

Methods in
Molecular Biology 2058

Springer Protocols

Christine E. Engeland *Editor*

Oncolytic Viruses

 Humana Press

METHODS IN MOLECULAR BIOLOGY

Series Editor

John M. Walker

School of Life and Medical Sciences

University of Hertfordshire

Hatfield, Hertfordshire, UK

For further volumes:

<http://www.springer.com/series/7651>

For over 35 years, biological scientists have come to rely on the research protocols and methodologies in the critically acclaimed *Methods in Molecular Biology* series. The series was the first to introduce the step-by-step protocols approach that has become the standard in all biomedical protocol publishing. Each protocol is provided in readily-reproducible step-by-step fashion, opening with an introductory overview, a list of the materials and reagents needed to complete the experiment, and followed by a detailed procedure that is supported with a helpful notes section offering tips and tricks of the trade as well as troubleshooting advice. These hallmark features were introduced by series editor Dr. John Walker and constitute the key ingredient in each and every volume of the *Methods in Molecular Biology* series. Tested and trusted, comprehensive and reliable, all protocols from the series are indexed in PubMed.

Oncolytic Viruses

Edited by

Christine E. Engeland

*Research Group Mechanisms of Oncolytic Immunotherapy, Clinical Cooperation Unit Virotherapy,
National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ), University Hospital
Heidelberg, Heidelberg, Germany*

 Humana Press

Editor

Christine E. Engeland
Research Group Mechanisms of
Oncolytic Immunotherapy
Clinical Cooperation Unit Virotherapy
National Center for Tumor Diseases (NCT)
German Cancer Research Center (DKFZ)
University Hospital Heidelberg
Heidelberg, Germany

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-9793-0 ISBN 978-1-4939-9794-7 (eBook)
<https://doi.org/10.1007/978-1-4939-9794-7>

© Springer Science+Business Media, LLC, part of Springer Nature 2020

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Humana imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.

The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

Anecdotal clinical reports of tumor remissions after viral infections laid the foundation for the field of oncolytic virotherapy. Advances in molecular virology, tumor biology, and immunology have enabled more refined studies of tumor-selective viruses. Concomitant with the resurgence of cancer immunotherapy and after the approval of Talimogene laherparepvec by the FDA and EMA, oncolytic virotherapy has gained unprecedented momentum. The field has flourished in recent years, yielding many notable preclinical studies and clinical trials. This book aims to provide a guide for basic virologists, translational researchers, and clinician scientists in the field by providing reference protocols from vector development to clinical translation.

The initial chapter provides an introductory review of the field, followed by a series of chapters describing virus modifications to enhance tumor specificity and anti-tumor efficacy. Reflecting the increasing interest in immunotherapeutic effects of oncolysis, a number of chapters address different strategies for immunomodulation and immunomonitoring. The third section of the book covers methodologies for different model systems to study oncolytic viruses, including mouse tumor models, patient-derived samples, and also mathematical modeling.

A number of virus platforms and approaches are represented, providing a survey of state-of-the-art methods for study of this unique treatment approach. Therefore, I would like to take this opportunity to thank all authors who have made this possible with their contributions. Hopefully this book will serve the research community as a useful resource to further enhance progress in the field of oncolytic virotherapy.

Heidelberg, Germany

Christine E. Engeland

Contents

<i>Preface</i>	<i>v</i>
<i>Contributors</i>	<i>ix</i>
1 Introduction to Oncolytic Virotherapy	1
<i>Christine E. Engeland and John C. Bell</i>	
2 Methods for Modification of Therapeutic Viruses	7
<i>Claudia A. P. Hill, Luca Bau, and Robert Carlisle</i>	
3 Tumor Targeting of Oncolytic Adenoviruses Using Bispecific Adapter Proteins	31
<i>Julia Niemann and Florian Kühnel</i>	
4 Development of Entry-Targeted Oncolytic Measles Viruses	51
<i>Michael D. Mühlbach and Roberto Cattaneo</i>	
5 Insert Stability and In Vivo Testing of MicroRNA-Detargeted Oncolytic Picornaviruses	77
<i>Autumn J. Schulze</i>	
6 Ex Vivo Virotherapy with Myxoma Virus to Treat Cancer	95
<i>Nancy Y. Villa, Lina S. Franco, and Grant McFadden</i>	
7 Immunomodulation in Oncolytic Measles Virotherapy	111
<i>Laura Dietz and Christine E. Engeland</i>	
8 A Functional Assay to Determine the Capacity of Oncolytic Viruses to Induce Immunogenic Tumor Cell Death	127
<i>Tiphaine Delaunay, Carole Achard, Marc Grégoire, Frédéric Tangy, Nicolas Boisgerault, and Jean-François Fonteneau</i>	
9 Design and Production of Newcastle Disease Virus for Intratumoral Immunomodulation	133
<i>Gayathri Vijayakumar and Dmitriy Zamarin</i>	
10 Analysis of Immunological Treatment Effects of Virotherapy in Tumor Tissue	155
<i>Krishna Das, Carles Urbiola, Bart Spiesschaert, Philipp Mueller, and Guido Wollmann</i>	
11 Immunohistochemistry for Tumor-Infiltrating Immune Cells After Oncolytic Virotherapy	179
<i>Dipongkor Saha and Samuel D. Rabkin</i>	
12 Detection of Tumor Antigen-Specific T-Cell Responses After Oncolytic Vaccination	191
<i>Jonathan G. Pol, Byram W. Bridle, and Brian D. Lichty</i>	
13 Evaluation of Oncolytic Virus-Induced Therapeutic Tumor Vaccination Effects in Murine Tumor Models	213
<i>Rūta Veinalde</i>	
14 Delivery of Oncolytic Reovirus by Cell Carriers	229
<i>Elizabeth J. Ilett</i>	

15 In Vivo Bioimaging for Monitoring Intratumoral Virus Activity 237
*Liesa-Marie Schreiber, Carles Urbiola, Patrik Erlmann,
and Guido Wollmann*

16 Oncolytic Immunotherapy for Bladder Cancer Using Coxsackie
A21 Virus: Using a Bladder Tumor Precision-Cut Slice Model
System to Assess Viral Efficacy 249
*Kate Relph, Nicola Annels, Chris Smith, Marcos Kostalas,
and Hardev Pandha*

17 Use of Liquid Patient Ascites Fluids as a Preclinical Model
for Oncolytic Virus Activity 261
*Eleanor M. Scott, Sally Frost, Hena Khalique, Joshua D. Freedman,
Len W. Seymour, and Janet Lei-Rossmann*

18 Generating Primary Models of Human Cancer to Aid
in the Development of Clinically Relevant Oncolytic Viruses 271
*Brian A. Keller, Marie-Ève Wedge, Abera Surendran,
and Carolina S. Ilkow*

19 Considerations for Clinical Translation of MG1 Maraba Virus 285
Caroline J. Breitbach

20 Fluorescence In Situ Hybridization (FISH) Detection of Viral
Nucleic Acids in Oncolytic H-1 Parvovirus-Treated Human Brain Tumors 295
*Irina Kiprianova, Alexandra Just, Barbara Leuchs,
Jean Rommelaere, and Assia L. Angelova*

21 Mathematical Modeling of Oncolytic Virotherapy 307
*Johannes P. W. Heidbuechel, Daniel Abate-Daga,
Christine E. Engeland, and Heiko Enderling*

Index 321

Contributors

- DANIEL ABATE-DAGA • *Department of Immunology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA*
- CAROLE ACHARD • *CRCINA, INSERM, Université d'Angers, Université de Nantes, Nantes, France; Immunology Graft Oncology, Labex IGO, Nantes, France*
- ASSIA L. ANGELOVA • *Division of Tumor Virology, German Cancer Research Center (DKFZ), Heidelberg, Germany*
- NICOLA ANNELS • *Targeted Cancer Therapy, Department of Clinical and Experimental Medicine, Faculty of Health and Medical Science, University of Surrey, Guildford, Surrey, UK*
- LUCA BAU • *Institute of Biomedical Engineering, University of Oxford, Oxford, UK*
- JOHN C. BELL • *Centre for Innovative Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, ON, Canada; Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, Canada*
- NICOLAS BOISGERAULT • *CRCINA, INSERM, Université d'Angers, Université de Nantes, Nantes, France; Immunology Graft Oncology, Labex IGO, Nantes, France*
- CAROLINE J. BREITBACH • *Turnstone Biologics, Ottawa, ON, Canada*
- BYRAM W. BRIDLE • *Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada*
- ROBERT CARLISLE • *Institute of Biomedical Engineering, University of Oxford, Oxford, UK*
- ROBERTO CATTANEO • *Department of Molecular Medicine, Mayo Clinic, Rochester, MN, USA*
- KRISHNA DAS • *Division of Virology, Medical University of Innsbruck, Innsbruck, Austria; Christian Doppler Laboratory for Viral Immunotherapy of Cancer, Innsbruck, Austria*
- TIPHAINE DELAUNAY • *CRCINA, INSERM, Université d'Angers, Université de Nantes, Nantes, France; Immunology Graft Oncology, Labex IGO, Nantes, France*
- LAURA DIETZ • *Faculty of Biosciences, Institute of Pharmacy and Molecular Biotechnology (IPMB), Heidelberg University, Heidelberg, Germany; Research Group Mechanisms of Oncolytic Immunotherapy, Clinical Cooperation Unit Virotherapy, National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ), University Hospital Heidelberg, Heidelberg, Germany*
- HEIKO ENDERLING • *Department of Integrated Mathematical Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA*
- CHRISTINE E. ENGELAND • *Research Group Mechanisms of Oncolytic Immunotherapy, Clinical Cooperation Unit Virotherapy, National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ), University Hospital Heidelberg, Heidelberg, Germany*
- PATRIK ERLMANN • *ViraTherapeutics GmbH, Innsbruck, Austria*
- JEAN-FRANÇOIS FONTENEAU • *CRCINA, INSERM, Université d'Angers, Université de Nantes, Nantes, France; Immunology Graft Oncology, Labex IGO, Nantes, France*
- LINA S. FRANCO • *Center for Immunotherapy, Vaccines and Virotherapy (B-CIVV), Biodesign Institute, Arizona State University, Tempe, AZ, USA*
- JOSHUA D. FREEDMAN • *Department of Oncology, University of Oxford, Oxford, UK*
- SALLY FROST • *Department of Oncology, University of Oxford, Oxford, UK*

- MARC GRÉGOIRE • *CRCINA, INSERM, Université d'Angers, Université de Nantes, Nantes, France; Immunology Graft Oncology, Labex IGO, Nantes, France*
- JOHANNES P. W. HEIDBUECHEL • *Research Group Mechanisms of Oncolytic Immunotherapy, Clinical Cooperation Unit Virotherapy, National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ), University Hospital Heidelberg, Heidelberg, Germany; Faculty of Biosciences, Heidelberg University, Heidelberg, Germany*
- CLAUDIA A. P. HILL • *Institute of Biomedical Engineering, University of Oxford, Oxford, UK*
- ELIZABETH J. ILETT • *Leeds Institute of Medical Research at St James', St James' University Hospital, Leeds, UK*
- CAROLINA S. ILKOW • *Centre for Innovative Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, ON, Canada; Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada*
- ALEXANDRA JUST • *Division of Tumor Virology, German Cancer Research Center (DKFZ), Heidelberg, Germany*
- BRIAN A. KELLER • *Department of Pathology and Laboratory Medicine, The Ottawa Hospital, Ottawa, ON, Canada; Centre for Innovative Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, ON, Canada*
- HENA KHALIQUE • *Department of Oncology, University of Oxford, Oxford, UK*
- IRINA KIPRIANOVA • *Division of Tumor Virology, German Cancer Research Center (DKFZ), Heidelberg, Germany; BIORON GmbH, Ludwigshafen, Germany*
- MARCOS KOSTALAS • *Targeted Cancer Therapy, Department of Clinical and Experimental Medicine, Faculty of Health and Medical Science, University of Surrey, Guildford, Surrey, UK*
- FLORIAN KÜHNEL • *Department of Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, Hannover, Germany*
- JANET LEI-ROSSMANN • *Department of Oncology, University of Oxford, Oxford, UK*
- BARBARA LEUCHS • *Division of Tumor Virology, German Cancer Research Center (DKFZ), Heidelberg, Germany*
- BRIAN D. LICHTY • *Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada; Turnstone Biologics, Ottawa, ON, Canada*
- GRANT MCFADDEN • *Center for Immunotherapy, Vaccines and Virotherapy (B-CIVV), Biodesign Institute, Arizona State University, Tempe, AZ, USA*
- PHILIPP MUELLER • *Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach a.d. Riss, Germany*
- MICHAEL D. MÜHLEBACH • *Section Product Testing of Immunological Veterinary Medicinal Products, Division of Veterinary Medicine, Paul-Ehrlich-Institut, Langen, Germany*
- JULIA NIEMANN • *Department of Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, Hannover, Germany*
- HARDEV PANDHA • *Targeted Cancer Therapy, Department of Clinical and Experimental Medicine, Faculty of Health and Medical Science, University of Surrey, Guildford, Surrey, UK*
- JONATHAN G. POL • *Gustave Roussy Comprehensive Cancer Institute, Villejuif, France; INSERM, U1138, Paris, France; Equipe I1 Labellisée par la Ligue Nationale Contre le Cancer, Centre de Recherche des Cordeliers, Paris, France; Université de Paris, Paris, France; Sorbonne Université, Paris, France*
- SAMUEL D. RABKIN • *Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*

- KATE RELPH • *Targeted Cancer Therapy, Department of Clinical and Experimental Medicine, Faculty of Health and Medical Science, University of Surrey, Guildford, Surrey, UK*
- JEAN ROMMELAERE • *Division of Tumor Virology, German Cancer Research Center (DKFZ), Heidelberg, Germany*
- DIPONGKOR SAHA • *Department of Immunotherapeutics and Biotechnology, School of Pharmacy, Texas Tech University Health Sciences Center, Abilene, TX, USA; Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
- LIESA-MARIE SCHREIBER • *Division of Virology, Medical University of Innsbruck, Innsbruck, Austria; Christian Doppler Laboratory for Viral Immunotherapy of Cancer, Innsbruck, Austria*
- AUTUMN J. SCHULZE • *Department of Molecular Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA*
- ELEANOR M. SCOTT • *Department of Oncology, University of Oxford, Oxford, UK*
- LEN W. SEYMOUR • *Department of Oncology, University of Oxford, Oxford, UK*
- CHRIS SMITH • *Targeted Cancer Therapy, Department of Clinical and Experimental Medicine, Faculty of Health and Medical Science, University of Surrey, Guildford, Surrey, UK*
- BART SPIESSCHAERT • *Division of Virology, Medical University of Innsbruck, Innsbruck, Austria; Christian Doppler Laboratory for Viral Immunotherapy of Cancer, Innsbruck, Austria; ViraTherapeutics GmbH, Innsbruck, Austria*
- ABERA SURENDRAN • *Centre for Innovative Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, ON, Canada; Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada*
- FRÉDÉRIC TANGY • *CNRS 3569, Institut Pasteur, Paris, France*
- CARLES URBIOLA • *Division of Virology, Medical University of Innsbruck, Innsbruck, Austria; Christian Doppler Laboratory for Viral Immunotherapy of Cancer, Innsbruck, Austria*
- RŪTA VEINALDE • *Latvian Biomedical Research and Study Centre, Riga, Latvia*
- GAYATHRI VIJAYAKUMAR • *Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Memorial Sloan Kettering Cancer Center, New York, NY, USA*
- NANCY Y. VILLA • *Center for Immunotherapy, Vaccines and Virotherapy (B-CIVV), Biodesign Institute, Arizona State University, Tempe, AZ, USA*
- MARIE-ÈVE WEDGE • *Centre for Innovative Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, ON, Canada; Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada*
- GUIDO WOLLMANN • *Division of Virology, Medical University of Innsbruck, Innsbruck, Austria; Christian Doppler Laboratory for Viral Immunotherapy of Cancer, Innsbruck, Austria*
- DMITRIY ZAMARIN • *Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA; Department of Medicine, Weil Cornell Medical College, New York, NY, USA; Parker Institute for Cancer Immunotherapy, Memorial Sloan Kettering Cancer Center, New York, NY, USA*



Chapter 1

Introduction to Oncolytic Virotherapy

Christine E. Engeland and John C. Bell

Abstract

Oncolytic viruses exploit key hallmarks of cancer for replication in malignant cells, leading to tumor cell lysis, modulation of the tumor microenvironment and in situ vaccination effects. Diverse virus platforms have been developed as oncolytic vectors and designed for improved tumor specificity, intratumoral spread, therapeutic gene delivery and especially as targeted cancer immunotherapeutics. This chapter provides a concise overview of the basic principles as well as current progress in preclinical and clinical studies of oncolytic virotherapy.

Key words Oncolytic viruses, Viral vectors, Cancer immunotherapy, Tumor targeting, Cancer gene therapy

1 Principles of Oncolytic Virotherapy: Exploiting Hallmarks of Cancer and Turning Cold Tumors Hot

Treating cancer patients with replicating viruses may seem an outrageous idea—which was actually inspired by clinical observations of tumor remissions after natural virus infections [1]. Indeed, these experiments of nature were followed up by clinicians and researchers, who deduced the following principles of oncolytic virotherapy (Fig. 1):

On a cellular level, viruses with oncolytic properties show tumor-selective infection, replication, and spread—supported by inherent characteristics of cancer cells, the “hallmarks of cancer.” As such, cancer cells show many properties conducive to viral replication including sustained proliferation, resistance to apoptosis, and immune evasion [2, 3]. Malignant transformation can include upregulation of viral entry receptors (e.g., CD46, a complement regulator) and proliferative signaling pathways usurped by viruses (e.g., Wnt/ β -Catenin and EGFR) as well as downregulation of antiproliferative and antiviral signaling (especially interferon) [4].

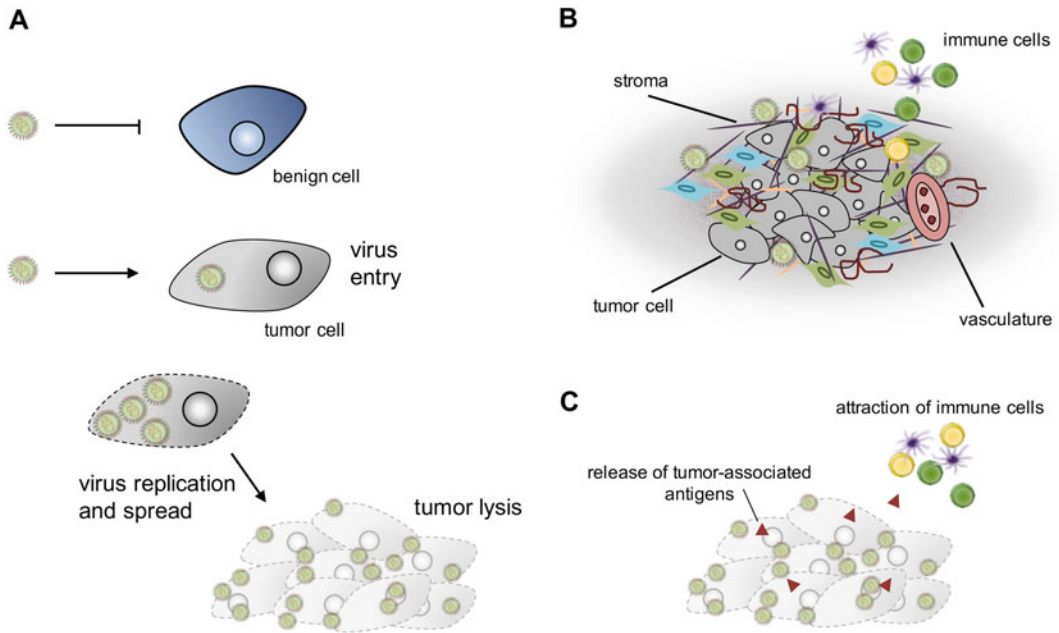


Fig. 1 Principles of oncolytic virotherapy. **(a)** Oncolytic viruses replicate selectively in malignant cells. **(b)** Oncolysis reshapes the tumor microenvironment. **(c)** Exposure of tumor antigens in the context of oncolysis can elicit tumor vaccination effects

A tumor comprises not only individual malignant cells but a complex microenvironment composed of stroma, vasculature, and leukocytes, typically characterized by immunosuppression. Oncolytic virotherapy can act to reshape the local milieu. An acute viral infection serves as a potent stimulus for the immune system. Local inflammation, innate immune activation, and danger signals (DAMPs and PAMPs) arise during viral replication which can change the immune contexture, thereby “turning cold tumors hot” [5].

During oncolysis, tumor-associated antigens are released in this context, which provides adjuvants for induction of adaptive anti-tumor immune responses. Thus, on a systemic level, oncolytic virotherapy can act as an *in situ* tumor vaccine, inducing therapeutic and protective antitumor immunity [6].

Preclinical and clinical data have provided proof of these principles. However, the role and contribution of these mechanisms of action to efficacy of oncolytic virotherapy has been a subject of debate. Moreover, this may depend on the specific oncolytic vector and the therapeutic setting.

2 Oncolytic Vector Platforms: From Adeno to Zika

These principle mechanisms of action outlined above are common to a diverse set of viruses which have been developed as oncolytic vector platforms (Fig. 2). These include the following:

- Small (e.g., parvovirus, approximately 25 nm and 5 kb), large (Vaccinia virus, 300 nm and 200 kb).
- Enveloped (herpes) and nonenveloped (PVSRIPO, derived from polio).
- DNA (adeno), RNA positive (Coxsackie) and negative (Maraba), and double-stranded (reovirus) RNA viruses as well as retroviruses (Toca 511, derived from amphotropic murine leukemia virus).
- Human (mumps), animal (Newcastle disease, vesicular stomatitis, myxoma).
- Pathogenic (influenza, Zika) and live-attenuated (measles) viruses.

These diverse viruses have been tested in preclinical studies and many have advanced to clinical trials. Overall, the clinical data have

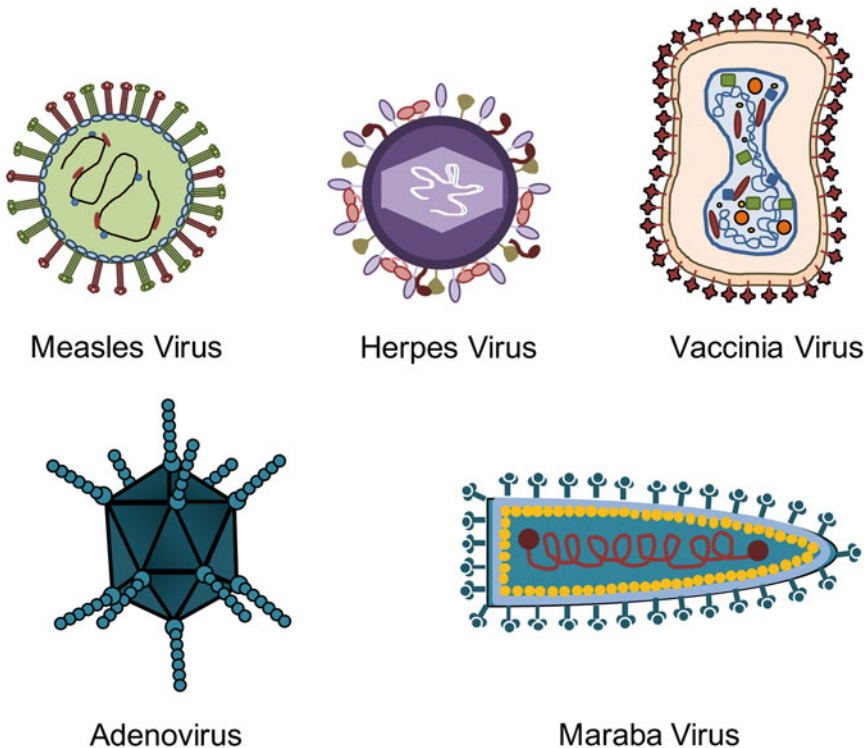


Fig. 2 Schematic depictions of five representative oncolytic viruses

demonstrated safety and typically mild, often flu-like symptoms as adverse events as well as some promising results in terms of anti-tumor efficacy [7]. While the adenovirus Oncorine H101 has been licensed for treatment of nasopharyngeal cancer in China since 2005, 2015 marked the approval of the herpes virus talimogene laherparepvec for treatment of advanced melanoma in the USA and Europe. Thus, the paradigm of using replicating viral vectors for cancer treatment has entered clinical practice.

To date, systematic head-to-head comparisons of these diverse viruses have not been performed. Viruses which have evolved a specific tissue tropism, conceivably, may be especially adapted to replicate in tumors originating from these tissues. In addition to the range of naturally occurring oncolytic viruses, the possibilities opened by genetic engineering offer a plethora of treatment options with vectors designed for specific therapeutic purposes.

3 Vector Design: Tumor Targeting and Spread, Tracers, Therapeutic Genes

Progress in molecular biology including the development of reverse genetics systems has enabled the design of oncolytic therapeutics with improved properties (Fig. 3) [8]. Main arenas of vector design include *tumor targeting* to increase specificity, which can be achieved on the entry level by modifying receptor tropism or incorporating matrix metalloproteinase cleavage sites into viral surface proteins. Targeting on the post-entry level can be achieved by placing viral genes under transcriptional control of a tumor-specific promoter, inserting target sites for microRNAs with differential expression in healthy and malignant cells or deletion of virulence

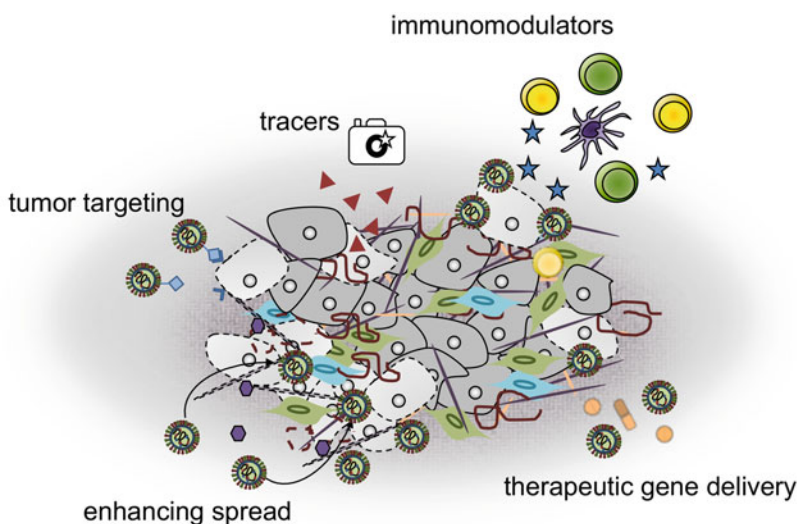


Fig. 3 Strategies to improve efficacy of oncolytic viruses by rational vector design