues

The tumor and tumor draining lymph nodes were collected for subsequent FACS analysis to study the immune response in mice. Cells suspensions were prepared from tissues either by mechanical dissociation or by enzymatic digestion. The antigens associated antibodies used for FACS analysis are described in Table 2. The stained cells were analyzed with a FACSCalibur® flow cytometer (BD Biosciences) equipped with 3 excitation lasers at wavelengths 488, 633, and 780 nm.

**Conclusions and perspectives**

A large panel of syngeneic mouse models is available for evaluation of immune checkpoint inhibitors such as CTLA-4 targeting therapies. In designing this panel, we chose tumor models known as part of the standard portfolio of CTLA-4 targeting therapies, allowing investigation of antitumor effect of combined treatments. The immunohistochemistry is the main method to evidence biomarkers of response to CTLA-4 targeting therapies and immunohistochemical analyses would be used as complementary readout. New available RNA sequencing data will help to understand how genomics information could be used as biomarker of response for these new therapies.

**Results**

Lung metastasis number from mice bearing 4T1 murine breast tumors. Mice were randomized based on tumor volume and treated IP with mAb against CTLA-4 at 10 mg/kg/inj. Individual and median values are from 5 mice per group. Median values for each group are also presented.