**ABSTRACT**

**TG4010** is a Modified Vaccinia virus Ankara (MVA) expressing human interleukin-2 and the human mucin (MUC1) tumor associated antigen. TG4010 has demonstrated clinical benefit for advanced non-small cell lung cancer (NSCLC) patients in combination with standard-of-care chemotherapy in two phase 1 randomized and controlled clinical trials (NCT00415818 and NCT1383448). Immunochemistry based on the use of immune checkpoint blockers (ICI) such as anti-PD-1 and anti-CTLA-4 has demonstrated efficacy in phase 2 and ongoing phase 3 trials. Hence, the combination of both approaches appears to be of great interest considering the high unmet medical need of this pathology.

**OBJECTIVES**

- Evaluate combinatorial treatment of MVA-based immunotherapy and murine immune checkpoint inhibitors (anti-PD-1 or anti-CTLA-4) in a β-galactosidase-positive murine tumor model (CT26-CL25).
- Develop CT26-based MUC1-positive murine tumor model (CT26-MUC1).
- Assess combinatorial treatment of TG4010 (MVA-MUC1-IL-2) and a murine anti-PD-1 antibody in MUC1-positive tumor model.
- Characterize tumor composition, lung-infiltrating leukocytes and specific immune responses.

**RESULTS**

1. **MVA-β-gal Immunotherapy combined with anti-CTLA-4 significantly improves survival in β-gal-lung tumor model**
   - Treatment with MVA-β-gal and anti-CTLA-4 increases β-gal-specific responses in the spleen and in a CD3⁺CD8⁺KLRG1⁺ cell population in the lung.

2. **Treatment with MVA-β-gal and anti-CTLA-4 increases β-gal-specific responses in the spleen and in a CD3⁺CD8⁺KLRG1⁺ cell population in the lung.**
   - Mice were treated i.p. with 1x10⁴ pfu MVA, iv with 1x10⁴ pfu MVA, and received 250 µg anti-CTLA-4 i.p. on days 10, 13, 15 and 17. Mice were sacrificed when the tumors reached the size of 2000 mm³.

3. **MVA-β-gal Immunotherapy combined with anti-PD-1 antibody significantly improves survival in s.c. β-gal tumor model**
   - Mice were treated i.p. with 1x10⁴ pfu MVA, iv with 1x10⁴ pfu MVA, and received 250 µg anti PDL1 i.v. on days 10, 13, 15 and 17. Mice were sacrificed when the tumors reached the size of 2000 mm³.

4. **s.c. CT26-CL25 or CT26-MUC1 tumors are PD-L2 positive, infiltrating leukocytes are PD-1 or PD-L1 positive.** Some infiltrating leukocytes in CT26-CL25, but not CT26-MUC1 tumors are PD-L2 positive.

5. **CT26-MUC1 cell line gives rise to MUC1-positive s.c. and lung tumors**
   - CT26-MUC1 cell line was developed by transducing CT26 cells with a retrovirus expressing the human MUC1 prepropeptide. Tumors were then generated after i.v. or s.c. injection of 1x10⁵ cells.

6. **Statistically significant effect of TG4010 on tumor growth or survival in MUC1-positive s.c. and lung tumor models**
   - TG4010 showed a significant effect on tumor growth or survival in MUC1-positive s.c. and lung tumor models.

7. **Combination of TG4010 and anti-PD-1 in a therapeutic s.c. MUC1-positive tumor model**
   - Mice were treated i.p. with 1x10⁴ pfu MVA, iv with 1x10⁴ pfu MVA, and received 250 µg anti PDL1 i.v. on days 10, 13, 15 and 17. Mice were sacrificed when the tumors reached the size of 2000 mm³.

**CONCLUSION**

- MVA-β-gal combined with anti-CTLA-4 antibody increased survival in therapeutic CT26-β-gal lung tumor model; combination was also shown to correlate with the appearance of CD8⁺CD3⁺KLRG1⁺ effector cells in the lung and an increase in β-gal-specific responses.
- MVA-β-gal combined with anti-PD-1 antibody increased survival in s.c. CT-26-β-gal lung tumor model.
- TG4010 immunotherapy alone increased survival in MUC1-positive s.c. and lung tumor models.
- First evidence of the benefits of the combination of TG4010 and an anti-PD-1 molecule in a therapeutic s.c. MUC1-positive tumor model.