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

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.
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.
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.
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.
Combination of RIT and anti-CTLA-4 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 $1 \times 10^6$ 66C14-M cells or $1 \times 10^6$ 66C14 parental cells. The number of mice that were challenged and the number of mice rejecting the new cells are indicated.

### Table S1
Combination of RIT and anti-CTLA-4 induces long-term anti-tumor immunity

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>66C14 Rejected/Challenged</th>
<th>66C14-M Rejected/Challenged</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μg/dose aCTLA-4 and SS1P</td>
<td>4/5</td>
<td>6/6</td>
</tr>
<tr>
<td>50 μg/dose aCTLA-4 and SS1P</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>25 μg/dose aCTLA-4 and SS1P</td>
<td>14/15</td>
<td></td>
</tr>
<tr>
<td>25 μg/dose aCTLA-4 and LMB-100</td>
<td>8/8</td>
<td></td>
</tr>
<tr>
<td>25 μg/dose aCTLA-4 and LMB-100</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>% mice rejecting the second cell challenge</td>
<td>98%</td>
<td>100%</td>
</tr>
</tbody>
</table>