586 INTRATUMORAL DNA-BASED GENE TRANSFER AS AN EFFICIENT DELIVERY APPROACH TO COMBINE CHECKPOINT-INHIBITING ANTIBODIES WITH INTERLEUKIN 12

Liesl Jacobs*,Elien De Smidt, Nick Geukens, Kevin Hollevoet, Paul Declerck. *KU Leuven – University of Leuven, Leuven, Belgium*

Background Checkpoint inhibitors have demonstrated clinical benefit for several types of cancer, but still a large proportion of patients do not respond to treatment. To improve response rates, many combination therapies are currently under clinical evaluation. One such example is the combination of anti-PD-1 monoclonal antibodies with intratumoral gene transfer of plasmid-based interleukin 12 (IL-12). Local expression of the cytokine IL-12 has been shown to increase immune cell infiltration in cold tumors, which can make them more responsive to anti-PD-1 antibodies.[1] The current study evaluates the efficacy of simultaneous delivery of checkpoint-inhibiting antibodies and IL-12 by intratumoral gene transfer. We recently demonstrated that intratumoral delivery of plasmid-based checkpoint inhibitors yielded systemic anti-tumor responses in a mouse tumor model, with only limited systemic antibody exposure and therefore improved biosafety.[2]

Methods C57BL/6J mice bearing a subcutaneous syngeneic MC38 tumor received a single intratumoral injection of plasmid DNA followed by in vivo electroporation. DNA-based IL-12 (p(IL-12), 2.5 µg) was administered alone or in combination with a DNA-based anti-PD-1 antibody (p(aPD-1), 60 µg) and/or DNA-based anti-CTLA-4 antibody (p(aCTLA-4), 60 µg). Abscopal effects were studied in mice bearing two contralateral tumors, of which only one received therapy.

Results The combined intratumoral delivery of p(IL-12) and p(aPD-1) resulted in 10% complete responders, in contrast to no complete tumor regressions with each individual treatment. Yet, differences in tumor growth or survival did not reach statistical significance between these groups. To improve anti-tumor efficacy, the combined gene transfer was expanded with a second DNA-based checkpoint inhibitor, p(aCTLA-4). While intratumoral delivery of this triple combination also led to 10% complete regressions, the response did result in significant tumor growth delay compared to p(IL-12) alone ($p<0.05$) and the combination of both checkpoint inhibitors ($p<0.01$). Moreover, in a dual MC38 tumor model, the triple combination enabled significant abscopal effects compared to untreated mice ($p<0.01$), which was not the case for the other treatments.

Conclusions This study demonstrates that intratumoral DNA-based gene transfer can be applied to efficiently combine different immunotherapeutics. This approach allows simplification of the treatment schedule, addresses the complex production of conventional protein-based therapeutics, and enables local drug expression, thereby minimizing systemic exposure and subsequent adverse events. Ongoing studies focus on the further validation of combined intratumoral delivery of plasmid-based checkpoint inhibitors and IL-12, by investigating the effect on tumor-infiltrating and peripheral immune cells as well as through evaluation of the triple combination in other tumor models.

Ethics Approval This study was approved by the KU Leuven Animal Ethics Committee, approval number P130/2017.

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587 TUMOR-ACTIVATED FC-ENGINEERED ANTI-CTLA-4 MONOCLONAL ANTIBODY, XTX101, DEMONSTRATES TUMOR-SELECTIVE PD AND EFFICACY IN PRECLINICAL MODELS

[1]Kurt Jenkins*, [1]Parker Johnson, [1]Minjie Zhang, [1]Wilson Guzman, [1]Ugur Eskiocak, [1]Megan McLaughlin, [1]Caitlin O'Toole, [1]Magali Pederzoli-Ribeil, [2]Miso Park, [2]John Williams, [3]Margaret Karow, [1]Jennifer O'Neil, [1]Timothy Clackson, [1]Ronan O'Hagan. *[1]Xilio Therapeutics, Waltham, MA, USA; [2]Beckman Research Institute, City of Hope, Duarte, CA, USA; [3]Tentarix Biotherapeutics, La Jolla, CA, USA*

Background The clinical benefit of CTLA-4 blockade to cancer patients has been well established. However, the promising antitumor activity shown by anti-CTLA-4 monoclonal antibodies (mAb) has been limited by the occurrence of immune-mediated adverse reactions, especially when CTLA-4 inhibition is used in combination with anti-PD-1 therapy. These dose-limiting toxicities restrict the therapeutic use of CTLA-4 blockade. To overcome these limitations, we have developed a potent anti-CTLA-4 antibody that is selectively active in the tumor microenvironment (TME). This antibody is engineered with an Fc region for enhanced FcγR binding and peptides that mask antigen-binding regions. The masking peptides are designed to be selectively cleaved and released by proteases that are more active in the TME, resulting in restoration of full activity of the antibody in the TME.

Methods A novel, fully-humanized anti-huCTLA-4 mAb was shown to bind human CTLA-4 with improved affinity compared to ipilimumab, as measured by SPR. Engineering of the Fc region enhanced FcγR binding and ADCC function. In addition, CDR-binding peptides identified by phage display were covalently linked to the antibody using a protease-sensitive polypeptide linker. This engineered anti-CTLA-4 antibody (XTX101) showed protease-dependent binding to CTLA-4 both with recombinant and tumor tissue derived proteases.

Results XTX101 demonstrated a 100-fold reduction in binding to human CTLA-4 by ELISA, compared to the non-masked antibody. Incubation with recombinant protease led to cleavage and release of the masking peptides and restored full binding to CTLA-4. Similarly, in vitro ADCC activity was impaired by masking and restored in a protease-dependent manner. SEB-stimulated human PMBCs were minimally responsive in vitro to XTX101, whereas PBMCs treated with proteolytically-activated XTX101 exhibited robust activation of T cell function. In human CTLA-4 knock-in mice with syngeneic MB49 tumors, XTX101 treatment led to complete tumor regression, enhanced CD8+ T cell proliferation, and depletion of tumor Tregs in the TME. By contrast, XTX101 had minimal pharmacodynamic effects in the periphery. In addition, XTX101 is effectively activated in culture supernatants from human solid tumor explants obtained from a broad range of tumor types.

Conclusions XTX101 is a tumor-selective anti-CTLA-4 mAb capable of: 1) effective CTLA-4 blockade, 2) depletion of intratumoral Tregs through enhanced antibody-dependent cellular cytotoxicity (ADCC) function, 3) minimization of systemic immune cell activation, and 4) potent anti-tumor activity. These pre-clinical data support the further evaluation of XTX101 in clinical studies.

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