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Commentary

Safety study supports clinical development of immunotherapeutic oncolytic measles vaccine

Christine E. Engeland[1,2]

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In a recent article in *Molecular Therapy – Methods and Clinical Development*, Galanis and co-authors presented pre-clinical safety data for a cancer immunotherapy derived from a measles vaccine strain, engineered to encode a bacterial immunomodulator.[1] These data supported a successful investigational new drug (IND) application for a phase I clinical trial for patients suffering from metastatic breast cancer that is now ongoing. Despite limitations of the mouse model, the pre-clinical data from the present study and prior work are encouraging. Now, clinical results are eagerly awaited.

Metastatic breast cancer remains a devastating disease with no curative treatment options. The recent triumph of novel cancer immunotherapies has spurred hopes that such treatments can improve outcomes for many previously intractable diseases. One modality of cancer immunotherapy is oncolytic viruses that selectively replicate in malignant cells, leading to tumor cell lysis and induction of anti-tumor immunity via tumor vaccination effects. Measles vaccine viruses are one type of oncolytic immunotherapies that are currently in clinical development.

Oncolytic measles vaccines have been developed as an adaptable vector platform. Various genetic engineering approaches have been employed to increase their therapeutic index. These approaches include encoding immunomodulatory transgenes within the viral genome to enhance anti-tumor immunity.[2] To this end, the group of Galanis has previously identified a secreted form of neutrophil-activating protein (s-NAP) from *Helicobacter pylori* as a candidate for immunotherapy in breast cancer. This protein induces acute inflammation and a Th1-polarized immune response, which is considered beneficial for immune-mediated tumor rejection. In a pleural effusion xenograft model, local administration of measles vaccine encoding s-NAP (MV-s-NAP) led to prolonged survival and production of cytokines.[3]

That study already highlighted the promise of this therapeutic for advanced breast cancer. However, it also draws attention to the challenge of selecting an appropriate pre-clinical model for evaluation of measles-derived immunotherapies. Measles is a primate-adapted virus that replicates poorly in murine cells. Thus, direct virus-mediated tumor lysis can only be assessed using human cells, commonly in xenograft models in immunodeficient mice. By contrast, immunocompetent models are mandatory to assess tumor immune infiltration, anti-tumor and anti-viral immunity, as well as immune-related adverse effects.

As the present study focused on safety, the authors used IFNARko-CD46Ge mice with an immunocompetent C57BL/6-C3H background.[4] These mice express the MV entry receptor CD46 with an expression pattern comparable to humans. Knockout of the alpha/beta interferon receptor enables replication of MV viruses, which are otherwise strongly restricted by type I interferon. Importantly, the FDA has accepted IFNARko-CD46Ge mice as a small animal toxicology model for assessment of oncolytic MVs.

In the current report, the authors present comprehensive biodistribution and toxicity data for MV-s-NAP in these mice. The authors assessed both locoregional (subcutaneous) and systemic (intravenous) application of MV-s-NAP. Though the actual clinical trial (ClinicalTrials.gov: NCT04521764) entails intratumoral injection, the latter route was intended to mimic a "worst case scenario"—inadvertent systemic exposure. Mice received either a single dose or three doses every 2 weeks of 1×10^6 or 1×10^7 $TCID_{50}$ of MV-s-NAP. This regimen results in relatively higher exposure compared with the dosing of 1×10^7 or 1×10^8 $TCID_{50}$ every 3 weeks intended in the clinical trial.

The findings indicate an overall favorable safety profile of MV-s-NAP: mice showed no clinical signs of toxicity, and body weight remained stable throughout the observation period of up to 56 days post treatment. Differential blood counts did not deviate significantly from controls. Clinical chemistry showed elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in individual mice, which was attributed to hemolysis during blood collection. Histopathological assessment after euthanasia on days 11/12 or 54/56 after the first treatment revealed minimal abnormalities. Focal lung hemorrhage, which was neither associated with administration route nor dose, was observed in individual animals, possibly caused by CO_2 use in euthanasia. Inflammation and leukocyte infiltration were found at the injection site after subcutaneous application of MV-s-NAP. Biodistribution, as assessed by qRT-PCR, demonstrated presence of viral genomes in

[1]Faculty of Health/School of Medicine, Institute of Virology and Microbiology – Experimental Virology, Center for Biomedical Education and Research (ZBAF), Witten/Herdecke University, Witten, Germany; [2]Research Group Mechanisms of Oncolytic Immunotherapy, Clinical Cooperation Unit Virotherapy, German Cancer Research Center (DKFZ), National Center for Tumor Diseases (NCT) and University Hospital Heidelberg, Department of Medical Oncology, Heidelberg, Germany

Correspondence: Christine E. Engeland, Faculty of Health/School of Medicine, Institute of Virology and Microbiology – Experimental Virology, Center for Biomedical Education and Research (ZBAF), Witten/Herdecke University, Witten, Germany.
E-mail: christine.engeland@uni-wh.de

multiple organs after systemic administration up to day 54. After subcutaneous injection, viral RNA was detected in regional lymph nodes of some animals on day 11. However, no samples were collected to test for the presence of replicating virus. A multiplex cytokine assay was employed to rule out a systemic inflammatory response, or "cytokine storm," in response to MV-s-NAP. Anti-drug antibodies were also measured. Interestingly, anti-measles antibodies were more readily detectable than anti-NAP antibodies, and overall antibody titers were higher after intravenous compared with subcutaneous application. The authors note that the present study was designed with input from the FDA. In light of successful trial initiation, this work may serve as blueprint or guidance for researchers in the field aiming at clinical translation of novel oncolytic immunotherapies.

Nevertheless, one may argue that the IFNARko-CD46Ge model with non-tumor-bearing mice, which were not pre-immunized with MV, cannot mimic the clinical situation of cancer patients with pre-existing anti-measles immunity. Type I interferon signaling, which is defective in this mouse strain, certainly impacts efficacy of cancer immunotherapies, especially also oncolytic measles viruses, in both deleterious and beneficial ways.[5] Further, the role of pre-existing anti-viral immunity in oncolytic virotherapy remains controversial.[6] Several approaches have been developed to circumvent or overcome anti-measles immunity,[7] but these may hamper safety. For other oncolytics, including reovirus, pre-existing anti-viral antibodies have been shown to enhance delivery.[8] In an immunocompetent mouse model, intratumoral injection of measles encoding a bispecific T cell engager in pre-vaccinated mice showed similar, if not better, efficacy compared with unvaccinated mice.[9] In this scenario, pre-existing anti-viral T cells may be harnessed as anti-tumor effectors. Overall, as also supported by lower antibody levels after subcutaneous administration in the present study, intratumoral administration may represent the most feasible therapeutic strategy.

The ongoing trial (ClinicalTrials.gov: NCT04521764) investigates intratumoral application of MV-s-NAP in patients with metastatic breast cancer. While primary endpoints of this phase I trial are related to safety and toxicity of single and multiple injections, secondary objectives aim at a preliminary assessment of anti-tumor efficacy. Notably, the trial includes correlative analyses. These are related to viremia, viral replication and shedding as well as anti-measles and anti-NAP antibodies, similar to the pre-clinical dataset in the present publication. Of special interest, the investigators intend to study anti-tumor immunity and determine PD-L1 expression on tumor and immune cells. Previous pre-clinical work has demonstrated upregulation of the PD-L1 immune checkpoint after measles virotherapy and potential synergy with PD-1/PD-L1 checkpoint blockade.[10–12] A recent paper by the Galanis group reported immunotherapeutic efficacy of MV-s-NAP in two immunocompetent glioblastoma models, GL261 and CT-2A, which was enhanced by addition of anti-PD-1 and JAK inhibition.[13]

In summary, the present study adds to the body of data attesting safety of oncolytic measles virotherapy. Previous early-phase clinical trials have demonstrated safety and tolerability of other MV derivatives in other tumor entities. These trials have shown exceptional responses in individual patients[14–16] and first promising signs of anti-tumor immunity.[16,17] The ongoing phase I trial (ClinicalTrials.gov: NCT04521764) will demonstrate whether the pre-clinical results for MV-s-NAP can be replicated in a real-world clinical setting. Although various measles-derived oncolytics encoding immunomodulators have been developed preclinically,[2] MV-s-NAP is the first to enter a clinical trial. It will be enlightening to see how immune modulation by NAP complements MV-mediated anti-tumor immune effects. If efficacy of MV-s-NAP monotherapy is limited, as is to be feared in advance-stage patients, combination immunotherapies, e.g., with checkpoint blockade, may prove beneficial. Moving forward, more correlative research identifying biomarkers of response to oncolytic immunotherapy will be key to identifying patients that benefit from these novel agents.

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