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Review

# Lighting a Fire in the Tumor Microenvironment Using Oncolytic Immunotherapy

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ABSTRACT

Oncolytic virus (OV) therapy is potentially a game-changing cancer treatment that has garnered significant interest due to its versatility and multi-modal approaches towards tumor eradication. In the field of cancer immunotherapy, the immunological phenotype of the tumor microenvironment (TME) is an important determinant of disease prognosis and therapeutic success. There is accumulating data that OVs are capable of dramatically altering the TME immune landscape, leading to improved antitumor activity alone or in combination with assorted immune modulators. Herein, we review how OVs disrupt the immunosuppressive TME and can be used strategically to create a *"pro-immune"* microenvironment that enables and promotes potent, long-lasting host antitumor immune responses.



**Contents**



## 1. Introduction

The wealth of insight into intra- and inter-tumoral heterogeneity and the supportive tumor microenvironment (TME) is shedding light on cancer survival mechanisms that contribute to treatment resistance and relapse. Broadly, as the host attempts to eradicate the tumor by

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generating an antitumor immune response, the tumor prompts a counteractive response by recruiting immunosuppressive immune cells and other TME components to build a physical and immunological fortress against attack. Current therapeutic efforts are directed towards harnessing principles of immunology to stimulate tumor-specific T-cell responses.

Oncolytic or "cancer-killing" viruses (OVs) are a class of self-replicating immunotherapeutic agents that present substantial potential to supplement the oncologist's cancer-fighting arsenal. In this review, we discuss a number of recent discoveries that demonstrate how OVs alone or in combination with other anticancer drugs act not only as direct tumor-killing weapons but also hold the capacity to promote *in situ* vaccination against the whole tumor. Indeed, compared to other immunotherapies that require patient-specific tumor-associated antigen (TAA) identification, OVs potently induce the release of the full range of TAAs into an inflammatory environment via tumor lysis and contribute to the establishment of tumor-specific T-cell immunity.

## 2. The Tumor Microenvironment

The original paradigm that tumors are a mass of proliferating cancer cells has now shifted to an in-depth understanding of tumors as complex entities. In addition to cancer cells, tumors harbour a variety of other cell types, including vascular endothelial cells (ECs), cancer-associated fibroblasts (CAFs) and various resident or migratory immune cell subsets [e.g. T cells, dendritic cells (DCs), Natural-Killer cells (NKs)]. Together, these various cell populations and the extracellular matrix that glues them together create an organized and dynamic community known as the tumor ecosystem or TME. It is now well established that the reciprocal crosstalk and cooperative interactions between cancer cells and these other cell types promote tumorigenesis and further sustain tumor growth, proliferation, and invasion [1]. For instance, certain biomolecules secreted by the immune cells within the TME can be critical to several of the established *"cancer hallmarks"* [2].

Although these general concepts apply to a wide range of tumors, individual tumors are unique ecosystems and are heterogeneous in the cellular composition of the TME within and between patients [1,2]. The TME's immune phenotypes are generally classified in three broad categories: immune desert, immune-excluded, and inflamed [3]. Inflamed tumors typically contain both cytokine-secreting $CD4^+$ and cytotoxic $CD8^+$ T cells and myeloid cells [4]. Unlike inflamed tumors, "immunologically cold" tumors contain less immune cells or cell subsets associated with immune suppression including regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs) and M2 macrophages. Whereas immune desert tumors are generally characterized by a very low number or even an absence of immune cell infiltrates, immune-excluded tumors contain immune cells that remain stuck in the surrounding stroma, thus unable to colonize the TME to exert their antitumor functions [5].

## 3. The Tumor Immune Microenvironment Shapes The Response to Anticancer Therapies

The exciting yet still limited success of immunotherapies to date highlights the need to better understand the unique characteristics of individual tumors for the rational design of treatment plans. For instance, identifying the type of immune landscape may predict therapeutic effectiveness of certain immunotherapies like immune checkpoint blockade [3]. In the case of non-inflamed tumors, there is a need for novel therapeutic strategies that change the TME landscape into an inflamed phenotype to promote the priming of antitumor immune responses [6].

Accumulating evidence indicates that type I interferons (IFNα/β) are crucial in the establishment of antitumor responses. In addition to their antiviral and antitumor properties, type I IFNs stimulate diverse immune cell subsets within the TME (e.g. the cytotoxic activity of NK and $CD8^+$ T cells, the secretion of pro-inflammatory cytokines by macrophages, and the cross-presentation activity of mature DCs) [7]. It has been described that the efficacy of many chemotherapies, radiotherapies, immunotherapies, and targeted anticancer agents depends upon major contribution of type I IFNs [7]. However, systemic administration of type I IFNs often has undesirable side-effects and as a result it has become of strong interest in the field of cancer therapy to select for therapeutic modalities that specifically induce type I IFNs expression in the TME. Recently, two studies have demonstrated that DNA methyltransferases inhibitors upregulate the expression of cytosolic dsRNAs derived from endogenous retroviral elements that subsequently activate viral sensors to induce type I and III IFN signaling associated with antitumor effects [8,9]. Alternatively, agonists of viral nucleic acid cellular sensors, such as RIG-I, STING or TLR3, elicit the production of type I IFNs and therefore promote tumor cell death and antitumor immunity [7]. In the following review, we argue that rather than using a viral mimetic, it is preferable to use a multi-functional replicating virus that directly attacks cancer cells while heating up the TME to stimulate antitumor immune responses.

## 4. Oncolytic Immunotherapy "Wakes Up" Tumors in an "Immunological Coma"

During their transformation, cancer cells acquire defects in numerous signaling pathways that simultaneously impinge on cellular growth control and innate antiviral defense systems [10]. As a result, many cancers are susceptible to a range of oncolytic virus therapeutics, a class of naturally occurring or genetically modified viruses that selectively replicate within and kill tumor cells without harming healthy tissues. The most advanced of these is Talimogene laherparepvec (T-Vec, Imlygic®), an engineered Herpes Simplex Virus (HSV) that was recently approved for the treatment of unresectable melanoma by the FDA and EMA [11]. Currently, numerous OV candidates are under extensive study, with several in late phases of clinical investigation (e.g. NCT02562755, NCT02879760, NCT02192775, NCT02364713). Due to space restrictions, we apologize that we cannot discuss all important pre-clinical and clinical studies that have been or are currently being studied in the context of various OV platforms. Additional information can be found in the following review article [12].

Several studies have highlighted the crucial role of tumor-specific T cells in OV-mediated therapeutic efficacy. For instance, it has been shown that the intratumoral injection of reovirus or vesicular stomatitis virus (VSV) potently primes adaptive antitumor immune responses playing a key role in primary and metastatic tumors regression [13,14]. *The question remains, how are OVs able to reverse TME immune suppression and facilitate T cell recognition of tumor antigens?* Although originally designed or selected to be cytolytic agents, it is now clear that OVs have pleotropic impacts on the TME (Fig. 1). While awakening of the immune system within the TME is initiated through OV-mediated cell killing, this is just the first of several events that ultimately culminate in the induction of a robust and long-lasting antitumor immune response [15]. One critical early event is the triggering of immunogenic cell death (ICD) of OV-infected cancer cells (Figs. 1 and 3) [12]. ICD is characterized in part by the expression and/or release of damage-associated molecular patterns (DAMPs) (i.e. ecto-calreticulin, ATP, and HMGB1) which attract and activate DCs in the TME [16]. In addition, pathogen-associated molecular patterns (PAMPs) in the tumor milieu are recognized by specific pathogen recognition receptors (PRRs) expressed by innate immune cells (Figs. 1 and 3). For example, it has been shown that the dsRNA genome of reovirus directly activates DCs through protein kinase receptor (PKR) signaling leading to the secretion of pro-inflammatory cytokines (e.g. IFN-α, IL-12, TNF-α and IL-6) [17]. Upon exposure to oncolytic MeV, two subsets of human blood DCs [plasmacytoid DCs (pDCs) and a subset of myeloid DCs] secrete IFN-α following activation of RIG-I-like receptors (RLRs) and/or Toll-like

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