#2499

LTX-315, an oncolytic peptide, increases anticancer immunity mediated by CTLA4 blockade in an interleukin-2 receptor beta-chain-dependent manner

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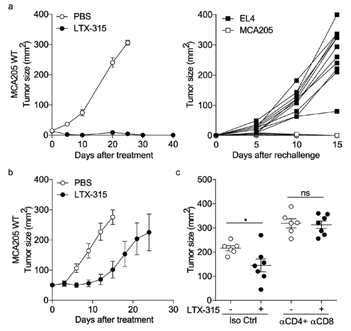
ABSTRACT

Intratumoral immunotherapies aim at reducing local immunosuppression as well as reinstating and enhancing systemic anticancer T cell functions without inducing side effects. LTX-315 is a first-in-class oncolytic peptide–based local immunotherapy that meets these criteria by inducing a type of malignant cell death that elicits anticancer immune responses. Here, we show that LTX-315 rapidly reprograms the tumor microenvironment by decreasing the local abundance of immunosuppressive Tregs and myeloid derived-suppressor cells and by increasing the frequency of polyfunctional Th1/Tc1 cells with a concomitant increase in CTLA4 and drop in PD-1 expression levels. Logically, in tumors that were resistant to intratumoral or systemic CTLA4 blockade, subsequent local inoculation of LTX-315 cured the animals or caused tumor regressions with abscopal effects. This synergistic interaction between CTLA4 blockade and LTX-315 was reduced upon blockade of the β-chain of the interleukin-2 receptor (CD122). This preclinical study provides a strong rationale for administering the oncolytic peptide LTX-315 to patients who are receiving treatment with the CTLA4 blocking antibody ipilimumab.
(Accepted by *Cell Death and Differentiation*)

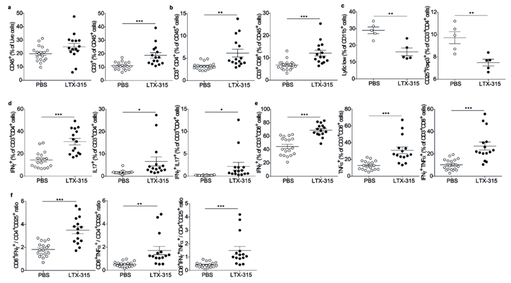
RESULTS

Fig. 1: **LTX-315 induced T-cell-dependent complete tumor regression**



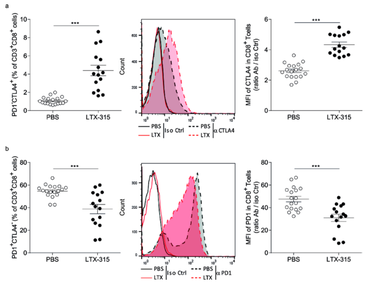
a. Three intratumoral daily consecutive injections of 300 µg of LTX-315 were performed in 20-25 mm² established MCA205 sarcomas evolving in C57BL/6 mice. After a rechallenge of tumor free mice with the MTD of EL4 or MCA205, the tumor growth were monitored over time (right panel). **b.** Idem as in a. but treating bigger tumors of 35-40 mm² size. **c.** Effects of depleting antibodies targeting CD4 and CD8a molecules on the tumoricidal activity of LTX-315 Student *t*-test : $*p < 0.05$, ns : not significant.

Fig. 2: **LTX-315 increased the intratumoral ratio of cytotoxic T lymphocytes (CTLs) over Tregs**



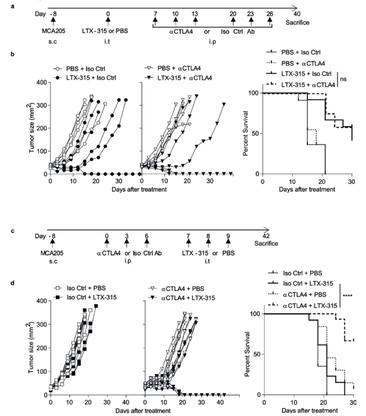
Flow cytometry determination of CD45⁺ leukocytes (**a**, left panel) in the gate of live cells after dissociation of fresh MCA205 sarcoma seven days post-LTX-315 (versus PBS), as well as CD3⁺ T cells (**a**, right panel), CD4⁺ T, CD8⁺ T cells in the CD45⁺ live cells (**b**), of CD4⁺ Treg defined as CD25⁺FoxP3⁺ cells (**c**, right panel) and MDSC defined as CD11b⁺Ly6C^{low} cells in the CD45⁺ gate (**c**, left panel), of cytokine producing CD4⁺ T cells in the CD4⁺ T cell gate (**d**), of cytokine producing CD8⁺ T cells in the CD8⁺ T cell gate (**e**). The ratio between Tc1 cells over Treg was calculated considering either IFNγ⁺ or TNFα⁺ or double positive CD8⁺ TILs (**f**, three panels). Student *t*-test: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns : not significant.

Fig. 3: **LTX-315 differentially modulated immune checkpoint receptors in TILs**



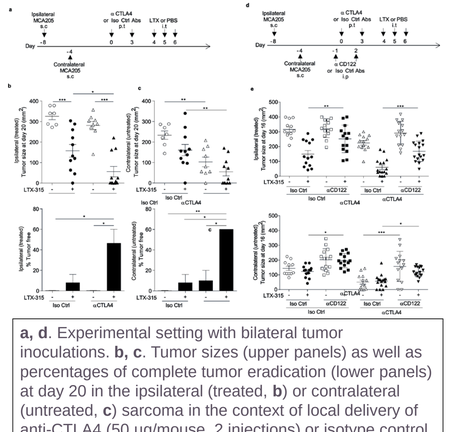
Flow cytometry determination of CTLA4 (**a**) or PD-1 (**b**) molecules on CD8⁺ TILs at day 7 post-LTX-315 (versus PBS) local injections. Percentages of positive cells (left panels) or mean fluorescence intensities (MFI) (middle and right panels) shown in overlay graphs or ratios of MFI between specific versus isotype control mAb are depicted. Each dot represented one mouse data and 2 experiments were gathered in each graph. Student *t*-test : $***p < 0.001$.

Fig 4: **Impact on the scheduling of LTX-315 and CTLA-4 blockade combination regimen**



a, c. Experimental setting. **b**. Tumor growth kinetics in the presence (right panel) or absence (left panel) of anti-CTLA4 (100 µg/mouse, 3 injections weekly for two weeks) or isotype control mAb injected **after** LTX-315. **d**. Tumor growth kinetics in the presence (right panel) or absence (left panel) of anti-CTLA4 (100 µg/mouse, 3 injections) or isotype control mAb injected **before** LTX-315. Comparison of Kaplan-Meier survival curves were performed using the Log-rank Mantel-Cox test : $**p < 0.01$, $****p < 0.0001$, ns, not significant.

Fig 5: **Role of CD122 in the synergistic effect of CTLA-4 blockade and LTX-315**



a, d. Experimental setting with bilateral tumor inoculations. **b, c**. Tumor sizes (upper panels) as well as percentages of complete tumor eradication (lower panels) at day 20 in the ipsilateral (treated, **b**) or contralateral (untreated, **c**) sarcoma in the context of local delivery of anti-CTLA4 (50 µg/mouse, 2 injections) or isotype control mAb injected before LTX-315. **e**. Tumor sizes at day 16 in the ipsilateral (treated, upper palne) or contralateral (untreated, lower panel) sarcoma in the context of local delivery of anti-CTLA4 (50 µg/mouse, 2 injections) or isotype control mAb injected before LTX-315 during a systemic neutralization of CD122 with specific mAb or isotype control mAb performed the day before CTLA4 blockade. One-way ANOVA followed by Tukey test : $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns : not significant. Comparison of Kaplan-Meier survival curves were performed using the Log-rank Mantel-Cox test : $* p < 0.05$, $***p < 0.001$, $****p < 0.0001$, ns, not significant.

CONCLUSION

LTX-315 is a cationic peptide with oncolytic properties following intralesional administration in mice and humans. It mediates its anticancer activity against a wide variety of histological tumor types in a T cell-dependent manner by inducing cell death endowed with immunogenic properties. In this preclinical study, we found that LTX-315 can be active against sarcomas that poorly respond to CTLA4 blockade, and that the sequential combination of anti-CTLA4 mAb followed by LTX-315 is synergistic, either using systemic or local delivery of anti-CTLA4 mAb, with a mechanism involving IL-2β receptor.

In conclusion, this work demonstrates that LTX-315 has the potential to stimulate therapeutically-relevant anticancer immune responses in several preclinical models. One of the salient features of LTX-315 is that it can be administered locally, by injection into malignant lesions to locally stimulate anticancer immune responses that suppress the growth of distant tumors, and hence mediate abscopal responses. Moreover, we have accumulated data suggesting that LTX-315 can be advantageously combined with CTLA4 blockade, in particular if CTLA4 blockade precedes or is concomitant to the local administration of LTX-315.