Abstract # 2497

Immune checkpoint inhibitors enhance benefits of modified vaccinia virus Ankara to improve survival in preclinical models of cancer

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ABSTRACT

TG4010 is a Modified Vaccinia virus Ankara (MVA) expressing human interleukin 2 and the human mucin1 (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 2 randomized and controlled clinical trials (NCT00415818 and NCT1383148).

Immunotherapy 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

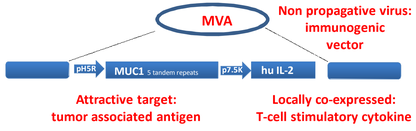
- Evaluate combinatorial treatment of MVA-based immunotherapy and murine immune checkpoint inhibitors (anti-PD-1 or anti-CTLA-4) in a β-galactosidase-positive murine tumor model (CT26.CL25).
- Develop CT26-based MUC1-positive murine tumor model (CT26-MUC1).
- Assess combinatorial treatment of TG4010 (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.

ABOUT TG4010

TG4010 is an immunotherapeutic vaccine consisting of Modified Vaccinia virus Ankara (MVA) encoding the tumor-associated antigen, MUC1, and human IL-2.

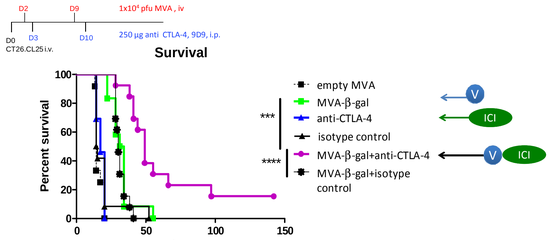
Key differences from other drug products targeting MUC1:

- TG4010 encodes the full cDNA sequence for MUC1, including all the epitopes
- IL-2 is a potent stimulant of T-cell response
- The viral vector itself is immunogenic, inducing expression of co-stimulatory signals by dendritic cells and the infiltration of CD8$^+$ lymphocytes.



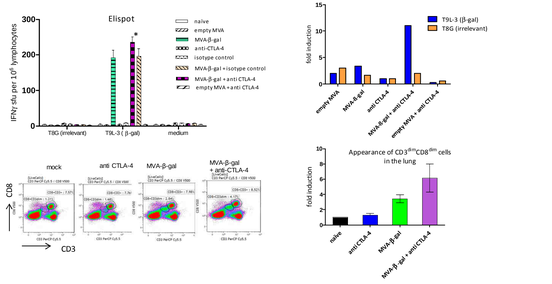
RESULTS

1: MVA-β-gal immunotherapy combined with anti-CTLA-4 significantly improves survival in β-gal$^+$ lung tumor model



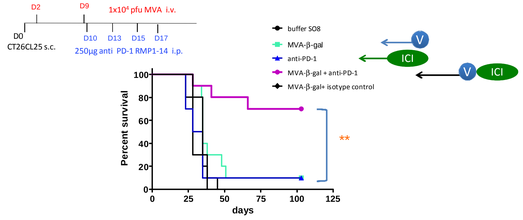
BALB/c mice were injected i.v. with 2.10^5 β-gal$^+$ CT26.CL25 cells. On days 2 and 9 after tumor challenge, mice were treated i.v. with MVA-β-gal or an empty MVA vector at 1.10^4 pfu. On days 3 and 10, mice received 250 µg anti-CTLA-4 (9D9, IgG2b, BioXCell). Mice were weighed twice per week and sacrificed when loosing 10% of weight.

2: Treatment with MVA-β-gal and anti-CTLA-4 increases β-gal-specific responses in the spleen and in a CD3^{dim}CD8^{dim} KLRG1$^+$ cell population in the lung



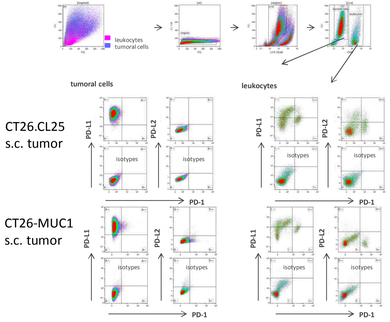
BALB/c mice were injected i.v. with MVA-β-gal or an emtpy control MVA at 1.10^4 pfu. On days 3 and 10, mice received 250 µg anti-CTLA-4 i.p.. Day 14, spleens (ELISPOT) and lungs were taken. Lungs were enzymatically dissociated and whole cells preparations were stimulated with anti CD28 , a β-gal specific peptide (T9L-3) or a control peptide (T8G) in the presence of anti CD107a. After 5h, cells were stained for CD8, CD3, KLRG1 and intracellular IFN-γ.

3: MVA-β-gal immunotherapy combined with anti-PD-1 antibody significantly improves survival in s.c. β-gal$^+$ tumor model



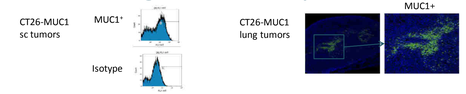
BALB/c mice were injected s.c. with 2.10^5 β-gal$^+$ CT26.CL25 cells. On days 2 and 9 after tumor challenge, mice were treated i.v. with MVA-β-gal at 1.10^4 pfu. On days 10, 13, 15 and 17, mice received 250 µg anti PD-1 (RMP1.14, IgG2a, BioXCell). Mice were sacrificed when the tumors reached the size of 2000 mm^3.

4: s.c. CT26.CL25 or CT26-MUC1 tumors are PD-L1 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.



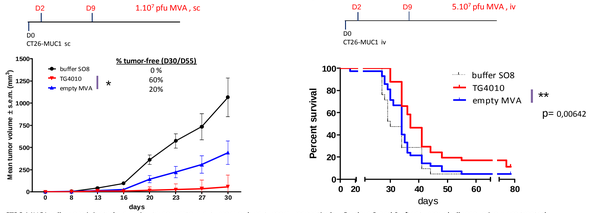
BALB/c mice were injected s.c. with 2.10^5 CT26.CL25 or CT26-MUC1 cells. Mice were sacrificed when the tumors reached 800 mm^3, tumors were excised, enzymatically dissociated , and cell suspensions were stained for flow cytometry analysis. Living cells, divided in CD45$^-$ tumoral cells and CD45$^+$ leukocytes were probed for PD-1, PD-L1 and PD-L2.

5: CT26-MUC1 cell line gives rise to MUC1-positive s.c. and lung tumors



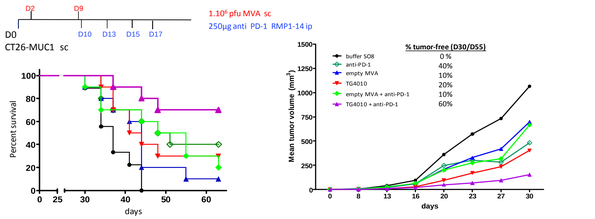
CT26-MUC1 cells were injected s.c. or i.v. to generate s.c. tumors or lung tumors, respectively. Subcutenous tumors were dissociated mechanically and MUC1 on the cell surface was probed with the monoclonal MUC1-specific antibody H23. Tumor-bearing lungs were immunohistochemically analysed using the MUC1 specific antibody H23.

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



CT26-MUC1 cells were injected s.c. or i.v. to generate s.c. tumors or lung tumors, respectively. On days 2 and 9 after tumor challenge, mice were treated s.c. or i.v. with TG4010 or an empty MVA control vector. Mean tumor volume or percent survival were monitored.

7: Combination of TG4010 and anti-PD-1 in a therapeutic s.c. MUC1-positive tumor model



BALB/c mice were injected s.c. with 2.10^5 CT26-MUC1 cells. On days 2 and 9 after tumor challenge, mice were treated s.c. with TG4010 or an empty control vector at the suboptimal dose of 1.10^5 pfu. On days 10, 13, 15 and 17, mice received 250 µg anti PD-1 (RMP1.14, IgG2a, BioXCell). Mice were sacrificed when the tumors reached the size of 2000 mm^{3}in size.

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^{dim}CD3^{dim} 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-positve tumor model.

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