Bacterial Ghosts: Immune-Adjuvant to Oxaliplatin in CRC

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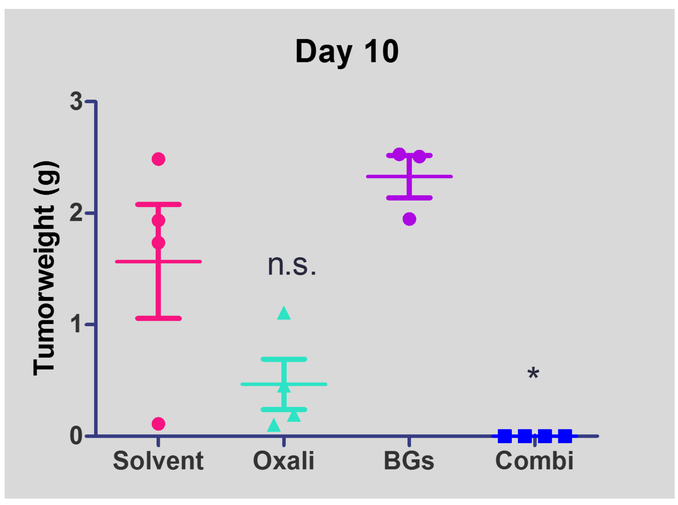
BGs from *E.coli* Nissle 1917

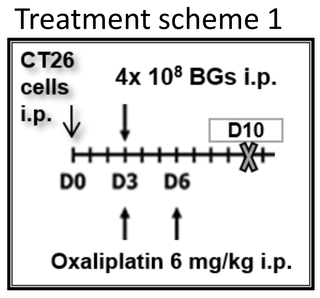
Introduction

Bacterial Ghosts (BGs) are empty envelopes of gram-negative bacteria, devoid of dangerous cytoplasmic or nucleic content. Due to immunogenic properties of their surface structures, they advance tumor-antigen recognition by immune cells. Consequently, this could be used to enhance the activity of several anticancer drugs -like oxaliplatin- which are characterized by a dependence on the immune system and immunogenic cell death induction. *In vivo* experiments were performed using Balb/c mice injected with CT26 cells i.p., an adequate immune-responsive model of peritoneal carcinomatosis.

1. BGs adjuvant to oxaliplatin induce complete remission *in vivo*



Treatment scheme 1

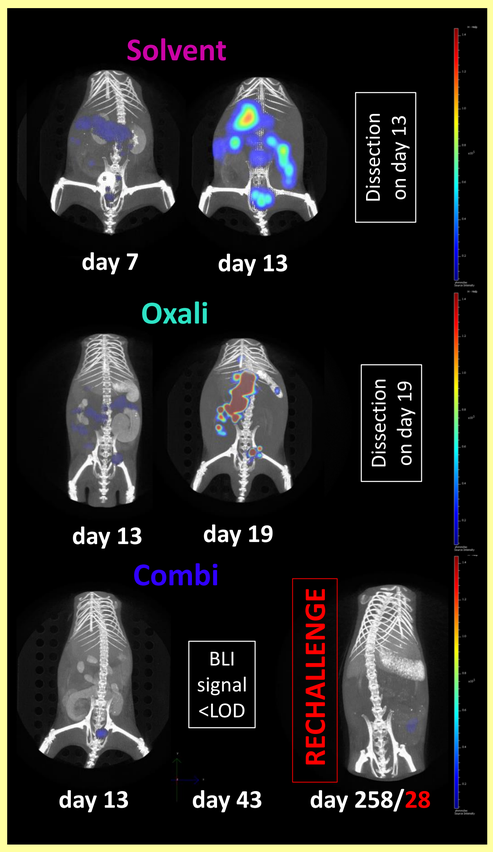


Following treatment scheme 1, dissection was done on day 10. Combination-treated animals were found to be tumor free, whereas tumor weights of the other groups are shown in the dot plot.

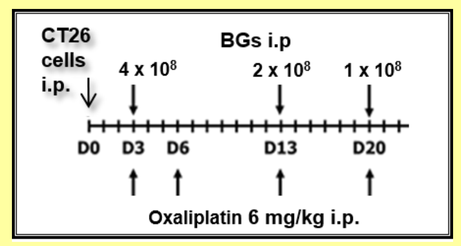
2. Prolonged survival and anti-tumor immunization shown by using DLIT

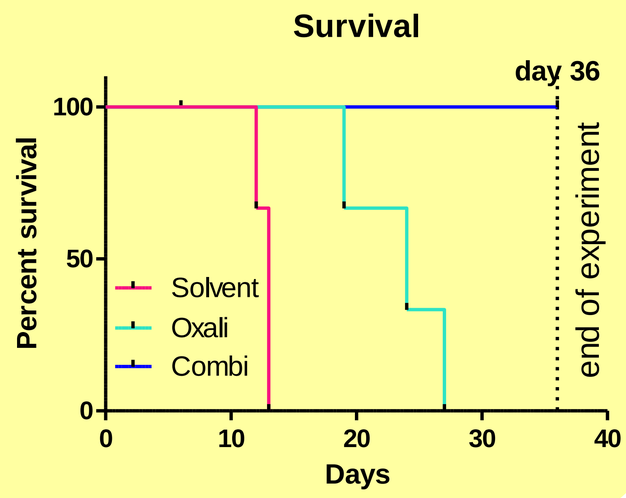
Luciferase-transfected CT26 cells were used for this experiment. Upon Luciferin application, 3D images of the tumors *in situ* could be generated by using diffuse light imaging tomography (DLIT). Treatment scheme 2 was applied, resulting again in complete remission after combination treatment, while in contrast oxaliplatin treatment alone lead to partial response, followed by resistance development.

Remarkably, cured mice were able to reject CT26 cells after re-challenge, indicating induction of a long-lasting **immunologic memory effect**.



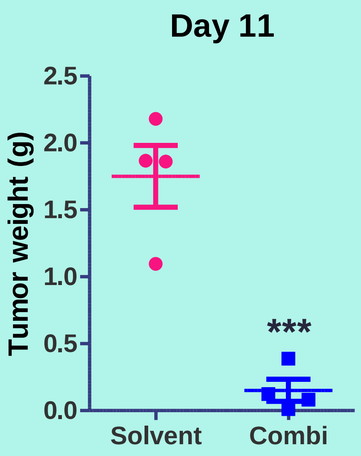
Treatment scheme 2

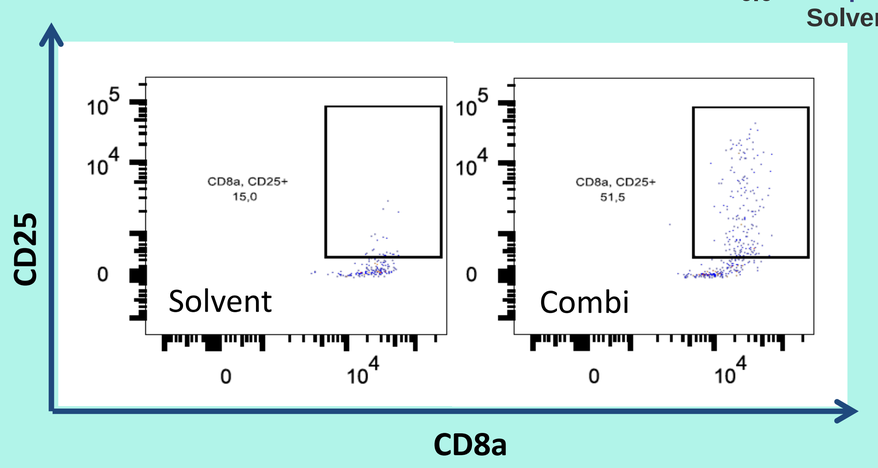




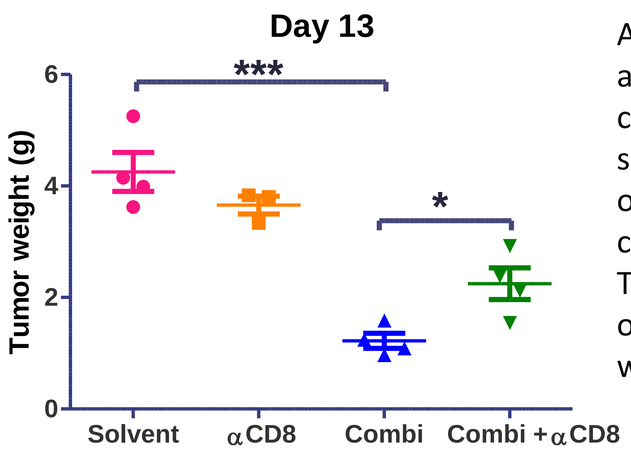
3. Infiltrating CTLs were found increased after combination treatment in the tumor

To reveal the mechanisms involved in treatment response, tumor immune cells were quantified by immuno-labeling and flow cytometry after treatment scheme 1. These experiments revealed a significantly decreased tumor weight together with a significantly increased proportion of cytotoxic T-cells upon BGs and oxaliplatin combination treatment.





4. Depletion of CTLs confirms their role in the immune-cellular MOA



Application of a CTL- depleting antibody in addition to the combination following treatment scheme 1 tested the importance of CTLs in the MOA. Blood was checked for CD8+ cell depletion. The experiment was terminated on day 13 and tumor weights were assessed.

Conclusion

BGs have proven to be a potent anticancer-adjuvant to clinically used oxaliplatin chemotherapy, based on their significantly increased *in vivo* response rates and long-term survival without enhancing side effects.

Acknowledgements: Many thanks to Gerhard Zeitler for helping with *in vivo* experiments and Alexandra Bogusch for CD8 antibody isolation.

Translational Cancer Therapy Research