Oncolytic Virotherapy for Cancer Treatment

Trends in Tumor Immunology
September 23, 2015
Oncolytic virotherapy mechanism of action: viral replication, cell killing, virus release and spread within cancer tissue but not normal tissues
Properties of the three best-studied family of oncolytic viruses used in clinical trials

<table>
<thead>
<tr>
<th>Properties:</th>
<th>pros</th>
<th>cons</th>
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<tbody>
<tr>
<td><strong>Adenovirus</strong></td>
<td>Wide host-cell range</td>
<td>Rapid virus sequestration by Kupffer cells</td>
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<td>Approved in China in 2006 (H101)</td>
<td>Large genomic capacity</td>
<td>Preexisting antiviral immunity</td>
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<td></td>
<td>Induction of danger signals</td>
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<tr>
<td><strong>Vaccinia virus</strong></td>
<td>Broad-spectrum infectivity</td>
<td>Preexisting antiviral immunity</td>
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<td>Phase IIb clinical trials with GM-CSF-expressing JX-594 virus in hepatocellular carcinoma</td>
<td>Large genomic capacity</td>
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<td>Replicates in cytoplasm</td>
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<td>Ability to travel through blood</td>
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<td>Available antiviral drugs</td>
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<td></td>
<td>Initiation of danger signals</td>
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<tr>
<td><strong>Herpes Simplex virus</strong></td>
<td>Wide host-cell range (neurotropism)</td>
<td>Poor intratumoral penetration/inefficient virus replication</td>
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<tr>
<td>Phase III clinical trials with GM-CSF-expressing T-Vect virus in advanced melanoma</td>
<td>Large genomic capacity</td>
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Limitations of Oncolytic Virotherapy

- Inefficient systemic delivery of the virus to tumors
- Inefficient replication in tumor cells and poor intratumoral spread
- Virus replication in normal cells
- Toxicity
- Immune clearance may reduce oncolytic effects
- Insufficient single agent potency due to limited understanding of the viral interaction with different elements within the context of tumor stroma

Future Goals:

- Improve clinical tolerability and efficacy
- Improve tumor selectivity with tropism-modifying strategies
- Improve systemic delivery for treatment of metastatic cancer (minimize virus sequestration, protect form neutralizing antibodies, increase cellular penetration)
- Attack and kill cancer stem cells and chemoresistant tumor cells
- Induce innate and adaptive antitumor immunity
Cancer Stem Cells (CSCs) or Cancer Initiating Cells (CICs)

A consensus of five defining criteria has been established to affirm the existence of CICs:

- Organize self-renewing, anchorage-independent spheres
- Restriction to a small minority of the total tumor population
- Reproducible tumor phenotype
- Expression of distinctive cell surface markers, permitting consistent isolation
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Immunogenic cell death

**Figure 1:** The figure illustrates the improved T cell priming in oncolytic virotherapy. Viral oncolysis of tumor cell induces immunogenic cell death by accumulation of PAMPs and accompanied by release of DAMPs. PAMPs and DAMPs activate antigen-presenting dendritic cells that can induce cytotoxic T cell responses against tumor-associated antigens or neoepitopes, respectively.

*Woller et al., Frontiers in Oncology, 2014, 4:1*
Box 1 | **Tumour-associated antigens**

A summary of the origins of tumour-associated antigens is provided below.

**The mutanome**
Advances in DNA sequencing have revealed the cellular mutanome — a comprehensive map of all of the somatic mutations in individual tumours. A subset of these somatic mutations occurs in protein-coding sequences and might create new antigenic structures that are recognized by the immune system of the patient. These novel antigens could arise from amino acid substitutions or deletions, protein truncations or fusions of two unique polypeptides.

**Oncofetal proteins**
Some gene products are only expressed during normal embryonic development and not in adult tissues. In cancers, the expression of these developmental genes can be reactivated and produce antigens that the adult immune system recognizes as foreign.

**Viral antigens**
More than 20% of cancers are known to arise as a result of infection with a cancer-causing virus. These oncoviruses drive the growth of the tumour through the expression of one or more oncoproteins. These virally encoded proteins are unique tumour-associated antigens that can be recognized by the immune system.

**Differentiation antigens**
Differentiation antigens are associated with the differentiation of a specific tissue and are overexpressed on tumour cells.

**Post-translational modifications**
An almost universal feature of cancer cells is altered glycosylation patterns that could be recognized by the immune system. For example, a loss of glycosylation can reveal protein peptide antigens that are not visible on normal cells.
Immunogenic cell death

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The endoplasmic reticulum stress-mediated cell surface marker calreticulin (ecto-CRT), has emerged as a hallmark DAMP of immunological cell death (ICD). Ecto-CRT binds to the low-density lipoprotein receptor-related protein on engulfing DCs to drive cellular activation.

Autophagy-dependent ATP that is released from dying cells binds to P2X purinoceptor 7 (P2X7) on DCs, activating the NALP3–ASC inflammasome and driving the secretion of interleukin-1β (IL-1β). This cytokine, together with antigen presentation, is required for the polarization of interferon-γ (IFNγ)-producing CD8⁺ T cells and the development of the adaptive antitumor immune response.

Similarly, the nuclear protein high mobility group protein B1 (HMGB1) is released during necrotic and apoptotic cell death and binds to toll-like receptor 4 (TLR4) on DCs to induce their activation.

DAMPs in cancer therapy

DAMPs are derived from different compartments of the cells. For example, they can come from mitochondria (DNA, formyl peptides and ATP), nucleus (HMGB1, high-mobility group box 1 protein; HMGN1, high mobility group nucleosome binding protein 1; histones), ER (calreticulin and ATP) and cytoplasm (ATP and F-actin)
Oncolytic viruses as therapeutic cancer vaccines

Bartlett et al., Mol. Cancer, 2013, 12:103
Apoptosis and necrosis

**Apoptosis**
This is accompanied by a rounding up of the cell, retraction of pseudopods, reduction of cellular volume, chromatin condensation, nuclear fragmentation, few or no ultrastructural modifications of cytoplasmic organelles, and plasma membrane blebbing, but the integrity of the cell is maintained until the final stages of the process.

Some forms of apoptosis are non-immunologic, while others are immunogenic. The pre-apoptotic surface exposure of CRT and HSP70/HSP90 may have a profound impact on the immune response. In addition, the release of HMGB1 during late apoptosis promotes antigen processing by DCs and hence contributes to cytotoxic T-cell activation.

**Necrosis**
Characterized by a gain in cell volume, swelling of organelles and rupture of plasma membrane, and subsequent loss of intracellular contents, including HMGB1, ATP, etc. This causes release of DAMPs and elicits substantial inflammation and affects local environment.
**Autophagy** is the basic catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes. The breakdown of cellular components promotes cellular survival during starvation by maintaining cellular energy levels. Autophagy allows the degradation and recycling of cellular components. During this process, targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membraned vesicle known as an autophagosome. The autophagosome then fuses with a lysosome and its cargo is degraded and recycled. There are three different forms of autophagy that are commonly described; macroautophagy, microautophagy and chaperone-mediated autophagy. In the context of disease, autophagy has been seen as an adaptive response to stress which promotes survival, whereas in other cases it appears to promote cell death and morbidity.

**Autophagic cell death** Occurs without chromatin condensation but is accompanied by massive autophagic vacuolization of the cytoplasm. The term ACD simply describes cell death with autophagy.

A) Diagram of autophagy; (B) Electron micrograph of autophagic structures in the fatbody of a fruit fly larva; (C) Fluorescently labeled autophagosomes in liver cells of starved mice.
Pyroptosis is a form of programmed cell death associated with antimicrobial responses during inflammation. In this process, immune cells that recognize certain danger signals within themselves produce cytokines, swell, burst and die. This releases the cytokines, attracts other immune cells to fight the infection and contributes to inflammation. Pyroptosis occurs for example in salmonella-infected macrophages and in HIV-infected T helper cells. The initiation of pyroptosis is caused by the recognition of flagellin components of Salmonella and Shigella species (and similar pathogen-associated molecular pattern in other microbial pathogens) by NOD-like receptors (NLRs). These receptors function like plasma membrane Toll-like receptors (TLRs), but recognise antigens located within the cell rather than outside of it.

In contrast to apoptosis, pyroptosis requires the function of the enzyme caspase-1. Recently, it was shown that caspase-1 is activated during pyroptosis by a large supramolecular complex termed the pyroptosome (also known as an inflammasome). Only one large pyroptosome is formed in each macrophage, within minutes after infection. Biochemical and Mass Spectroscopic analysis revealed that this pyroptosome is largely composed of dimers of the adaptor protein ASC (apoptosis-associated speck protein containing a CARD or Caspase activation and recruitment domain).

Pyroptosis results in the release of pathogen associated molecular patterns (PAMPs) and cytokines that activate pro-inflammatory immune cell mediators.
## Types of cell death and their immunological consequence

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<th>Immunogenicity</th>
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<td><strong>Apoptosis</strong> (type 1 cell death)</td>
<td>This is accompanied by a rounding up of the cell, retraction of pseudopods, reduction of cellular volume, chromatin condensation, nuclear fragmentation, few or no ultrastructural modifications of cytoplasmic organelles, and plasma membrane blebbing, but the integrity of the cell is maintained until the final stages of the process. Some forms of apoptosis are non-immunologic, while others are immunogenic. The pre-apoptotic surface exposure of CRT and HSP70/HSP90 may have a profound impact on the immune response. In addition, the release of HMGB1 during late apoptosis promotes antigen processing by DCs and hence contributes to cytotoxic T-cell activation.</td>
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<tr>
<td><strong>Autophagic cell death</strong> (ACD; type 2 cell death)</td>
<td>Occurs without chromatin condensation but is accompanied by massive autophagic vacuolization of the cytoplasm. The term ACD simply describes cell death with autophagy. High. It may release DAMPs (HMGB1, ATP, and others) and elicit substantial inflammation.</td>
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<tr>
<td><strong>Necrosis</strong> (type 3 cell death)</td>
<td>Characterized by a gain in cell volume, swelling of organelles and rupture of plasma membrane, and subsequent loss of intracellular contents, including HMGB1, ATP, etc. High. This causes release of DAMPs and elicits substantial inflammation and affects local environment.</td>
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<tr>
<td><strong>Pyroptosis</strong> (or caspase 1-dependent cell death)</td>
<td>It is a highly inflammatory form of cell death mediated by the inflammasome and caspase-1 activation, and triggered by various pathological stimuli, such as microbial infection, or stroke, heart attack and cancer. High. It is a highly inflammatory form of cell death due to cytokine release and escape of cytoplasmic contents (DAMPs). However, some pathogens encode immunosuppressive proteins.</td>
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<tr>
<td><strong>Secondary necrosis</strong></td>
<td>This is the dissolution of the cell following apoptosis. Some remaining cellular contents are released. High. It is quite immunogenic due to necrosis occurring in apoptotic cells at the late stage.</td>
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Summary

- Oncolytic virotherapy is a new strategy to reduce tumor burden through selective virus replication in proliferating cells.

- Oncolytic viruses belong to many families. They have undergone different modifications to enhance tumor selectivity, improve intratumoral replication, modify immune responses, enhance vascular delivery, and express antitumorigenic genes that function independently of viral replication.

- Recent advances include preclinical proof of feasibility, the development of strategies to monitor virus spread in micrometastases, and clinical trials that document tolerability and effectiveness.

- Current efforts have favored intratumoral delivery, but systemic delivery is required for the treatment of metastatic disease. Strategies to increase specific tumor penetration, minimize virus sequestration in the liver and spleen, evade neutralization by serum factors, and enhance vascular penetration are under investigation.
An overview on critical components to be included in multimodale virotherapy-based therapies that work like prime-boost strategies.
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