Background Checkpoint blockade immunotherapy has dramatically changed cancer treatment; however, these therapies depend on the presence of a pre-existing immune infiltrate. Unfortunately, some patients have few to no infiltrating immune cells, highlighting the need for therapies that can generate antigenic stimuli. Oncolytic viruses, which infect and lyse tumor cells while leaving healthy tissue unharmed, are an attractive means to provide these signals, although the mechanisms of action of these engineered viral therapies remain incompletely understood. Virally induced immunogenic death causes an influx of tumor- and virus- specific effector CD8+ T cells. Many oncolytic viruses also decrease tumor-infiltrating suppressive immune populations, such as regulatory T cells (Treg), however the mechanism for this is unknown. Here we show that an oncolytic strain of vaccinia virus (VV) infects tumor infiltrating Tregs, in contrast to the prevailing idea that oncolytic viruses only infect tumor cells. Infection leads to viral-mediated Treg depletion that is required for tumor regression.

Methods Using a mouse model of head and neck squamous cell carcinoma (MEER), a VV-resistant line was generated through serial treatment of a VV-sensitive MEER line. At varied time points post-intratumoral treatment with VV, tumor infiltrating lymphocytes (TIL) were isolated from both the VV-resistant and VV-sensitive lines and analyzed by flow cytometry.

Results One day post-treatment of VV-sensitive MEER tumors, tumor isolated Tregs were infected by VV as determined by viral GFP expression. Infection was confirmed in vitro with purified Tregs. Four days post-treatment, tumor infiltrating Treg counts were reduced, and active caspase 3 staining was increased, suggesting that infection lead to Treg death. At 7 days post-treatment, the remaining Tregs in the VV-sensitive tumors acquired a fragile phenotype (IFN?+ Nrp1-). This was not observed in the VV-resistant MEER line. Fragile Tregs are less suppressive and indeed we observed an increase in pro-inflammatory cytokine production from CD8+ and Tconv (CD4+ Foxp3-) T cells in the VV-sensitive tumors compared to VV-resistant. We then engineered oncolytic VV to be susceptible to Cre mediated inactivation. Infection of various murine transgenic Cre lines confirmed the importance of non-tumoral immune infection for therapeutic efficacy, with a particular emphasis on Treg infection.

Conclusions These data reveal a previously unappreciated mechanism of action of oncolytic virus immunotherapy, in which new tumor immunity accompanies the viral mediated loss and phenotypic change of regulatory populations. Importantly, as this treatment is delivered intratumorally the loss of Tregs is tumor specific, resulting in targeted Treg deletion without systemic autoimmunity.

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590 PRE-CONDITIONING OF THE TUMOR MICROENVIRONMENT WITH ONCOLYTIC REOVIRUS CONVERTS CD3-BISPECIFIC ANTIBODY TREATMENT INTO EFFECTIVE IMMUNOTHERAPY

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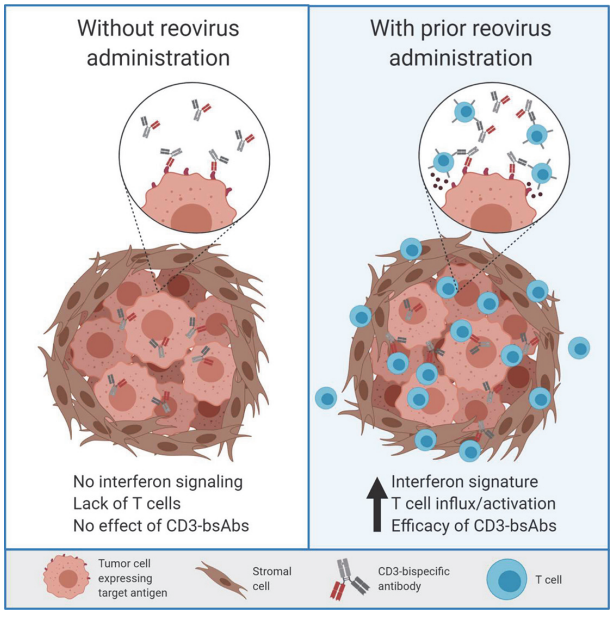
Background The use of T cell-engaging CD3-bispecific antibodies (CD3-bsAbs) is a promising immunotherapeutic strategy for cancer. Although this therapy has reached clinical practice for hematological malignancies, the absence of sufficient infiltrating T cells is a major barrier for efficacy in solid tumors.[1] Oncolytic viruses are emerging as anti-cancer therapeutics, and accumulating evidence demonstrates their applicability to sensitize tumors for immune checkpoint immunotherapy.[2] In this study, we exploited oncolytic reovirus as a strategy to enhance the efficacy of CD3-bsAbs in immune-silent, solid tumors.

Methods The mutant p53 and K-ras induced murine pancreatic cancer model KPC3 resembles human pancreatic ductal adenocarcinomas with a desmoplastic tumor microenvironment, low T cell density, and resistance to immunotherapy. Immune-competent mice with established, subcutaneous KPC3 tumors were intratumorally injected with an optimized regimen of oncolytic reovirus (type 3 Dearing strain) and the reovirus-induced changes in the tumor microenvironment and lymphoid organs were analyzed over time by NanoString analysis, RT-qPCR and multicolor flow cytometry. The efficacy of combination with systemically injected CD3-bsAbs was evaluated in KPC3 and B16.F10 murine tumor models and the close-to-patient HER2+ BT474 breast cancer model with cell surface-expressed TRP1 and HER2 as target antigens, respectively. Primary outcome was tumor size, measured with caliper three times a week in a blinded-manner.

Results Replication-competent reovirus induced an early IFN-signature, followed by a strong influx of CD8+ T cells (2.6-fold increase, p=0.0092). Viral replication declined after seven days and was associated with systemic activation of lymphocytes. Tumor-infiltrating T cells were mostly reovirus-specific and served as effector cells for the subsequently systemically administered CD3-bsAbs. The combination of reovirus and CD3-bsAbs induced regressions up to 70% in all mice with large, established KPC3, B16.F10, and BT474 tumors and significantly prolonged survival. Importantly, the employment of



Abstract 590 Figure 1 Reovirus sensitizes tumors for CD3-bsAb therapy
Reovirus-induced interferon signaling leads to increased T cell influx and subsequent effective CD3-bispecific antibody therapy in solid tumors

reovirus as a pre-conditioning regimen performed significantly better than the simultaneous or preceding administration of bsAbs. This combination treatment also induced regressions of non-injected distant lesions, suggesting that this therapy might be effective for metastatic disease.

Conclusions Oncolytic reovirus administration represents an effective strategy to induce a local IFN response and strong T cell influx, thereby sensitizing the tumor microenvironment for subsequent CD3-bsAb therapy (figure 1). Our data advocate for the inclusion of oncolytic viruses as a pre-conditioning strategy in T cell engaging antibody trials for solid tumors. Since both CD3-bispecific antibodies and oncolytic viruses are in advanced clinical development as monotherapies, efficient translation of this combination seems feasible.

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Ethics Approval All mouse studies were approved by the institutional Animal Welfare Body of Leiden University Medical Center and carried out under project licenses AVD1160020187004 or AVD116002015271, issued by the competent authority on animal experiments in the Netherland (named CCD).

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591 COMPARISON OF TWO OHSV VECTORS FOR THE TREATMENT OF GLIOBLASTOMA

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Background Glioblastoma multiforme (GBM) is the most common human brain cancer. Despite a well-established standard of care, the 5-year mortality rate of GBM patients is 95%, highlighting the need for innovative therapeutic interventions. A variety of oncolytic viruses, including those derived from herpes simplex virus (oHSV), have been designed for GBM therapy, but early-phase clinical trials have reported few complete responses and no evidence of durable anti-tumor immunity. Potential reasons for the lack of efficacy are limited vector potency (i.e., virulence) and the presence of a highly immunosuppressive tumor microenvironment (TME) comprised of few activated lymphocytes, large numbers of immunosuppressive myeloid cells (macrophages, myeloid derived suppressor cells [MDSCs], microglia), and an agglomerate of immunosuppressive cytokines (IL-10, VEGF, MIF, etc.).[1] Herein we explore these obstacles by comparing the anti-tumor activity two different oHSV designs, an HSV-1 KOS strain derivative designated KG4:T124, and an F strain derivative designated rQNestin34.5v.1 (a similar oHSV, rQNestin34.5v.2, is currently in a phase I clinical trial for GBM).[2]

Methods Using the murine syngeneic GBM models, GL261N4 and CT2A, we compared the anti-tumor activity of KG4:T124 and rQNestin34.5v1. In vitro, we evaluated the viral entry, replication capacity, and cytotoxicity of both oHSVs. In vivo, we measured the impact of both vectors on tumor progression, TME immune cell composition, and animal survival.

Results Virus entry into cancer cells of KG4:T124 or rQNestin34.5v1 was relatively similar, but rQNestin34.5v.1 replicated more effectively and generally induced greater viral mediated cytotoxicity. In syngeneic mice, rQNestin34.5v.1 reduced orthotopic GL261N4 tumor burden and enhanced animal survival compared to KG4:T124. However, preliminary data indicate that multiple injections of KG4:T124 but not rQNestin34.5 enhance GL261N4 survival outcome. Neither oHSV impacted survival outcomes in the more pernicious CT2A model. Analysis, of either the GL261N4 or CT2A TME two days post virus administration revealed that both viruses had reduced microglia cell frequency, induced the influx of tumor associated macrophages and polymorphonuclear cells, but did not alter the frequency of monocytic MDSCs, natural killer cells, CD8+ or CD4+ T-cells.

Conclusions rQNestin34.5 had greater oncolytic activity In vivo and in vitro, but did not benefit from multiple oHSV injections. Both viruses induced similar changes in the TME immune cell composition. However, the presence of vital adaptive immune cell types within the TME was not observed at 2 days post oHSV treatment.

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592 ENHANCING THE THERAPEUTIC POTENTIAL OF ONCOLYTIC ADENOVIRUSES WITH VSENS™ TECHNOLOGY

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Background Oncolytic viro-therapeutics is a promising treatment for cancer. Among the different strains of oncolytic viruses currently being developed, potent cytolytic activity, manageable safety profiles, large genomic capacity for addition of transgenes and available advanced manufacture processes make adenovirus (Ad) a great choice.[1] However, the delivery of Ad for clinical application is limited due to 1) neutralization by pre-existing neutralizing antibodies (nAb) in bloodstream and 2) receptor restricted tumor-cellular entry.[2] To overcome these limitations, we developed a novel proprietary