Development of an Irradiated Whole Tumor Cell (rWTC) Vaccine Against Metastatic Tumors

Rogelio Medina, BA[1,2]; Herui Wang, PhD[2]; Veronika Caisová, PhD[3]; Jing Cui, PhD[2]; Ondra Uher, PhD[3]; Mark Gilbert, MD, PhD[2]; Masaki Terabe, PhD[2]; Karel Pacak, MD, PhD, D.Sc[3]; Zhengping Zhuang, MD, PhD[2]
[1]David Geffen School of Medicine at UCLA, [2]NCI - Neuro-Oncology Branch, [3]NICHD – Medical Neuroendocrinology

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Introduction

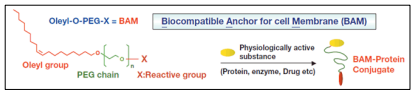
Primary and metastatic tumors commonly present with immunosuppressive microenvironments characterized by relatively low tumor infiltrating lymphocytes (TIL). The extent of T-cell infiltration within the tumor microenvironment has important prognostic and therapeutic implications. A T-cell inflamed tumor phenotype suggests the presence of a pre-existing antitumor immune response, one that could potentially be enhanced via immune checkpoint inhibitors (ICI) and other immunotherapeutic strategies. Hence, conversion of non-T-cell inflamed tumors to more immunogenic phenotypes is an important immunotherapeutic aim.

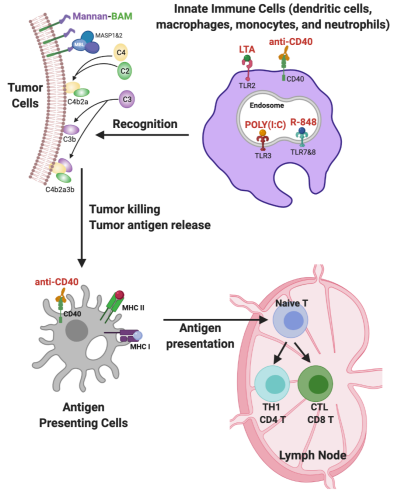
Vaccination with rWTC mixed with immunostimulatory adjuvants is a promising immunotherapeutic strategy against solid tumors. Optimization of the rWTC vaccine strategy with amphiphilic phagocytic agonists and immunostimulatory adjuvants has not been explored. Hence, we assessed the therapeutic efficacy of an rWTC vaccine, pulsed with a phagocytic agonist (Mannan-BAM), TLR ligands and anti-CD40 antibody (collectively abbreviated as rWTC-MBTA) in mice with representative metastatic lesions.

MBTA Immunotherapy

Theory behind MBTA Therapy

1. **Mannan –** Biocompatible Anchor for Cell Membrane **(BAM)**: Phagocytosis stimulating ligand directed towards tumor cell surface
2. **TLR Agonists**: Immunostimulatory adjuvants enhance acute inflammatory response
3. **Anti-CD40 ab**: Enhances APC activation

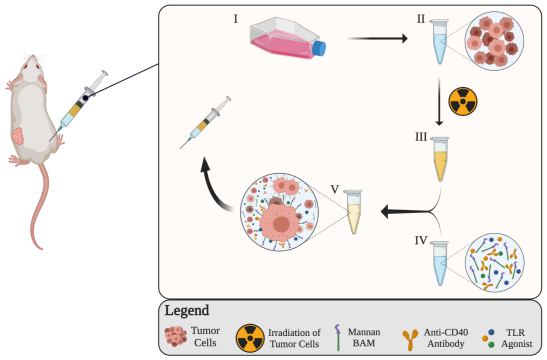




Methods

Animal Model

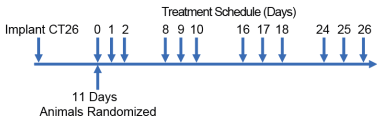
- A syngeneic colon carcinoma (CT26) model was established to assess the rWTC-MBTA vaccine efficacy in generating immune responses against representative metastatic colon carcinoma tumors.
- Mice were inoculated in the left flank with 2.5 x10^5 CT26 cells. After 11 days, mice bearing tumors (average tumor volume—42.4 mm^3) were randomized into three treatment arms – normal saline (control), radiated CT26 cells (rCT26) or rCT26-MBTA vaccine.
- Mice received a total of 12 vaccines according to their respective treatment group. Vaccines were delivered subcutaneously into the right flank and tumor growth was assessed twice a week until survival end point.



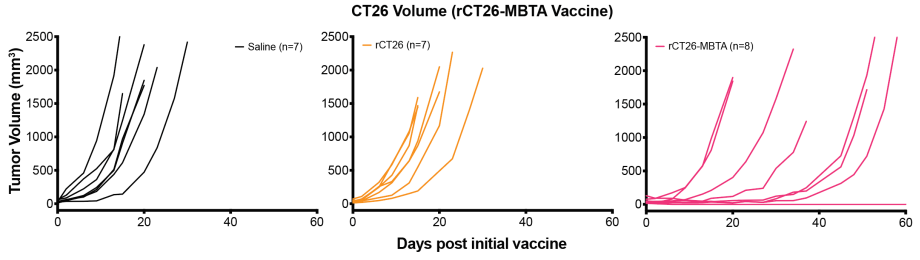
Development of rCT26-MBTA Vaccine

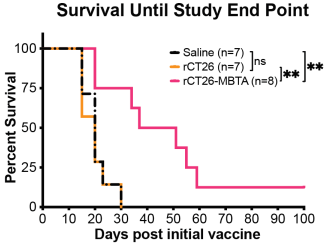
- CT26 cells are expanded in vitro (I) and subsequently aliquoted into 1 × 10^6 million cells per vaccine dose (II).
- CT26 cells are sublethally irradiated using a ^{137}Cs MARK I model irradiator to induce tumor cell apoptosis and prevent engraftment of tumor cells at the vaccine site (III).
- Irradiated tumor cells (III) are subsequently incubated with MBTA (IV) for an hour, facilitating the in vitro integration of Mannan-BAM into tumor cell membranes (V).
- The therapeutic mixture consisting of irradiated CT26 tumor cells and MBTA is subcutaneously injected into CT26 tumor bearing animals.

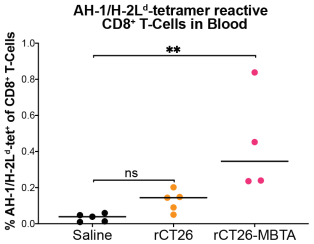
Treatment Schema for the rCT26-MBTA Vaccine experiments

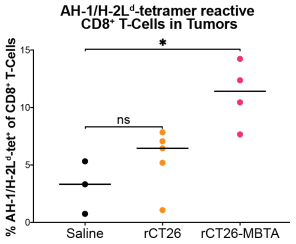


Results





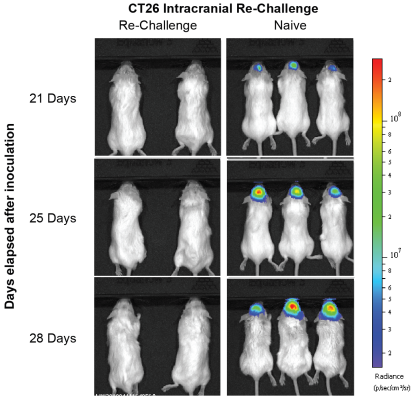




Results (Continued)

CT26 Re-Challenge

- Mice cured of CT26 colon carcinoma were re-challenged intracranially with 1 × 10^4 CT26-Luc cells in the right frontal lobe (1 mm rostral of the bregma, 2 mm right of midline and 2 mm deep from the skull surface). MBTA Pre-treated (left) and Naïve (right).



Results Summary

- Subcutaneous injection of irradiated CT26 cells pulsed with MBTA (rCT26-MBTA) significantly reduced metastatic CT26 tumor growth rates and induced complete remission (CR) in 28.6% (2/7) of treated animals.
- Tumor infiltrating leukocyte analyses demonstrated significantly increased CD8+ cytotoxic T-lymphocytes (CTL) in metastatic tumors.
- Assessments with MHC I tetramers revealed significantly increased CT26-associated peptide (AH1) specific CTLs in the blood and tumors of rCT26-MBTA Vaccine treated animals.
- All animals that achieved complete remission of colon carcinoma tumors resisted subsequent challenges with CT26 cells, confirming the induction of immunological memory against CT26 tumors.

Conclusions

Collectively, immunotherapy with rWTC-MBTA Vaccines addresses two challenges in cancer immunology:

- The effective recognition of tumor specific neoantigens
- The induction of more favorable T-cell signatures within the tumor microenvironment

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References

1. Quail DF, Joyce JA. The Microenvironmental Landscape of Brain Tumors. *Cancer Cell*. 2017;31(3):326–341. doi:10.1016/j.ccell.2017.02.009
2. Darvin P, Toor SM, Sasidharan nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. 2018;50(12):165.
3. Caisová V, Uher O, Nedbalová P, et al. Effective cancer immunotherapy based on combination of TLR agonists with stimulation of phagocytosis. Int Immunopharmacol. 2018;59:86-96.
4. Kato K, Itoh C, Yasukouchi T, Nagamune T. Rapid protein anchoring into the membranes of Mammalian cells using oleyl chain and poly(ethylene glycol) derivatives. Biotechnol Prog. 2004;20(3):897-904.
5. Hassan SB, Sørensen JF, Olsen BN, Pedersen AE. Anti-CD40-mediated cancer immunotherapy: an update of recent and ongoing clinical trials. Immunopharmacol Immunotoxicol. 2014;36(2):96-104.
6. Sims S, Willberg C, Klenerman P. MHC-peptide tetramers for the analysis of antigen-specific T cells. Expert Rev Vaccines. 2010;9(7):765-74.
7. Curry WT, Gorrepati R, Piesche M, et al. Vaccination with irradiated autologous tumor cells mixed with irradiated gm-k562 cells stimulates antitumor immunity and t lymphocyte activation in patients with recurrent malignant glioma. Clin Cancer Res. 2016;22(12):2885-2896.
8. Medina, R., Wang, H., Caisová, V., Cui, J., Indig, I.H., Uher, O., Ye, J., Nwankwo, A., Sanchez, V., Wu, T., Nduom, E., Heiss, J., Gilbert, M.R., Terabe, M., Ho, W., Zenka, J., Pacak, K. and Zhuang, Z. (2020), Induction of Immune Response against Metastatic Tumors via Vaccination of Mannan-BAM, TLR Ligands, and Anti-CD40 Antibody (MBTA). Adv. Therap., 3: 2000044.

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