Combining Local Immunotoxins Targeting Mesothelin with CTLA-4 Blockade Synergistically Eradicates Murine Cancer by Promoting Anti-Cancer Immunity

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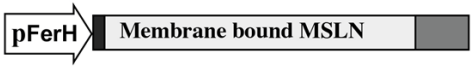
SUPPLEMENTARY INFORMATION

Supplementary figure S1

A

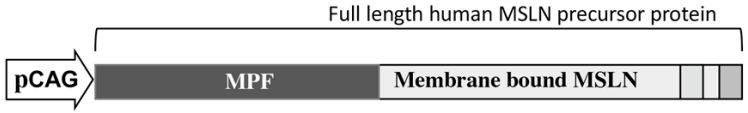


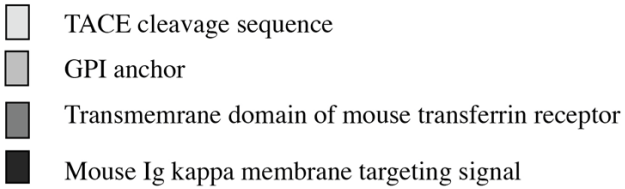
B

METDTLLLWVLLLWVPGSTG*EVEKTACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIP*
FTYEQLDVLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLEVNKGHEMSPQV
ATLIDRFVKGRGQLDKDTLDTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKA
RLAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKLLGPHVE
*GLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNG*LCFAAIALVIFFLIGFMSGYLGYG

Bold letters: Murine IgK leader domain.
Italics: Membrane bound human mesothelin domain.
Underlined: Murine transferrin receptor transmembrane domain.

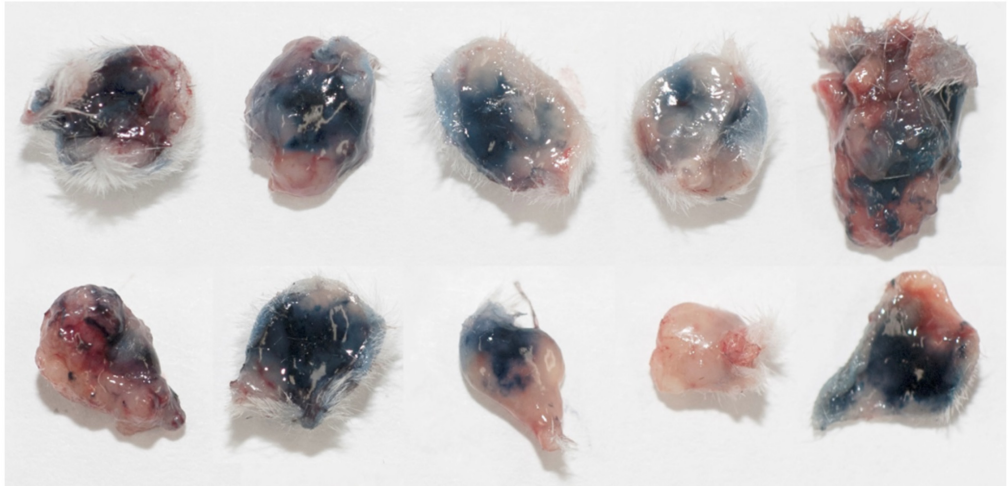
C





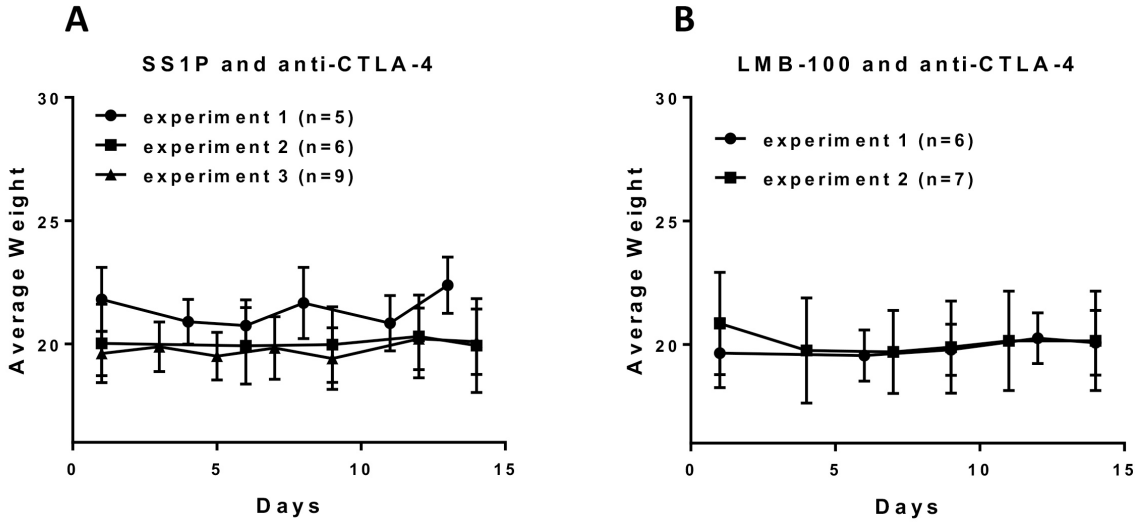
Description of DNA constructs. A-B. Diagram of the DNA construct used to generate 66C14-M cell line (A) and its sequence (B). **C.** Diagram of the DNA construct used to generate human mesothelin transgenic mice.

Supplementary figure S2



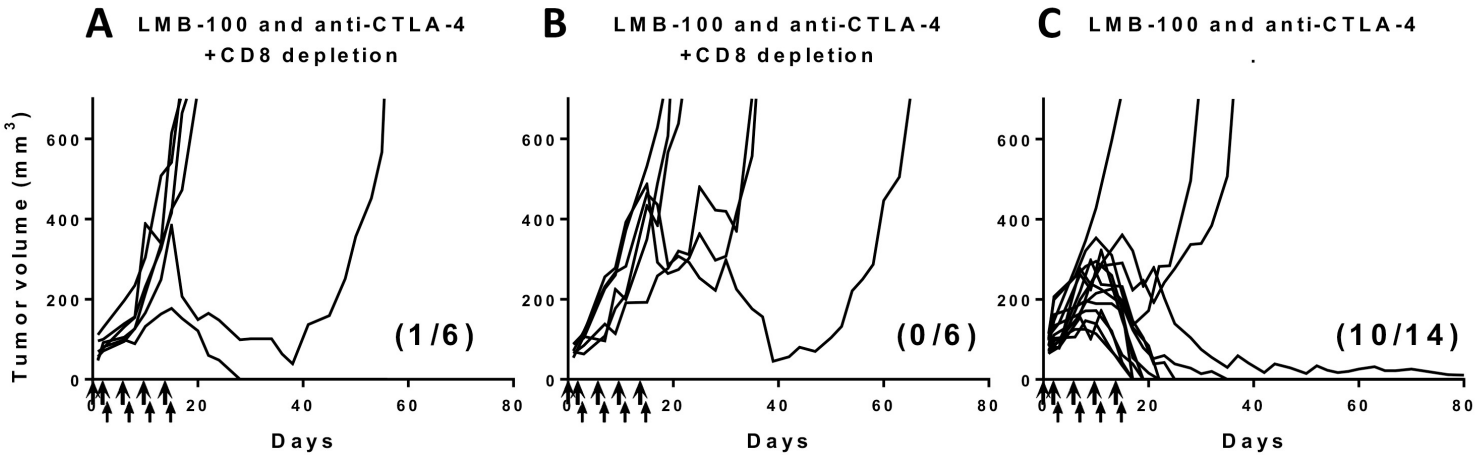
Distribution of intra-tumoral injected trypan blue. Five 66C14 tumors (72 to 117mm^3) were injected with 30 µl trypan blue . One hour after the injection, tumors were harvested, cut to half and photographed.

Supplementary figure S3



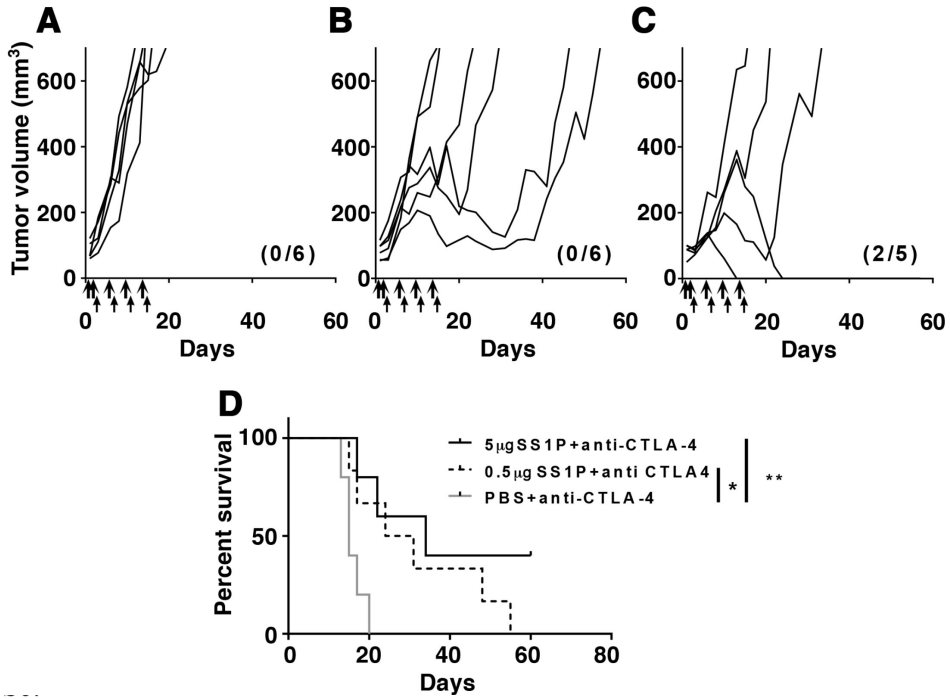
Combination of intra-tumoral immunotoxins with anti-CTLA-4 is well tolerated by the mice. Shown are average body weights measured over the course of treatment with 25µg/dose anti-CTLA-4 and **(A)** 5µg /dose SS1P or **(B)** 30µg /dose LMB-100

Supplementary figure S4



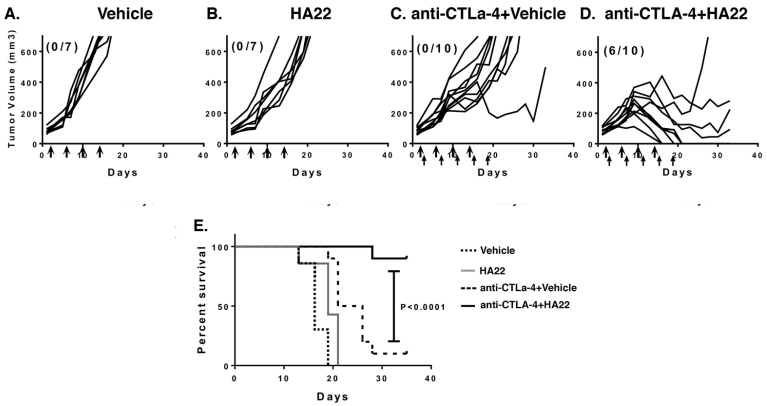
Anti-tumor effect of anti-CTLA-4 and LMB-100 depends on CD8+ cells. A-B Individual growth curves of 66C14-M tumors treated with anti-CTLA-4 (thin arrows), LMB-100 (thick arrows) and **(A)** 200 µg or **(B)** 100 µg anti-CD8 antibodies. **(C)** shows pooled data from two experiments. The number of mice in complete remission and the total mice per group is shown in parentheses.

Supplementary figure S5



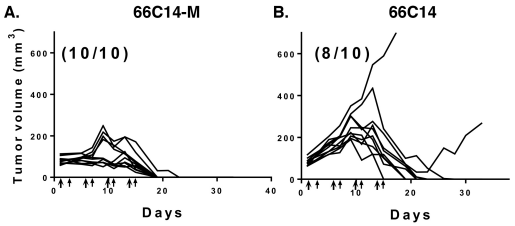
High dose of SS1P is needed for induction of complete remission. A-B. Individual growth curves of 66C14-M tumors treated with (**A**) anti-CTLA-4 (thin arrows) and PBS (thick arrows), (**B**) anti-CTLA-4 (thin arrows) and 0.5 μg SS1P (thick arrows) or (**C**) anti-CTLA-4 (thin arrows) and 5 μg SS1P (thick arrows). (**D**) Survival of mice described in (**A-C**). * $P<0.05$, ** $P<0.01$.

Supplementary figure S6A



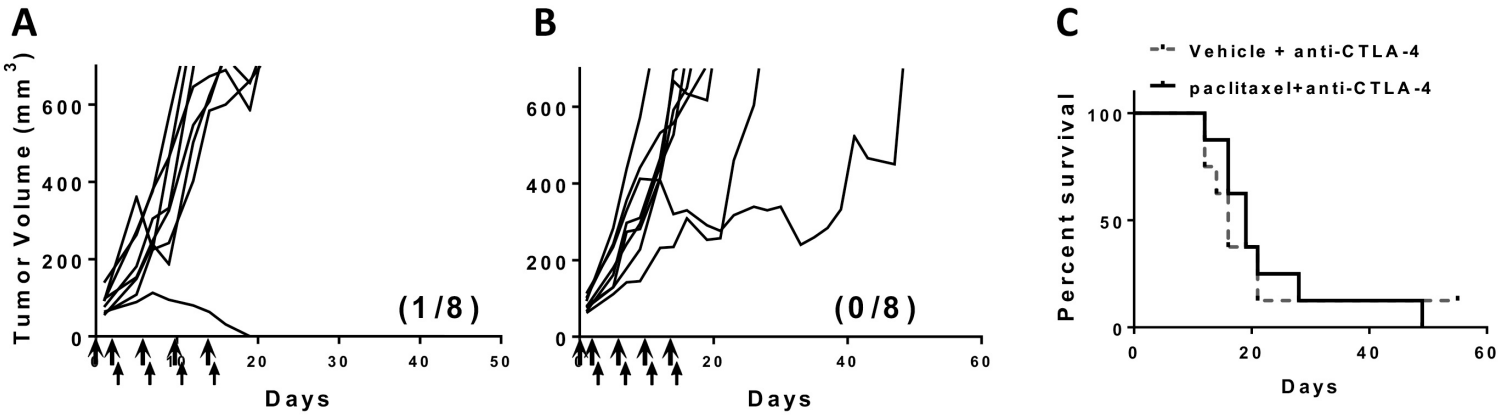
Anti-tumor effect of an immunotoxin targeting human CD22. Individual growth curves of 66C14-M tumors treated with **(A)** vehicle alone (thick arrows), **(B)** 10 µg HA22 alone (thick arrows), **(C)** vehicle and anti-CTLA-4 (thin arrows) or **(D)**10 µg HA22 and anti-CTLA-4. **(E).** Survival of mice described in **(A-D)**. The number of mice in CR and total mice per group is shown in parentheses.

S6B



Combination of SS1P with anti-CTLA-4 lead to tumor regression of 66C14 tumors not expressing MSLN. Individual growth curves of **(A)** 66C14-M tumors or **(B)** 66C14 parental tumors treated identically with SS1P (10 µg, thick arrows) and anti-CTLA-4 (thin arrows). The number of mice in CR and total mice per group is shown in parentheses. The graph shows a representative experiment out of two done.

Supplementary figure S7



Intra-tumors injection of paclitaxel does not improve the anti-tumor activity of anti-CTLA-4. Individual growth curves of 66C14-M tumors treated with 25µg anti-CTLA-4 (thin arrows) and **(A)** vehicle (thick arrows) or **(B)** 30 µg paclitaxel (thick arrows). **(C).** Survival of mice described in **(A-B).** The graph shows a representative experiment out of two done.

Table S1

Combination of RIT and anti-CTLA-4 induces long-term anti-tumor immunity

Treatment group	66C14 Rejected/Challenged	66C14-M Rejected/Challenged
100 µg/dose aCTLA-4 and SS1P	4/5	6/6
50 µg/dose aCTLA-4 and SS1P	5/5	
25 µg/dose CTLA-4 and SS1P	14/15	
25 µg/dose aCTLA-4 and LMB-100	8/8	
25 µg/dose aCTLA-4 and LMB-2	5/5	
% mice rejecting the second cell challenge	98%	100%

Combination of RIT and anti-CTLA-4 induces long term anti-tumor immunity. Mice that reached complete remission after RIT and anti-CTLA-4 treatment received an injection with tumor cells 45 days after complete remission using either $1x10^6$ 66C14-M cells or $1x10^6$ 66C14 parental cells. The number of mice that were challenged and the number of mice rejecting the new cells are indicated.